BIG DATA, PHARMACOGENOMICS AND REAL-WORLD RESEARCH IN PHARMACOLOGY

EDITED BY: James Cheng-Chung Wei, Taisei Mushiroda and Wei-Chiao Chang
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BIG DATA, PHARMACOGENOMICS AND REAL-WORLD RESEARCH IN PHARMACOLOGY

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EDITORIAL
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Editorial: Big Data, Pharmacogenomics and Real-World Research in Pharmacology

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Editorial on the Research Topic

Big Data, Pharmacogenomics and Real-World Research in Pharmacology

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INTRODUCTION

Big data is characterized by large volume, velocity of volume increase, and variety of information that requires specific technology and analytical methods to derive useful knowledge for clinical applications. Big data research in biomedical science has the potential to directly affect personalized and precision medical care, reduce costs of treatment, predict out breaks of epidemics, avoid preventable diseases, and improve the quality of life and clinical practice. Through advances in bioinformatics and medical information systems, big data research is now a hot topic in omics approaches and epidemiological studies.

A parallel trend with big data and clinical researches is the development of real-world studies (RWS). The health authorities had increasingly recognized the critical role of high-volume, real-world data, including electronic medical data, post-marketing surveillance, and claim-based databases, as an important reference of drug approval and pharmacovigilance. With the increasing volume, velocity, and variety of information, the trend of RWS is reaching the big data level.

In this topic "Big Data, Pharmacogenomics and Real-World Research in Pharmacology", we aimed for studies of pharmacogenomics and pharmacogenetics using big data approaches, studies of real-world registry or cohort studies in therapeutics, claim-based health database, and omics-level big data studies. We received 29 manuscripts globally from March 2019 to January 2020. Finally, 16 manuscripts were accepted for publication and 13 were rejected. The acceptance rate was 55%. We herein thank all authors and reviewers' great contributions to this important topic. Some manuscripts are highlighted below.

REAL-WORLD STUDIES

Real-world evidence on a big data level can compensate the deficit of clinical trials, especially on the long-term effectiveness and safety of drugs. In this issue, Wessie et al. described the use of opioids

increases with age in older adults from the Nivel Primary Care Database, which includes 283,600 patients in the Netherlands from 2005 to 2017. Fernandez et al. reported the off-label use of antineoplastic in oncology is limited but has notable scientific support in a university hospital setting. Tsai T-L et al. disclosed the association between the usage of colchicine and pneumonia in a nationwide, population-based cohort study. Yeh et al. reported the relationship of the usage of statin and vital organ failure in patients with asthma-chronic obstructive pulmonary disease overlap in a time-dependent population-based study. They nicely demonstrated that statin use was associated with vital organ failure, including the heart, lung, and renal failures in patients with asthma-chronic obstructive pulmonary disease overlap. We appreciated Ji et al. who demonstrated the effectiveness of subcutaneous tumor necrosis factor inhibitors in patients with ankylosing spondylitis (AS) from 804 patients with AS in China.

BIG DATA AND REGISTRY STUDIES

Several big data studies were retrieved from the National Taiwan Insurance Research Database (NHIRD) (Davis and Huang, 2008), a 20-year nationwide, population-based dataset (Hsing and Ioannidis, 2015; Wu and Lee, 2016). Wei et al. reported an increased risk of sulpiride-induced parkinsonism in patients with peptic ulcer and gastroesophageal reflux disease. Lin et al. found that flunarizine use might induce parkinsonism in patients with migraine. Tsai S-H et al. described a long-term evidence of incidence of hypothyroidism associated with surgical procedures for thyroid disorders. Big data is also a powerful tool to investigate healthcare costs and utilization (Hsu et al., 2018). For example, Chen et al. published a comparison of healthcare costs and utilization in rheumatoid arthritis (RA) patients receiving biological and conventional synthetic disease-modifying drugs, which shows a solid pharmaco-economic evidence of RA therapies.

BIG DATA AND ARTIFICIAL INTELLIGENCE STUDIES

Merging big data and artificial intelligence would be an even more powerful tool in biomedical research. For example, Mo et al. successfully used machine learning technique to predict clinical response of methotrexate treatment in juvenile idiopathic arthritis patients. Noguchi et al. did a nice review of statistical methodologies for detecting drug–drug interactions by using spontaneous reporting systems. Lee et al. developed a proteotranscriptomic-based computational drug-repositioning method for Alzheimer's disease (AD) that might be a shortcut to discover new efficacy of drugs for AD. Zamami et al. searched for therapeutic agents for cardiac arrests by using "TargetMine", a drug discovery tool and large-scale medical information database. They extracted data from the Japan Medical Data Center (JMDC) claims database and found that isosorbide dinitrate, nitroglycerin, and nicardipine may be novel therapeutic agents to improve prognosis of cardiac arrest patients.

Overall, we believed the topic, "Big Data, Pharmacogenomics and Real-World Research in Pharmacology", is a fruitful collection of big data and real-world studies. We wish the readers of Frontiers in Pharmacology would enjoy it.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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The Association Between Usage of Colchicine and Pneumonia: A Nationwide, Population-Based Cohort Study

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Objectives: A previous study suggested that colchicine may cause leukopenia and increase the risk of infection, such as pneumonia. Thus, we investigated the potential relationship between colchicine use and risk of developing pneumonia.

Methods: Data were collected from Taiwan's National Health Insurance Research Database (NHIRD), a nationwide, population-based database. A 13-year retrospective cohort study was conducted, and all investigated subjects were identified by International Classification of Disease, Ninth Revision, Clinical Modification, codes between 2000 and 2012. Propensity score matching was applied to adjust for potential confounding variables, and then Cox proportional hazard model was used to evaluate the hazard ratio (HR) of pneumonia in gout patients and its associations with colchicine use, colchicine dosage, and days of colchicine use.

Results: A total of 24,410 gout patients were enrolled in this study, including 12,205 cases who were treated with colchicine (colchicine group) and 12,205 cases who did not receive colchicine (non-colchicine group). The overall incidence rates of pneumonia in the colchicine group and non-colchicine group were 18.6 and 12.6 per 1,000 person-years, respectively. The colchicine group had a higher risk of pneumonia as compared with the non-colchicine group [adjusted HR, 1.42; 95% confidence interval (CI), 1.32 to 1.53; $P < 0.05$]. High cumulative dose and days of colchicine use notably increased the risk of contracting pneumonia.

Conclusion: This nationwide population-based cohort study reveals that gout patients taking colchicine are at increased risk of developing pneumonia compared with gout patients who do not use colchicine. Therefore, it is crucial that gout patients being treated with colchicine be given the minimally effective dosage for the shortest possible duration to minimize their risk of pneumonia.

Keywords: gout, pneumonia, colchicine, cohort, population-based study

INTRODUCTION

Gout is the most common form of inflammatory arthritis and has a considerable deleterious impact on daily life (Roddy and Choi, 2014; Kuo et al., 2015b). According to a nationwide population study, the prevalence of gout in Taiwan was reported to be 6.24% in 2010 (Kuo et al., 2015a). Colchicine is an anti-inflammatory drug that is effective for treating and preventing gouty arthritis (Hainer et al., 2014).

Recent studies have suggested that colchicine may inhibit neutrophil function (Asako et al., 1992; Cronstein et al., 1995; Chia et al., 2008). It has also been reported that colchicine may exert an immuno-suppressive effect (Dalbeth et al., 2014). Moreover, *in vivo* research on myocarditis revealed that colchicine may not be suitable for treating patients with viral myocarditis because it can exacerbate the severity of viral infection in both the heart and the pancreas (Smilde et al., 2016). Moreover, an early case report showed that neutropenia may occur in patients using the recommended colchicine dosage (Dixon and Wall, 2001). Another case report showed that a patient with colchicine administration developed leukopenia, a disease that renders patients prone to infectious disease, such as pneumonia (Beggs et al., 2012). Furthermore, a cohort study showed that infections in gout patients may be attributable to colchicine (Spaetgens et al., 2017).

Pneumonia is the most serious infectious disease of the respiratory system and was the third highest cause of mortality in Taiwan in 2017 (Shen et al., 2016). Since colchicine might impair normal immunity, it is crucial to gain a better understanding of the relationship between colchicine and pneumonia using a long-term population-based database (Spaetgens et al., 2017).

METHODS

Database

Taiwan's National Health Insurance Research Database (NHIRD) was established in 1995, and currently contains comprehensive health care data for almost all Taiwanese citizens. The database is composed of all National Health Insurance (NHI) claims data, and includes information on hospitalization, emergency care, and medical visits. Taiwan's NHI program had a coverage rate >99% in 2010, and thus the NHIRD contains data for approximately 23 million beneficiaries (Hsing and Ioannidis, 2015; Su et al., 2018). In Taiwan, 93% of medical institutes were contracted by the NHI. The NHIRD releases anonymized data for medical research and is one of the world's largest medical databases of its kind.

The Longitudinal Health Insurance Research Dataset 2000 (LHIRD 2000) contains the clinical information of 1 million beneficiaries randomly selected from the NHIRD during the period 2000 to 2013 (Chen et al., 2018). Moreover, the diagnosis in the LHIRD is made by physicians using the International Statistical Classification of Diseases and Related Health Problems, 9th Revision, Clinical Modification (ICD-9-CM). In Taiwan, patients' medical data include drug items and their corresponding NHI code, dosage, frequency of use, and number of days prescribed. This information can be used for detecting drug interactions and potential duplicate medications when patients visit multiple hospitals. Every NHI code corresponds to a code in the five-level ATC classification system recommended by the World Health Organization (WHO) for studies on drug utilization (Hsu et al., 2011).

Study Design

This population-based nationwide retrospective cohort study analyzed data from Taiwan's National Health Insurance Research Database from 2000 to 2013. The study was approved by the institutional review board of Chung Shan Medical University Hospital, with IRB number CS17114.

Patients Selection

We identified 1 million people from the database. First, we selected patients 20 years or older with newly diagnosed gout, based on the ICD-9-CM code 274, from 2000 to 2012. To ensure disease code accuracy, we selected patients whose clinical history included at least three outpatient visits or one hospitalization. Second, individuals were divided into two groups.

The colchicine group comprised individuals who had used colchicine within 1 year after diagnosis of gout. The non-colchicine group comprised individuals who had never used colchicine. We excluded gout patients who did not take colchicine within 1 year. This process makes our patient groups more specific to the relation between gout and colchicine. Therefore, we set 1 year after gout diagnosis as our index date. Next, to confirm new-onset pneumonia, we excluded cases diagnosed with pneumonia before the index date in both groups. Then, we matched the two groups based on propensity score in a 1:1 ratio by age, gender, hypertension, chronic liver disease (CLD), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), and gout diagnosis year. After performing propensity score matching, gout patients were precisely distributed into two groups, i.e., with and without colchicine use. Hence, it was possible to observe differences in the rates of new-onset pneumonia between the two groups and to determine factors associated with pneumonia in gout patients taking colchicine.

Endpoints

Pneumonia was defined as a diagnosis with one or more of the following ICD-9-CM codes: 481, 482, 483, 485, and 486. To ensure the diagnosis, an inpatient or emergency diagnosis of pneumonia was also required for inclusion in the study. To find the relation between colchicine and pneumonia, the diagnosis date of pneumonia had to be at least 1 year after the diagnosis of gout. Patients were followed until pneumonia was diagnosed, withdrawal from the NHI, or until the end of 2013. The abovementioned definitions are for the dependent variables, and

Abbreviations: aHR, adjusted HR; CKD, chronic kidney disease; CLD, chronic liver disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HR, hazard ratio; ICD-9-CM, The International Statistical Classification of Diseases and Related Health Problems, 9th Revision Clinical Modification; LHIRD 2000, Longitudinal Health Insurance Research Dataset 2000; NHI, National Health Insurance; NHIRD, National Health Insurance Research Database; WHO, World Health Organization.

the following definitions are for the independent variables. The study period was January 1, 2000, to December 31, 2013. All gout patients were diagnosed with three outpatient or one inpatient (ICD-9-CM = 274) during the period 2000 to 2012.

Colchicine use was defined as a prescription with one of the following National Health Insurance (NHI) codes: A005225100, A0217541G0, A022077100, A022534100, A021754100, A030 396100, A0303961G0, A041316100, A0413161G0, A046680100, A048749100, A0487491G0, A054643100, AC54643100, B022 246100, N006271100, and N0062711G0 in the study period.

The age of the patients was defined on the index date. For the sex variable, 0 represented females and 1 represented males. We selected the comorbidities of pneumonia. Two outpatient visits or one inpatient diagnosis of comorbidities of pneumonia a year after the index date was required to ensure diagnostic accuracy. The baseline characteristics were age, gender, hypertension (ICD-9-CM = 401-405), CLD (ICD-9-CM = 571), CKD (ICD-9-CM = 585), COPD (ICD-9-CM = 490-492, 494, 496), and DM (ICD-9-CM = 250).We defined that 0 represent patients who had no comorbidities were represented by 0, those who developed a comorbidity were represented by 1. Then, we adjusted for age, gender, hypertension, CLD, CKD, COPD, and DM by applying propensity score matching to eliminate any bias that might confound the identification of differences between gout patients with and without colchicine use.

Statistical Analysis

The comparison of incidental pneumonia between the colchicine group and non-colchicine group was done by Chi-square test and independent t-test. Kaplan-Meier analysis was applied to evaluate the cumulative incidence of pneumonia in the two groups and the log-rank test was to test whether it was significant. Cox proportional hazard model was used to evaluate the hazard ratio (HR) of pneumonia in relation to colchicine dosage as well as days of colchicine use, after adjustment for potential confounding variables (Austin, 2011). Subgroup analysis by age, gender, hypertension, CLD, CKD, COPD, and DM was performed after applying the Cox proportional hazard model. SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) statistical software was used for the analyses. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 24,410 gout patients with and without colchicine use, who had been selected from the NHIRD, were included in the final analysis after propensity score matching. The flowchart of the study population selection protocol is shown in **Figure 1**.

Table 1 shows that the colchicine group and non-colchicine group both had similar age and gender distributions after propensity score matching. Moreover, we found that males and

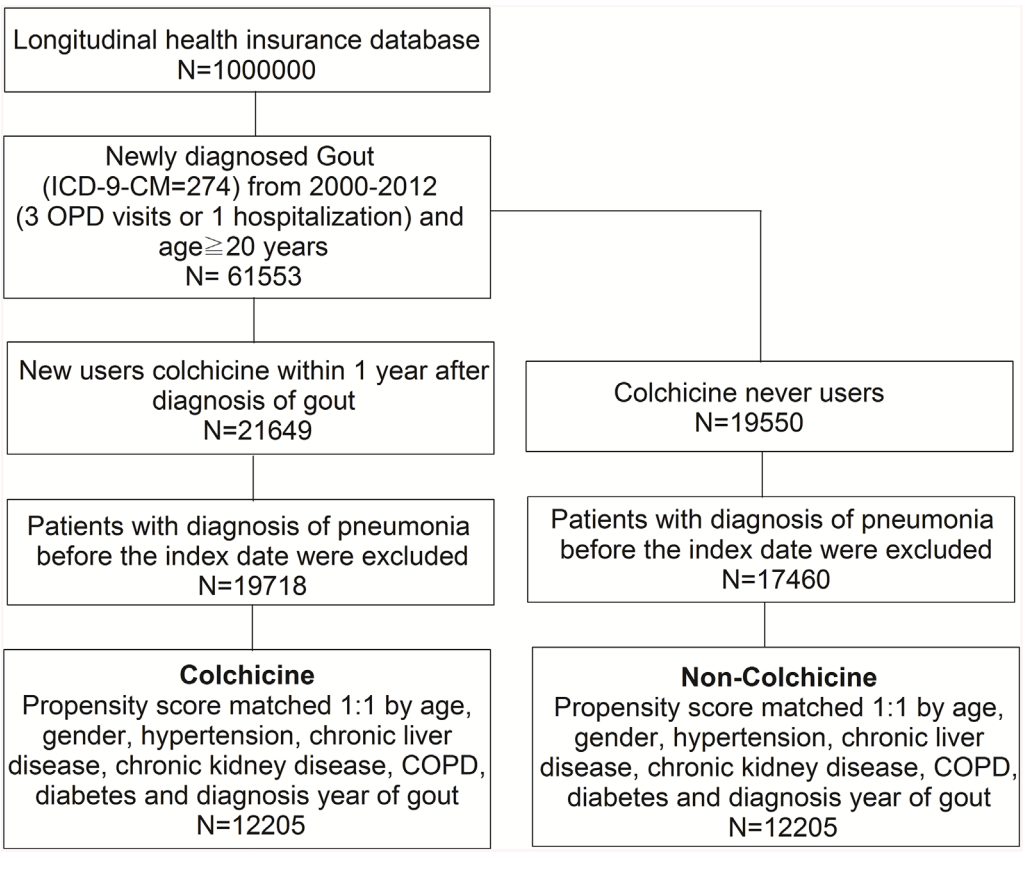

FIGURE 1 | The study population selection protocol.

patients aged 20 to 65 years predominated. The mean ages of the colchicine and non-colchicine groups were 55 (SD = 16) and 54.1 (SD = 15.3) years, respectively. As compared with the non-colchicine group, the colchicine group had a higher prevalence of hypertension at baseline ($p < 0.05$). The prevalence rates of the other comorbidities, i.e., CLD, CKD, COPD, DM, did not show significant differences between the two groups ($p > 0.05$).

As shown in **Figure 2**, the results of Kaplan-Meier analysis revealed that the cumulative incidence of pneumonia rose over time. The trend can be observed in both the colchicine and non-colchicine groups. Furthermore, the colchicine group showed a higher cumulative incidence of pneumonia than the non-colchicine group (log-rank test, $p < 0.001$).

Table 2 demonstrates that the overall incidence rates of pneumonia, in the colchicine group and non-colchicine group, were 18.6 and 12.6 per 1,000 person-years, respectively. After adjustment for age, gender, and comorbidities, the colchicine group exhibited a higher risk of pneumonia compared with the non-colchicine group [adjusted HR (aHR), 1.42; 95% confidence interval (CI), 1.32 to 1.53]. The incidence of pneumonia was higher in gout patients 65 years or older than in those aged 20 to 65 years and was higher in males than in females. Using a Cox proportional hazard model, the adjusted HR of pneumonia was not only 4.41-fold higher in gout patients 65 years or older than in those aged 20 to 65 years (95% CI, 4.06–4.80), but was also 1.26-fold higher in males than in females (95% CI, 1.16–1.37). Moreover, the risk of pneumonia was higher in patients with hypertension, CKD, COPD, and DM ($p < 0.05$).

In **Table 3**, the result of the subgroup analysis revealed that the aHRs of the colchicine group compared with the non-colchicine group based on demographic factors and comorbidities. Patients aged 20 to 65 years and 65 years or older in the colchicine group were more likely to develop pneumonia than their respective counterparts in the non-colchicine group. Moreover, with respect to gender, there was a significant difference in the incidence of pneumonia between the colchicine group and the non-colchicine group. In the colchicine group, all of the selected comorbidities were associated with increased risk of pneumonia. Moreover, the greatest magnitude of aHR could be observed in patients with chronic liver disease.

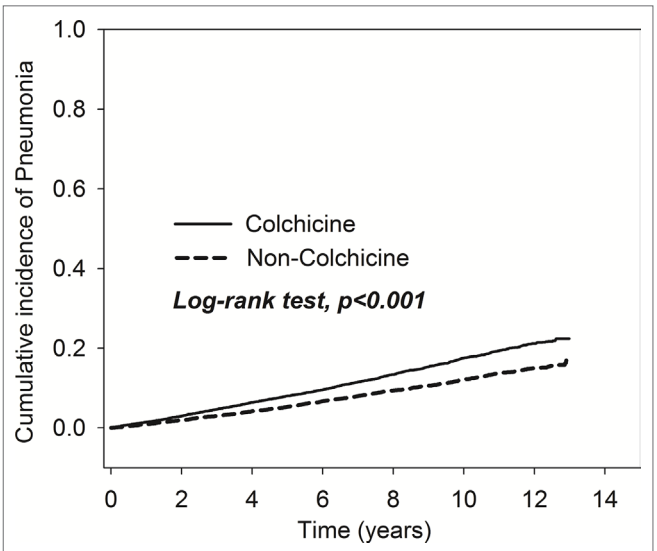

FIGURE 2 | Comparison of cumulative incidences of pneumonia in the patients with and without taking colchicine.

Table 4 shows the risk of developing pneumonia based on cumulative days of colchicine use. Individuals taking colchicine for less than 8 days, more than 8 days but less than 32 days, and more than 33 days had aHRs of 1.33 (95% CI, 1.20-1.48), 1.45 (95% CI, 1.31-1.60), and 1.47 (95% CI, 1.33-1.62), respectively. That is to say, the risk of developing pneumonia increased with duration of colchicine use. Cumulative dose of colchicine was divided into three groups: less than 9 mg, more than 9 mg but less than 24 mg, and more than 24 mg. The aHRs were 1.38 (95% CI, 1.25-1.53), 1.43 (95% CI, 1.29-1.58), and 1.45 (95% CI, 1.31-1.60), respectively. The risk of pneumonia increased with cumulative dose of colchicine.

TABLE 1 | Demographic characteristics of gout patients treated with and without colchicine.

	Before PS matched		*p*-value	After PS matched		*p*-value[a]
	Colchicine (N = 19,718), n (%)	Non-Colchicine (N = 17,460), n (%)		Colchicine (N = 12205), n (%)	Non-Colchicine (N = 12205), n (%)	
Age, year			<0.001			**<0.001**
20–65	15,548 (78.9)	12,543 (71.8)		8,560 (70.1)	8,978 (73.6)	
≧65	4,170 (21.1)	4,917 (28.2)		3,645 (29.9)	3,227 (26.4)	
Mean ± SD	49.8 ± 16.6	55.4 ± 14.8	<0.001	55 ± 16	54.1 ± 15.3	**<0.001**
Gender			<0.001			0.108
Female	3,582 (18.2)	7,359 (42.1)		3,582 (29.3)	3,697 (30.3)	
Male	16,136 (81.8)	10,101 (57.9)		8,623 (70.7)	8,508 (69.7)	
Hypertension	5,530 (28)	7,458 (42.7)	<0.001	4,908 (40.2)	4,738 (38.8)	**0.026**
Chronic liver disease	1,860 (9.4)	3,139 (18)	<0.001	1,836 (15)	1,765 (14.5)	0.200
Chronic kidney disease	343 (1.7)	332 (1.9)	0.243	248 (2)	246 (2)	0.928
COPD[b]	943 (4.8)	1057 (6.1)	0.688	751 (6.2)	690 (5.7)	0.098
Diabetes	1,793 (9.1)	3,482 (19.9)	<0.001	1,743 (14.3)	1,835 (15)	0.096

[a]*Bold font represents statistical significance (p< 0.05).*
[b]*COPD, chronic obstructive pulmonary disease.*

TABLE 2 | Analysis of factors affecting pneumonia risk in gout patients treated with and without Colchicine by Cox proportional hazard model.

	No. of Pneumonia event	Observed Person-Years	Incidence Density (Per 1000 Person-Years)	Crude HR (95% CI) [a]	Adjusted HR[c] (95% CI) [a]
Colchicine					
No	1,127	89,638	12.6	1	1
Yes	1,687	90,748	18.6	**1.48 (1.37-1.59)**	**1.42 (1.32-1.53)**
Age					
20-65	1,057	136,667	7.7	1	1
≧65	1,757	43,720	40.2	**5.37 (4.98-5.80**)	**4.41 (4.06-4.80)**
Gender					
Female	828	54,199	15.3	1	1
Male	1,986	126,187	15.7	1.03 (0.95-1.12)	**1.26 (1.16-1.37)**
Hypertension	1,575	64,894	24.3	**2.31 (2.14-2.49)**	**1.33 (1.22-1.44)**
Chronic liver disease	364	28,135	12.9	0.80 (0.71-0.89)	1.01 (0.90-1.13)
Chronic kidney disease	102	2,416	42.2	**2.90 (2.38-3.53)**	**1.75 (1.43-2.13)**
COPD[b]	404	9,566	42.2	**3.02 (2.71-3.35)**	**1.80 (1.61-2.00)**
Diabetes	643	23,187	27.7	**2.04 (1.87-2.23)**	**1.63 (1.49-1.78)**

[a]*Bold font represents statistical significance (p < 0.05).*

[b]*COPD, chronic obstructive pulmonary disease.*

[c]*Adjusted for age, gender, hypertension, chronic liver disease, chronic kidney disease, COPD and diabetes.*

TABLE 3 | Subgroup analysis of pneumonia risk in gout patients with and without colchicine treatment by Cox proportional hazard model.

	Colchicine		Non-Colchicine		
	N	No. of Pneumonia	N	No. of Pneumonia	HR[b] (95% CI)[a]
Age (years)					
20-65	8,560	639	8,978	418	**1.58 (1.39-1.78)**
≧65	3,645	1,048	3,227	709	**1.36 (1.23-1.49)**
Gender					
Female	3,582	505	3,697	323	**1.50 (1.31-1.73)**
Male	8,623	1,182	8,508	804	**1.40 (1.28-1.53)**
Hypertension					
No	7,297	735	7,467	504	**1.46 (1.31-1.64)**
Yes	4,908	952	4,738	623	**1.41 (1.27-1.56)**
Chronic liver disease					
No	10,369	1,456	10,440	994	**1.41 (1.30-1.53)**
Yes	1,836	231	1,765	133	**1.57 (1.27-1.94)**
Chronic kidney disease					
No	11,957	1,629	11,959	1083	**1.42 (1.32-1.53)**
Yes	248	58	246	44	1.37 (0.91-2.08)
					p for interaction = 0.869
COPD					
No	11,454	1,451	11,515	959	**1.43 (1.32-1.55)**
Yes	751	236	690	168	**1.40 (1.14-1.71)**
Diabetes					
No	10,462	1,299	10,370	872	**1.39 (1.28-1.52)**
Yes	1,743	388	1,835	255	**1.53 (1.30-1.79)**

[a]*Bold font represents statistical significance (p < 0.05).*

[b]*Adjusted for age, gender, hypertension, chronic liver disease, chronic kidney disease, chronic obstructive pulmonary disease (COPD), and diabetes.*

DISCUSSION

This is the largest real-world study on the risk of pneumonia in gout patients using colchicine. We found that colchicine increased the risk of contracting pneumonia in gout patients. Our results showed a 42% greater hazard of pneumonia in gout patients using colchicine compared with gout patients not using colchicine. The risk of pneumonia risk was higher in older gout patients than in younger gout patients. It was also higher in males than in females, and in people with comorbidity compared with those without comorbidity. In addition, risk of developing pneumonia increased in proportion to colchicine dosage and duration of use.

Spaetgens et al. (2017) investigated infections in gout patients, including risk of pneumonia in colchicine users. Their results showed that patients with gout who used colchicine >31 days had a higher risk of contracting pneumonia. In contrast, gout patients who used colchicine ≦30 days had a lower risk, which was not consistent with our results. However, in the present study, the studied population was far larger than that in the UK study, and

TABLE 4 | Analysis of pneumonia risk in gout patients based on Colchicine dose and duration using Cox proportional hazard model.

	N	No. of Pneumonia events	Observed Person-Years	Incidence Density (Per 1000 Person-Years)	Crude HR (95% CI)[a]	Adjusted HR[b] (95% CI)[a]
Cumulative days of Colchicine use						
No	12,205	1,127	89,638	12.6	1	1
<8 days	3,890	481	29,962	16.1	**1.27 (1.15-1.42)**	**1.33 (1.20-1.48)**
8-32 days	4,207	587	31,204	18.8	**1.50 (1.35-1.65)**	**1.45 (1.31-1.60)**
≧33 days	4,108	619	29,583	20.9	**1.66 (1.51-1.84)**	**1.47 (1.33-1.62)**
Cumulative dose of Colchicine use						
None	12,205	1,127	89,638.43	12.6	1	1
Low (<9 mg)	3,916	529	29,599.42	17.9	**1.42 (1.28-1.58)**	**1.38 (1.25-1.53)**
Median (9-24 mg)	4,198	552	31,262.56	17.7	**1.40 (1.27-1.55)**	**1.43 (1.29-1.58)**
High (≧24 mg)	4,091	606	29,886.03	20.3	**1.61 (1.46-1.78)**	**1.45 (1.31-1.60)**

[a]*Bold font represents statistical significance ($p < 0.05$).*

[b]*Adjusted for age, gender, hypertension, chronic liver disease, chronic kidney disease, chronic obstructive pulmonary disease (COPD), and diabetes.*

therefore, our findings may have more accurately reflected the relationship between colchicine and pneumonia.

The mechanism of colchicine to relieve gout involves the inhibition of microtubule formation (Andreu and Timasheff, 1982; Luduena and Roach, 1991). However, microtubule polymerization is related to many cell functions, such as intracellular vesicle transport, secretion of cytokines and chemokines, cell migration, cell division, and regulation of gene expression (Caviston and Holzbaur, 2006). Disruption of microtubule formation may cause adverse effects. A number of reactions resulting from colchicine usage may explain the increased risk of pneumonia infection in gout. First, NADPH oxidase mediates the production of superoxide anions by neutrophils. The assembly of the NADPH oxidase complex is disrupted by interference of microtubule polymerization. Therefore, by using low doses of colchicine, superoxide produced by neutrophils can be inhibited (Chia et al., 2008). Moreover, superoxide is a factor used by neutrophils to fight against viruses and bacteria (Winterbourn et al., 2016). Thus, if the NADPH oxidase-superoxide system is weakened, there is a greater likelihood of pneumonia infection. Second, by decreasing neutrophil L-selectin expression and changing the distribution of E-selectin on endothelial cells, colchicine can decrease neutrophil recruitment and adhesion to inflamed tissues (Cronstein et al., 1995). Accordingly, as the effectiveness of neutrophils passing through blood vessels has been reduced, they may not be able to reach tissue and fight against bacteria. Third, leukotriene B4 (LTB4) is a mediator of inflammation and a chemo-attractant. LTB4 also takes part in promoting the adhesion and mobility of neutrophils. Colchicine dramatically decreases leukocyte adherence and emigration induced by LTB4 (Asako et al., 1992). Paschke et al. investigated the effect of colchicine on the regulation of cell motility. They postulated that colchicine could modulate the stiffness, elasticity, and viscosity of neutrophils through the reorganization of subcellular compartments (Paschke et al., 2013). Hence, colchicine is thought to affect the motility and deformability of neutrophils in specific places at therapeutic doses. To date, the mechanism of the relationship between colchicine and pneumonia remains unclear, although intriguing recent evidence has shed light on the apparent immuno-suppressive characteristic of colchicine. It is known that the cytochrome P450 (CYP) 3A4 enzyme can metabolize colchicine. Recently, Dalbeth et al. confirmed that combination of colchicine and CYP3A4 inhibitors, such as cyclosporin, tacrolimus, and imidazole, may increase intracellular accumulation of colchicine, which can lead to increased infections (Dalbeth et al., 2014; Stack et al., 2015). The abovementioned findings support our hypothesis that colchicine, an immunosuppressive drug widely used in the treatment of gout, weakens the immune system, rendering the patient prone to pneumonia infection.

This is the first study to investigate the relationship between cumulative doses of colchicine and pneumonia. We postulated that colchicine was a risk factor and applied 1:1 propensity score matching to adjust for demographic factors and comorbidities. A major strength of this study was the use of a nationwide population-based database (NHIRD). The NHIRD allows researchers to readily determine the incidence and correlations of selected factors for virtually the entire population of Taiwan. Also, the retrospective nature of the study design minimized any potential selection bias, reference bias, and participant bias in this population-based study. The analysis of data obtained from the NHIRD for the period 2000 to 2013 in Taiwan revealed that gout patients taking colchicine had a significantly greater risk of developing pneumonia, and there was also a significant dose-dependent effect.

Our study had several limitations. First, information about lifestyle, such as smoking habit and alcohol consumption, are not collected in the claims-based insurance database. To reduce this bias, we adjusted for COPD, which would cover smoking habit, chronic liver disease, which would reflect alcohol consumption, and other comorbidities. Second, there is no genomic variables in this study because the NHIRD does not record related data. Third, all of the diagnoses in the NHIRD were made by physicians using ICD-9-CM codes. Thus, the severity or pathogen of pneumonia was not available in this database. Patients with mild pneumonia may not have been included in our analysis because we selected pneumonia patients diagnosed from emergency visits or admissions as well as gout patients diagnosed with at least three outpatient visits or one admission to ensure that only patients with an accurate diagnosis were selected. Finally, our study design was retrospective so additional prospective studies are needed to elucidate the causal relationship between colchicine and pneumonia. Furthermore,

risk factors may vary among different countries, and thus further research is necessary to confirm the association found in this study.

In conclusion, this nationwide population-based cohort study revealed that colchicine use was associated with higher risk of pneumonia in gout patients. Therefore, it is crucial that gout patients taking colchicine be prescribed the minimally effective dosage for as short a duration as possible to minimize the risk of developing pneumonia.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

T-LT, JC-CW, Y-TW, Y-HK, K-LL, and Y-HW participated in the design of the study. Y-HW was involved in collecting data and producing tables. T-LT, Y-TW, Y-HK, and K-LL produced the initial draft of the manuscript that was further revised by JC-CW and J-YC. All co-authors reviewed and approved the final version of the manuscript.

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Statin use and Vital Organ Failure in Patients With Asthma–Chronic Obstructive Pulmonary Disease Overlap: A Time-Dependent Population-Based Study

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Objective: The effects of statins on the risk of hepatic, renal, respiratory, and heart failure among patients with asthma–chronic obstructive pulmonary disease overlap (ACO) have not been reported.

Design: Time-dependent population-based study.

Setting: Patient data from 2000 to 2010 were retrieved from the Taiwan National Health Insurance Research Database.

Patients: We divided patients with ACO into cohorts of statin use (N = 1,211) and nonuse (N = 7,443).

Measurements and Main Results: The cumulative incidence rates of hepatic, renal, respiratory, and heart failure were analyzed through Cox proportional regression analysis with time-dependent variables. After adjustment for multiple confounding factors, including age, sex, comorbidities, and medications [statins, inhaled corticosteroid (ICS), or oral steroid (OS)], the adjusted hazard ratios (aHRs) [95% confidence intervals (CIs)] for hepatic, renal, respiratory, and heart failure were 0.50 (0.40–0.64), 0.49 (0.38–0.64), 0.61 (0.27–2.21), and 0.47 (0.37–0.60), respectively. The aHRs (95% CIs) for statin use with [ICS, OS] for hepatic, renal, and heart failure were [0.36 (0.20–0.66), 0.52 (0.39–0.70)]; [0.82 (0.51–1.34), 0.46 (0.33–0.63)]; and [0.66 (0.40–1.07), 0.48 (0.37–0.64)], respectively.

Conclusions: The ACO cohort with statin use exhibited lower risk of hepatic, renal, and heart failure than any other cohort, regardless of age, sex, comorbidities, or ICS or OS use. Regarding the combined use of statins and ICS, the risks of hepatic failure were lower. For the combined use of statins and OS, hepatic, renal, and heart failure were less frequent.

Keywords: asthma–chronic obstructive pulmonary disease overlap (ACO), hepatic failure, renal failure, heart failure, respiratory failure

INTRODUCTION

Multiple organ failure is a serious concern in hospitals (Valley et al., 2015). It can include respiratory failure (hypoxemia), reduction in the ration of oxygen pressure in arterial blood (PaO2) to inspired oxygen fraction (FiO2), a requirement for mechanical ventilation, cardiac failure (hypotension unresponsive to adequate fluid resuscitation and requiring vasopressors), renal failure (diminished urine output), increased serum creatinine, and hepatic failure (bilirubin increased, liver function impaired, prothrombin time prolonged). Respiratory failure is usually followed by cardiac, renal, and hepatic failure (Valley et al., 2015). Multiple organ failure is a clinical syndrome characterized by the functional deterioration of two or more organs or organ systems. Evidence suggests that sepsis is the most frequent cause of organ function deterioration. The pathophysiology is the following: 1) Monocyte stimulation by exotoxins and endotoxins results in the release of proinflammatory cytokines [e.g., tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)], increased susceptibility of monocytes to aggregation on the endothelium, and enhanced release of other inflammatory and anti-inflammatory cytokines. 2) Stimulation of platelet aggregation and thrombin formation result in thrombosis in microvasculature and impaired circulation in individual organs or organ systems (Chaudhry et al., 2013). Therefore, this process causes a cascade of events in multiple organ failure, and multiple organ failure may be considered as systemic inflammation.

High-density lipoprotein cholesterol decreases and low-density lipoprotein cholesterol increases before heart failure, renal failure, and liver failure. This dyslipidemia was also found in chronic obstructive pulmonary disease (COPD) and asthma.

Statins are cholesterol-lowering drugs used for primary or secondary prevention of cardiovascular diseases (Afilalo et al., 2007). In addition to the primary effect, statins act beneficially through different pleiotropic mechanisms on inflammation, fibrosis, endothelial function, thrombosis, and coagulation to ameliorate chronic liver diseases (Jones, 2006). Statins also affect the airways (Wang et al., 2015) and pulmonary vessels (Zhang et al., 2017). However, this effect in multiple organ failure is speculative (Omar et al., 2017).

Asthma–COPD overlap (ACO) is a disorder combining the components of both COPD and asthma. A higher frequency of cardiovascular disease, stroke, pneumonia, pulmonary embolism, and tuberculosis was associated with acute exacerbation of COPD or asthma (Yeh et al., 2018). Consequently, the incidence of cardiac, hepatic, renal, and respiratory failure increased (Llanos et al., 2018). The roles of statins, inhaled corticosteroid (ICS), and oral steroid (OS) in these vital organ failures are unclear from the English literature. Therefore, we identified this topic based on the general population.

Abbreviation: ACO, asthma–chronic obstructive pulmonary disease overlap; ICS, inhaled corticosteroid; OS, oral steroid; aHR, adjusted hazard ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

MATERIAL AND METHODS

Data Source

This retrospective cohort study based on the Longitudinal Health Insurance Database selected 1 million insurants from the Taiwan National Health Insurance (NHI) program from 2000 to 2011. Implemented in 1995, the NHI program supplies comprehensive medical care embodying ambulatory and inpatient care for almost all of Taiwan's population (Database NHIR). Details of the NHI program are available in a previous study (Wang et al., 2018). Diseases were defined by the *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) codes. The Research Ethics Committee of China Medical University and Hospital in Taiwan approved the study (CMUH104-REC2-115-CR3).

Sampled Participants

This retrospective cohort study analyzed the effects of statins, ICS, and OS exposure on patients with ACO. The prevalence of ACO among young adults was found in many studies in the real world (de Marco et al., 2013; de Marco et al., 2015; Ekerljung et al., 2018), especially in the aboriginal population (Koleade et al., 2018). We identified patients aged 20 years and above with ACO and diagnosed with COPD (*ICD-9-CM* codes 491, 492, and 496) from January 1, 2000, to December 31, 2010. The date of the first diagnosis of asthma (*ICD-9-CM* code 493) was selected as the index date. These patients had experienced at least one COPD or asthma-related examination such as a pulmonary function test (PFT), immunoglobulin E (IGE), eosinophil count with chest X-ray (CXR), or chest computer tomography (CT). In Taiwan, the demographic characteristics reveal that 82.9% of COPD patients were smokers, 84.7% had abnormal CXR (e.g., emphysema or chronic bronchitis), and 58.7% had a PFT (Ho et al., 2018). The sensitivity of the diagnosis of asthma and COPD were high, up to 92.0% and 86.2%. Meanwhile, we used the ICS and OS use status of the patients for confirming asthma–chronic pulmonary disease overlap syndrome (ACOS). Thus, deriving the ACO from the COPD cohort in the National Health Insurance Research Database (NHIRD) is reasonable (Yeh et al., 2019). Patients with a record of multiple vital organ failure including hepatic failure (*ICD-9-CM* codes 570, 571, and 572), renal failure (*ICD-9-CM* codes 584, 585, 586, 403.01, 403.11, 403.91, 404.02, 404.03, 404.12, 404.13, 404.92, 404.93, 582, 588, V42.0, V45.1, V56.2, 570, 571, and 572), respiratory failure (*ICD-9-CM* codes 5181, 5182, 5183, and 5184), or heart failure (*ICD-9-CM* code 428) prior to the asthma diagnosis were excluded. Each patient was followed up from the initial asthma diagnosis date until one of the following: the patient was removed from health insurance; hepatic, renal, respiratory, and heart failure occurred; or December 31, 2011. We were primarily interested in the distinction between ACO patients with and without statin exposure. Because the medical follow-up period was dynamic, we computed medicine exposure every 6 months (Zhang et al., 2017; Yeh et al., 2019). Patients who received the medicine at least 30 days after the date of ACO diagnosis were enrolled, and these patients received the statin before multiple vital organ failure (Zhang et al., 2017; Yeh et al., 2019).

Considering the influence of comorbidity and medication, we defined a person as having a certain comorbidity if the disease had been confirmed by an end point of multiple vital organ failure such as hepatic, renal, respiratory, and heart failure. Comorbidities consisted of sleep disorder (*ICD-9-CM* 780.50), diabetes (*ICD-9-CM* 250), hypertension (*ICD-9-CM* 401–405), hyperlipidemia (*ICD-9-CM* 272.0 272.4), coronary artery disease (CAD) (*ICD-9-CM* 410–414), stroke (*ICD-9-CM* 430–438), hepatitis B (*ICD-9-CM* 070.20, 070.22, 070.30, 070.32), and hepatitis C (*ICD-9-CM* 070.40, 070.44, 070.51, 070.54, 070.70, 070.71). Medications were ICS and OS. Patients who received ICS or OS over 30 days from January 2000 to December 2010 were included in this study (Ko et al., 2019). The statin cohort and nonstatin cohort were matched at a 1:1 ratio based on propensity scores (Byrne et al., 2019; Ko et al., 2019) (**Supplement Table 1**).

The World Health Organization (WHO) recommends the use of the defined daily dose (DDD) and prescribed daily dose (PDD) methodology in drug utilization studies. The definition of the DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults. DDDs are assigned by the WHO. For instance, the DDD for atorvastatin is 20 mg. The PDD is defined as the average dose prescribed according to a representative sample of prescriptions. The DDDs for statins remained unchanged from 1997. However, in January 2009, alterations were made to the DDD for five of the six statins in order to better reflect the current daily dosages. The DDDs for atorvastatin and simvastatin have been doubled, whereas for pravastatin, lovastatin, and fluvastatin the DDDs, have been increased by 50% (Godman et al., 2014). There are different DDDs in different stages during this study. To avoid this dosage bias, we used the length of use in days (>30 days) for the analysis (Pedan et al., 2007; Ko et al., 2019).

Statistical Analysis

Student's t-test was used for calculating the mean age, and the chi-square test was used for age, sex, strata of comorbidity, and strata of medication for the statin use and nonuse cohorts. The Kaplan–Meier method was applied to derive the cumulative incidences of hepatic failure, renal failure, and heart failure between statin users and nonusers and to detect differences through log-rank testing.

We defined the incidence rate (IR) as the number of events divided by the total person-years. Because those ACO patients taking statins fluctuated in terms of follow-up, we set statin as a time-dependent covariate in the Cox proportional hazards model to demonstrate the effects as hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). The time-dependent covariate of statins indicated that intrapatient comparison may be performed. The drug exposure variables were quantified as a binary (yes/no) variable every 6 months. The adjusted HRs were estimated after controlling for age, sex, comorbidity, and medication. Furthermore, we dissected the IRs and HRs of multiple vital organ failure including hepatic failure, renal failure, and heart failure according to sex, age, ICS status, and OS status for statin users and nonusers. Analyses were conducted and data collected using SAS 9.4 software (SAS Institute, Cary, NC, USA). Statistical significance was set at $P < 0.05$.

Results

Among 8,654 patients with ACO, 1,211 had been exposed to statins. **Table 1** indicates that the mean age of statin users was less than that of nonusers (62.9 vs. 64.1 years, $P = 0.001$). The distributions of statin users and nonusers differed according to age, sex, strata of comorbidity (except hepatitis B and C), and strata of medication [age, $P < 0.001$; sex, $P < 0.001$; strata of comorbidity (except hepatitis B and C), all $P < 0.001$; strata of medication: ICS, $P = 0.02$; OS, $P < 0.001$].

Table 2 demonstrates that statin users with multiple vital organ failure were respectively 0.58, 0.68, and 0.44 times less likely to experience hepatic, renal, and heart failure than were nonusers (IR per 1,000 person-years: hepatic failure, 8.61 vs. 16.2; renal failure, 7.64 vs. 11.3; heart failure, 7.99 vs. 19.3), with adjusted HRs of 0.5, 0.49, and 0.47 (hepatic failure, 95% CI = 0.40–0.64; renal failure, 95% CI = 0.38–0.64; heart failure, 95% CI = 0.37–0.60). Comparing statin use with nonuse, the adjusted HR for respiratory failure for statin use was 0.61 (0.17–2.21).

Table 3 reveals that women who took statins with multiple vital organ failure were respectively 0.57, 0.66, and 0.36 times less likely to experience hepatic, renal, and heart failure than women who did not (IR: hepatic failure, 7.88 vs. 15.0; renal failure, 6.00 vs. 8.96; heart failure, 5.81 vs. 17.5), with adjusted HRs of 0.47, 0.48, and 0.40 (hepatic failure, 95% CI = 0.33–0.67; renal failure, 95% CI = 0.33–0.72; heart failure, 95% CI = 0.27–0.58). Men who took statins were respectively 0.59 and 0.54 times less likely to experience hepatic and heart failure than men who did not (IR: hepatic failure, 9.45 vs. 17.1; heart failure, 10.5 vs. 20.8), with adjusted HRs of 0.54 and 0.55 (hepatic failure, 95% CI = 0.38–0.75; heart failure, 95% CI = 0.40–0.75). Men who took statins were half as likely to experience renal failure as were men who did not after adjustment for age, comorbidity, and medication (95% CI = 0.36–0.70). Statin users aged ≥50 years were respectively 0.58, 0.62, and 0.40 times as likely to experience hepatic, renal, and heart failure as nonusers aged ≥50 years (IR: hepatic failure, 8.78 vs. 16.5; renal failure, 8.50 vs. 13.8; heart failure, 9.18 vs. 24.7), with adjusted HRs of 0.53, 0.50, and 0.47 (hepatic failure, 95% CI = 0.40–0.68; renal failure, 95% CI = 0.38–0.65; heart failure, 95% CI = 0.37–0.60). Among patients aged <50 years, statin users were 0.55 times as likely to experience hepatic failure as nonusers (IR: hepatic failure, 7.76 vs. 15.2), with an adjusted HR of 0.29 (95% CI = 0.15–0.54). Among patients aged <50 years, statin users were 0.27 times as likely to experience renal failure as nonusers after sex, comorbidity, and medication were held constant (95% CI = 0.10–0.74).

Table 4 indicates that among takers of ICS, those who also took statins were 0.36 times as likely to experience hepatic failure as those who did not, after age, sex, and comorbidity were controlled (95% CI = 0.20–0.66). Likewise, among those who did not take ICS, statin users were respectively 0.59, 0.54, and 0.40 times as likely to experience hepatic, renal, and heart failure as nonusers (IR: hepatic failure, 10.0 vs. 18.8; renal failure, 6.79 vs. 12.9; heart failure, 7.97 vs. 21.7), with adjusted HRs of 0.53,

TABLE 1 | Distribution of demographic and clinical comorbidity data in study cohorts.

Variables	ACO						p-value
	Statin						
	All (N = 8,654)		No (N = 7,443)		Yes (N = 1,211)		
	n	%	n	%	n	%	
Age, years							<0.001***
<50	1,599	(18.5)	1,418	(19.1)	181	(15.0)	
50–64	2,418	(27.9)	1,962	(26.4)	456	(37.7)	
65+	4,637	(53.6)	4,063	(54.6)	574	(47.4)	
Mean (SD)[a]	64.0	14.8	64.1	15.3	62.9	11.9	0.001**
Gender							<0.001***
Women	3,722	43.0	3,090	41.5	632	52.2	
Men	4,932	57.0	4,353	58.5	579	47.8	
Comorbidity							
Sleep disorder	3,578	41.4	2,962	39.8	616	50.9	<0.001***
Diabetes	1,455	16.8	1,093	14.7	362	29.9	<0.001***
Hypertension	5,628	65.0	4,648	62.5	980	80.9	<0.001***
Hyperlipidemia	2,825	32.6	1,898	25.5	927	76.6	<0.001***
CAD	3,576	41.3	2,917	39.2	659	54.4	<0.001***
Stroke	1,415	16.4	1,205	16.2	210	17.3	<0.001***
Hepatitis B	326	3.77	281	3.78	45	3.72	0.92
Hepatitis C	135	1.56	116	1.56	19	1.57	0.98
Medication							
Inhaled corticosteroids (ICSs)	2,064	23.9	1,743	23.4	321	26.5	0.02*
Oral steroids (OSs)	6,319	73.0	5,377	72.2	942	77.8	<0.001***

Chi-square test, [a] t-test.
**P < 0.05, **P < 0.01, ***P < 0.001.*

0.40, and 0.43 (hepatic failure, 95% CI = 0.41–0.69; renal failure, 95% CI = 0.29–0.55; heart failure, 95% CI = 0.32–0.57). Among patients on OS, statin takers were respectively 0.65, 0.61, and 0.47 times as likely to experience hepatic, renal, and heart failure as nontakers (IR: hepatic failure, 8.01 vs. 13.1; renal failure, 6.15 vs. 10.1; heart failure, 7.91 vs. 17.6), with adjusted HRs of 0.52, 0.46, and 0.48 (hepatic failure, 95% CI = 0.39–0.70; renal failure, 95% CI = 0.33–0.63; heart failure, 95% CI = 0.37–0.64). Without OS, statin takers were respectively 0.48 and 0.38 times as likely to experience hepatic and heart failure as nontakers (IR: hepatic failure, 10.9 vs. 25.1; heart failure, 8.28 vs. 24.0), with adjusted HRs of 0.45 and 0.42 (hepatic failure, 95% CI = 0.29–0.71; heart failure, 95% CI = 0.25–0.70); additionally, they were 0.57 times as likely to experience renal failure as nonusers, after adjustment for age, sex, and comorbidity (95% CI = 0.37–0.88).

Propensity score matching for sensitive analysis is shown in **Supplement Table 1** and **Supplement Table 2** (Zhang et al., 2017; Ko et al., 2019). The incidences for hepatic failure in the statin cohort and the propensity score–matched nonstatin cohort were 8.77 and 17.3 per 1,000 person-years, respectively. Statin users had a 0.51-fold lower risk compared with propensity score–matched nonstatin patients (95% CI = 0.38–0.66). Statin users also had a 0.45-fold lower risk of renal failure compared with propensity score–matched nonstatin patients (95% CI = 0.34–0.61). Statin users also had a 0.46-fold lower risk of heart failure compared with propensity score–matched nonstatin patients (95% CI = 0.34–0.61).

Figure 1 illustrates that the cumulative incidences of statin users and nonusers differed significantly for hepatic, renal, and heart failure (log-rank test: hepatic failure, P < 0.001; renal failure, P < 0.001; heart failure, P < 0.001).

Validation of the ACO Cohort

In their study based on the NHIRD in Taiwan, Su et al. reported that the frequency of ICS use among an ACOS cohort was 53.48% at follow-up. In addition, the ACO cohort of Shantakumar et al. (Shantakumar et al., 2018) received ICS in 46.1% of cases and OS in 85.5% of cases at 1-year follow-up. The validity of the *ICD-9-CM* codes for the diagnosis of COPD, as issued from an NHIRD report, was verified by physicians in 63.5% of patients. A high percentage (up to 58.7%) of patients received a PFT. Among the patients who received PFT, three were outpatients, and two were inpatients. In our study, the ACO cohort was derived from the COPD cohort (Ho et al., 2018). In addition, we identified the ACO cohort based on the *ICD-9-CM* code of COPD, asthma with ICS and OS (Cooke et al., 2011). The ACO cohort using statins had 26.5% on ICS and 77.8% on OS (Cooke et al., 2011). Physicians' decision regarding ICS treatment should not only follow the treatment guidelines for the specific diseases but also observe the payment regulations stipulated by the NHI. These policies may prevent an indication bias.

Validation of Hepatic, Renal, Heart, and Respiratory Failure

NHIRD data are de-identified and contain basic demographic information, disease diagnoses, prescriptions, operations,

TABLE 2 | Overall incidence of hepatic failure, renal failure, respiratory failure, and heart failure (per 1,000 person-years) and estimated HRs in ACO patients taking statins compared with ACO patients without statins using a time-dependent regression model.

	Statin	
Variables	**No (N = 7,443)**	**Yes (N = 1,211)**
Hepatic failure		
Person-years	42822	9519
Follow-up time (y), mean ± SD	5.75 ± 3.45	7.86 ± 2.94
Event, n	694	82
Rate	16.2	8.61
cHR (95% CI)	1 (reference)	0.58 (0.46, 0.72)***
aHR (95% CI)[a]	1 (reference)	0.50 (0.40, 0.64)***
Renal failure		
Person-years	45,034	9,684
Follow-up time (y), mean ± SD	6.05 ± 3.45	8.00 ± 3.41
Event, n	510	74
Rate	11.3	7.64
cHR (95% CI)	1 (reference)	0.68 (0.53, 0.87)**
aHR (95% CI)[a]	1 (reference)	0.49 (0.38, 0.64)***
Respiratory failure		
Person-years	46,898	9,897
Follow-up time (y), mean ± SD	6.30 ± 3.42	8.17 ± 2.78
Event, n	20	3
Rate	0.43	0.30
cHR (95% CI)	1 (reference)	0.68 (0.20, 2.30)
aHR (95% CI)[a]	1 (reference)	0.61 (0.17, 2.21)
Heart failure		
Person-years	43,215	9,461
Follow-up time (y), mean±SD	5.81 ± 3.50	7.96 ± 2.88
Event, n	835	77
Rate	19.3	7.99
cHR (95% CI)	1 (reference)	0.44 (0.35, 0.56)***
aHR (95% CI)[a]	1 (reference)	0.47 (0.37, 0.60)***

[a]*Adjusted for age; sex; comorbidity of sleep disorder, diabetes, hypertension, hyperlipidemia, CAD, stroke, hepatitis B, and hepatitis C; and ICS and OS.*
cHR, crude hazard ratio; aHR, adjusted hazard ratio; ICS, inhaled corticosteroid; OS, oral steroid.
***P < 0.01, ***P < 0.001.*

and investigations (Nan-Ping et al., 2013). While only a small number of validation studies with small sample sizes have been undertaken, they have generally reported positive predictive values of over 70% for various diagnoses (Lin et al., 2018). The catastrophic diseases included renal failure; respiratory failure and respiratory failure–related diseases pneumonia (sensitivity 94.7%) and tuberculosis (sensitivity 98.3%); heart failure–related disease (positive predictive value 96.6%) CAD (sensitivity 88.0%) (Cheng et al., 2014; Hsieh et al., 2019); and hepatic failure–related diseases liver tumor (sensitivity 91.5%) and cirrhosis (Hsieh et al., 2019). Issuance of catastrophic illness certificates was strict (Nan-Ping et al., 2013). Currently, patients cannot opt out of inclusion in the database, although this requirement is under review. In conclusion, the NHIRD is a large, powerful data source for biomedical research.

Sensitivity Analysis and Healthy Use Bias

A higher health awareness and healthier lifestyle were found in patients using statins compared with nonusers (Shrank et al., 2011). Accordingly, statin users should be more likely to seek out preventive health services. In addition, the ACO cohort received more medical advice during regular follow-up. Moreover, we compared the ICS and OS effects on the risk of hepatic, renal, and heart failure in the ACO cohort between statin users and nonusers (Giovanni et al., 2014). We also stratified the ACO cohort into subgroups by sex, age (<50 or ≥50 years), IC use, and OS use for further sensitivity analysis. These sensitivity tests could help us to avoid healthy use bias. In addition, the frequency of the ACO cohort receiving CXR, PFT, sputum culture, and vaccinations was higher than the COPD cohort during the follow-up course. Meanwhile, regarding the severity of the late course of ACO with multiple vital organ failure (Chung et al., 2015; Shantakumar et al., 2018), a large portion, up to 98.7%, of patients with ACO (8554/8654) underwent eosinophil count, IGE, spirometry, and thoracic imaging as mentioned before (Yeh et al., 2019).

Measuring lifestyle factors, disease prevention behaviors, and drug compliance is difficult in observational studies. Sleep disorders are associated with lifestyle, income, and urbanization level (Cyril et al., 2013). To reduce the effect of confounding from healthy user bias, we used diseases such as sleep disorder, hypertension, and diabetes in individual insurance as a proxy to adjust for socioeconomic status (Cyril et al., 2013). In addition, vital organ failures were associated with nutritional and immunological statuses, such as diabetes, hyperlipidemia, stroke, and hepatitis. We included these factors for analysis. This statistical methodology also enabled observational study to simulate randomized control trials.

DISCUSSION

This study revealed that statins were associated with a lower incidence of hepatic, renal, and heart failure but not respiratory failure. These results applied to the ACO cohort, regardless of age, sex, comorbidities, or ICS or OS use, in the late course of the study (Zeki et al., 2013; Tse et al., 2014). Past statin use may attenuate system inflammations, (Malik and Kashyap, 2003; Schierwagen et al., 2017) such as sepsis (TNF-α and IL-6) or acute lung injury (Bajwa et al., 2012; Wang et al., 2016), with multiple organ failure as demonstrated in a previous study supporting our result (Jones, 2006). Statins may be beneficial in the case of acute hepatic failure, renal failure, (Gupta et al., 2007) and heart failure. Statins significantly reduced the development of sepsis and infection-related organ dysfunction in older patients in hospitals; however, it did not reduce intensive care unit (ICU) admission incidence with respiratory failure, supporting our findings (Gui et al., 2017).

The major concerns regarding adverse statin reaction are hepatic function impairment (Jones, 2006) in chronic liver diseases and renal function impairment (Kovesdy et al., 2007). Another concern is statin use with acute lung injuries (Huang et al., 2013). Jason et al. conducted a meta-analysis of randomized trials and revealed that patients rarely discontinued statins in the event of an adverse reaction when using a high dose (Josan et al., 2008). In addition, the benefits of statins outweighed the adverse reaction in a recent report by Collins et al. (2016); this effect holds even when

TABLE 3 | Overall incidence (per 1,000 person-years) and hazard ratio for hepatic failure, renal failure, and heart failure stratified by sex and age using a time-dependent regression model.

Variables	Men		Women	
	Statin		Statin	
	No (N = 4,353)	Yes (N = 579)	No (N = 3,090)	Yes (N = 632)
Hepatic failure				
No. of events	415	42	279	40
Incidence rate	17.1	9.45	15.0	7.88
cHR (95% CI)	1 (Reference)	0.59 (0.43, 0.81)**	1 (Reference)	0.57 (0.41, 0.80)**
aHR (95% CI)[a]	1 (Reference)	0.54 (0.38, 0.75)***	1 (Reference)	0.47 (0.33, 0.67)***
Renal failure				
No. of events	334	43	176	31
Incidence rate	13.2	9.52	8.96	6.00
cHR (95% CI)	1 (reference)	0.74 (0.54, 1.01)	1 (reference)	0.66 (0.45, 0.97)*
aHR (95% CI)[a]	1 (reference)	0.50 (0.36, 0.70)***	1 (reference)	0.48 (0.33, 0.72)***
Heart failure				
No. of events	508	47	327	30
Incidence rate	20.8	10.5	17.5	5.81
cHR (95% CI)	1 (Reference)	0.54 (0.40, 0.72)***	1 (reference)	0.36 (0.25, 0.52)***
aHR (95% CI)[a]	1 (Reference)	0.55 (0.40, 0.75)***	1 (reference)	0.40 (0.27, 0.58)***
	Age < 50		Age ≧ 50	
	Statin		Statin	
	No (N = 1,418)	Yes (N = 181)	No (N = 6,025)	Yes (N = 1,030)
Hepatic failure				
No. of events	145	12	549	70
Incidence rate	15.2	7.76	16.5	8.78
cHR (95% CI)	1 (Reference)	0.55 (0.30, 0.99)*	1 (Reference)	0.58 (0.45, 0.74)***
aHR (95% CI)[a]	1 (Reference)	0.29 (0.15, 0.54)***	1 (Reference)	0.53 (0.40, 0.68)***
Renal failure				
No. of events	33	5	477	69
Incidence rate	3.19	3.19	13.8	8.50
cHR (95% CI)	1 (Reference)	1.02 (0.40, 2.62)	1 (Reference)	0.62 (0.48, 0.80)***
aHR (95% CI)[a]	1 (Reference)	0.27 (0.10, 0.74)*	1 (Reference)	0.50 (0.38, 0.65)***
Heart failure				
No. of events	25	3	810	74
Incidence rate	2.41	1.90	24.7	9.18
cHR (95% CI)	1 (Reference)	0.79 (0.24, 2.60)	1 (Reference)	0.40 (0.32, 0.51)***
aHR (95% CI)[a]	1 (Reference)	0.35 (0.10, 1.26)	1 (Reference)	0.47 (0.37, 0.60)***

[a]*Adjusted for comorbidity of sleep disorder, diabetes, hypertension, hyperlipidemia, CAD, stroke, hepatitis B, and hepatitis C as well as ICS and OS use.*
HD, cHR, crude hazard ratio; aHR, adjusted hazard ratio; ICS, inhaled corticosteroid; OS, oral steroid.
**P < 0.05, **P < 0.01, ***P < 0.001.*

combined with other cholesterol-reducing drugs, such as niacin, as reported by Landry et al. (Group et al., 2014).

The lungs, heart, kidneys, and liver interact. These multiple cross-reactions make vital organ failure more complex (Vaz Fragoso et al., 2018). Those with severe forms of ACO usually received a higher frequency of ICS and OS. ICS did not present a lower risk of renal or heart failure among statin users with ACO. The ACO cohort may develop heart failure, as we noted in our previous report. Chin et al. indicated that statin therapy conferred no particular benefits to patients with heart failure undergoing percutaneous coronary intervention (Chin et al., 2018). If the ACO–statins status was steroid-resistant, ICS would not exhibit additive effects for attenuating the risk of heart failure. However, patients with ACO using both statins and ICS had the lowest risk of hepatic failure, and patients with ACO using statins and OS had the lowest risk of hepatic, renal, and heart failure. These findings are explained by statins' additive effects on the anti-inflammatory properties of ICS and OS (Maneechotesuwan et !al., 2010; Maneechotesuwan et al., 2013).

Our study also discovered that the ACO cohort had higher risks of respiratory failure and no response to statins. One explanation is that the ACO cohort may be associated with malnutrition–inflammation complex syndrome (Levin et al., 2007). Thus, hyperlipidemia and obesity protect against the development of respiratory failure. The higher body mass index and normal albuminuria in the ACO cohort indicated a lower risk of mortality than the COPD cohort. This hypothesis was supported by Bai et al. (Bai et al., 2017). Therefore, statin use in the ACO cohort did not affect the risk of respiratory failure. However, this speculation warrants further research.

TABLE 4 | Overall incidence (per 1,000 person-years) and hazard ratio for hepatic failure, renal failure, and heart failure by ICS and OS status using a time-dependent regression model.

Variables	With ICSs		Without ICSs	
	Statin		Statin	
	No (N = 1743)	Yes (N = 321)	No (N = 5,700)	Yes (N = 890)
Hepatic failure				
No. of events	99	13	595	69
Incidence rate	8.81	4.95	18.8	10.0
cHR (95% CI)	1 (Reference)	0.57 (0.32, 1.02)	1 (Reference)	0.59 (0.46, 0.75)***
aHR (95% CI)[a]	1 (Reference)	0.36 (0.20, 0.66)***	1 (Reference)	0.53 (0.41, 0.69)***
Renal failure				
No. of events	79	26	431	48
Incidence rate	6.88	9.95	12.9	6.79
cHR (95% CI)	1 (eference)	1.38 (0.89, 2.15)	1 (Reference)	0.54 (0.40, 0.73)***
aHR (95% CI)[a]	1 (reference)	0.82 (0.51, 1.34)	1 (Reference)	0.40 (0.29, 0.55)***
Heart failure				
No. of events	140	21	695	56
Incidence rate	12.5	8.04	21.7	7.97
cHR (95% CI)	1 (Reference)	0.65 (0.41, 1.02)	1 (Reference)	0.40 (0.30, 0.52)***
aHR (95% CI)[a]	1 (Reference)	0.66 (0.40, 1.07)	1 (Reference)	0.43 (0.32, 0.57)***
	With OSs		Without OSs	
	Statin		Statin	
	No (N = 5377)	Yes (N = 942)	No (N = 2,066)	Yes (N = 269)
Hepatic failure				
No. of events	414	60	280	22
Incidence rate	13.1	8.01	25.1	10.9
cHR (95% CI)	1 (Reference)	0.65 (0.49, 0.85)**	1 (Reference)	0.48 (0.31, 0.75)**
aHR (95% CI)[a]	1 (Reference)	0.52 (0.39, 0.70)***	1 (Reference)	0.45 (0.29, 0.71)***
Renal failure				
No. of events	332	47	178	27
Incidence rate	10.1	6.15	14.5	13.2
cHR (95% CI)	1 (Reference)	0.61 (0.45, 0.82)**	1 (Reference)	0.93 (0.62, 1.40)
aHR (95% CI)[a]	1 (Reference)	0.46 (0.33, 0.63)***	1 (Reference)	0.57 (0.37, 0.88)*
Heart failure				
No. of events	555	60	280	17
Incidence rate	17.6	7.91	24.0	8.28
cHR (95% CI)	1 (Reference)	0.47 (0.36, 0.62)***	1 (Reference)	0.38 (0.23, 0.61)***
aHR (95% CI)[a]	1 (Reference)	0.48 (0.37, 0.64)***	1 (Reference)	0.42 (0.25, 0.70)***

[a]*Adjusted for age; sex; and comorbidity of sleep disorder, diabetes, hypertension, hyperlipidemia, CAD, stroke, hepatitis B, and hepatitis C.*

vHD, cHR, crude hazard ratio; aHR, adjusted hazard ratio; ICS, inhaled corticosteroid; OS, oral steroid.

**P < 0.05, **P < 0.01, ***P < 0.001.*

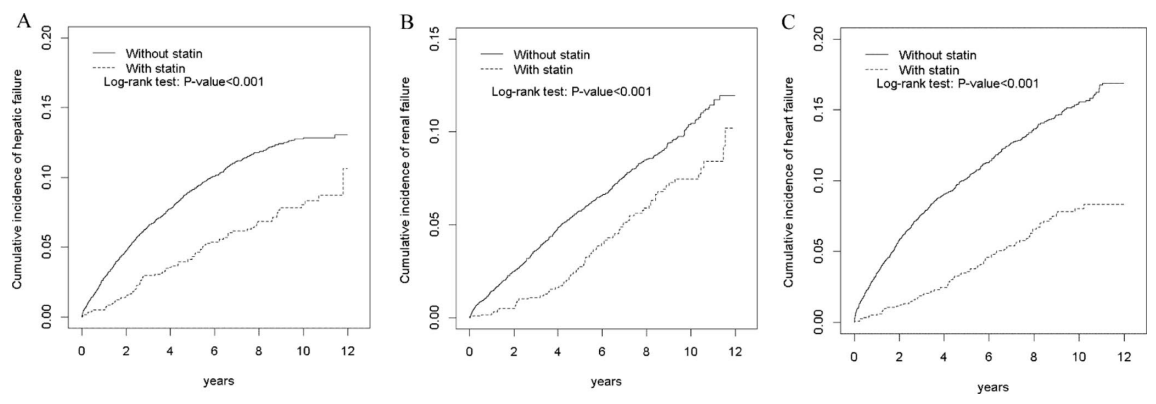

FIGURE 1 | Cumulative incidence of hepatic failure **(A)**, renal failure **(B)**, and heart failure **(C)** compared between patients with and without statin use through the Kaplan–Meier method.

The risks of hepatic, renal, and heart failure were lower among the ACO–statins status cohort. Furthermore, ICS and OS formula may attenuate the risk of hepatic, renal, and heart failure. In agreement with our result, Tsai et al. discovered that the ICS formula was associated with the lowest frequency of ICU admission based on the NHIRD (Tsai et al., 2017). Statin use in the early course of ACO may attenuate these risks in the later course of this disease.

With statins, the reported rate of adverse events differs widely, between 1–2% in randomized clinical trials (RCTs) and 10–20% in observation studies (real world). One possible explanation is the claim that RCTs mostly use a run-in period with a statin. This may exclude intolerant patients from remaining in the trial. Thus, the result may have a bias towards lower rates of intolerance. A study by Vonbank et al. includes data from RCTs with >1,000 participants with and without a run-in period who were included in the Cholesterol Treatment Trialists Collaboration. They found: 1) a majority of RCTs without a test dose of a statin in the run-in phase and 2) a test dose in the run-in phase without association of a significantly improved adherence rate within that trial comparison of the trials without a test dose. In summary, the RCTs of statins reviewed here suggest a bias towards an artificially higher adherence rate because of a run-in period with a test dose of the statin. In the review study of Vonbank et al., the apparent disparity between RCTs and observation studies are also included, albeit mostly not supported by scientific data (Vonbank et al., 2018).

Taking these together, the objective treatment of multiple organ failure should be based on the right action (e.g., blocking the inflammation-antibiotic or anti-tuberculosis drug with adjunctive therapy with a statin or steroid) at the right time (e.g., early in the course of ACO with ICS and statin before multiple vital organ failure) (Kruger and Venkatesh, 2014) in the right patients (e.g., early detection of pneumonia or pulmonary tuberculosis individualized per patient—precise medicine) (Tzovaras et al., 2011; De Loecker and Preiser, 2012; Kruger and Venkatesh, 2014; Yeh et al., 2018; Yeh, 2019). However, it should be underlined that these data come mainly from observational retrospective investigations, and randomized prospective studies are warranted to confirm these encouraging results.

STRENGTHS

The confirmation of the ACO cohort was established in a previous study (Yeh et al., 2018). The time-dependent model accurately presents the prescription status and correctly classifies the event-free person-time of the users before their first prescription as the unexposed follow-up time. The time-dependent model was the most informative (Levesque et al., 2010). The major cause of multiple organ failure is sepsis. In Taiwan, diagnoses of sepsis-induced vital organ failure were validated by Shin et al. (Shih et al., 2017). In addition, among Taiwan's older population utilizing ambulatory medical services, the prevalence of certificated catastrophic illness such as hepatic, renal, heart, and respiratory failure was high. Statins were administered in accordance with Taiwanese regulations. Therefore, the diagnosis and treatment of these vital organ failures were methodical (Nan-Ping et al., 2013).

The statin effect on cardiovascular diseases based on the NHIRD has been well addressed. These studies were incorporated into the 2017 Taiwan lipid guidelines for high-risk patients (Chung et al., 2017; Li et al., 2017). The database of these previous studies is the same as in our study. Therefore, our result can provide baseline trends useful for further research on statin effect on vital organ failure (Hsieh et al., 2017; Byrne et al., 2019; Ko et al., 2019).

LIMITATIONS

The identification of ACO challenged the researchers. PFT may increase the sensitivity to COPD (Ho et al., 2018). However, the diagnosis of ACO was based on clinical manifestation, imaging, PFT, and therapeutic response to the aforementioned bronchodilators—ICS and OS. Lanos et al. discovered that 27.1% of patients with ACO received PFT (Llanos et al., 2018). Shantakumar et al. found that 21.1% of patients with ACO received PFT after the index date in Taiwan. These laboratory data were unavailable in the NHIRD.

In our previous study, we performed propensity score matching to avoid baseline bias in the analysis of the effect of the statin on the vital organ failure (e.g., heart, hepatic, and renal failure–related diseases) such as cardiovascular diseases, pneumonia, and pulmonary tuberculosis. In this re-analysis, we found that the statin has a protective effect on cardiovascular disease, pneumonia, and pulmonary tuberculosis. Similar to these findings, the ACO cohort with statin use has a lower risk of vital organ failure, in line with our previous finings. Beta-blockers may play a role in heart failure, as in our previous study. Meanwhile, beta-blockers have an effect on heart rate control and may have a benefit with hemodynamic and clinical outcomes in patients having sepsis with respiratory failure (Coppola et al., 2015; Yeh et al., 2017). Owing to the ACO cohort with an asthma component, beta-blockers may not have an optimal role in this cohort. In addition, the ACO cohort has the experience of more severity of multiple organ failure than the non-ACO cohort (Chung et al., 2015). The drug effect on the development of multiple vital organ failure in the late course of this cohort may be more complex. These points of the study warrant further randomized control trials. Furthermore, there will be many new drugs and new strategies in the future. These points were the limitations of this retrospective study. Finally, details of the laboratory data, clinical procedure, or medical treatment records to approve the definition of ACO and organ failure were unavailable in the NHIRD. This is another limitation of our study.

In summary, the results of this study may be different in a different cohort with a different treatment strategy. However, we used a time-dependent population-based study and propensity method in this study (Yeh et al., 2019). The propensity method revealed the ACO–statin use cohort having

a lower risk of hepatic (aHR = 0.51), renal (aHR = 0.45), and heart failure (aHR = 0.46) also. These methods may avoid bias in this retrospective study. Thus, our findings may contribute to the trends in the optimal use of statins in personalized medicine as part of the future of precise medicine (Godman et al., 2014; Hsieh et al., 2017; Li et al., 2017; Byrne et al., 2019; Hsieh et al., 2019).

CONCLUSION

The ACO cohort using statins exhibited a lower risk of hepatic, renal, and heart failure than any other cohort, regardless of age, sex, comorbidities, or ICS or OS use of patients. The risk of hepatic failure was lower for the combined use of statins and ICS, and the risk of hepatic, renal, and heart failure was less frequent for the combined use of statins and OSs.

DATA AVAILABILITY

The datasets for this manuscript are not publicly available because the dataset used in this study is held by the Taiwan Ministry of Health and Welfare (MOHW). The Ministry of Health and Welfare must approve our application to access this data. Any researcher interested in accessing this dataset can submit an application form to the Ministry of Health and Welfare requesting access. Please contact the staff of MOHW (Email: stcarolwu@mohw.gov.tw) for further assistance. Taiwan Ministry of Health and Welfare Address: No.488, Sec. 6, Zhongxiao E. Rd., Nangang Dist., Taipei City 115, Taiwan (R.O.C.). Phone: +886-2-8590-6848. All relevant data are within the paper. Requests to access the datasets should be directed to please contact the staff of MOHW (Email: stcarolwu@mohw.gov.tw) for further assistance. Taiwan Ministry of Health and Welfare Address: No.488, Sec. 6, Zhongxiao E. Rd., Nangang Dist., Taipei City 115, Taiwan (R.O.C.). Phone: +886-2-8590-6848. All relevant data are within the paper.

ETHICS STATEMENT

The Research Ethics Committee of China Medical University and Hospital in Taiwan approved the study (CMUH104-REC2-115-CR3).

AUTHOR CONTRIBUTIONS

The authors' individual contributions are mentioned as follows. Conception and design: J-JY and C-HK. Administrative support: C-HK. Data collection and organization: all authors. Data analysis and interpretation: all authors. Manuscript writing: all authors. Final approval of the manuscript: all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00889/full#supplementary-material

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frontiers in Pharmacology

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Early and Accurate Prediction of Clinical Response to Methotrexate Treatment in Juvenile Idiopathic Arthritis Using Machine Learning

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Background and Aims: Accurately predicting the response to methotrexate (MTX) in juvenile idiopathic arthritis (JIA) patients before administration is the key point to improve the treatment outcome. However, no simple and reliable prediction model has been identified. Here, we aimed to develop and validate predictive models for the MTX response to JIA using machine learning based on electronic medical record (EMR) before and after administering MTX.

Materials and Methods: Data of 362 JIA patients with MTX mono-therapy were retrospectively collected from EMR between January 2008 and October 2018. DAS44/ESR-3 simplified standard was used to evaluate the MTX response. Extreme gradient boosting (XGBoost), support vector machine (SVM), random forest (RF), and logistic regression (LR) algorithms were applied to develop and validate models with 5-fold cross-validation on the randomly split training and test set. Data of 13 patients additionally collected were used for external validation.

Results: The XGBoost screened out the optimal 10 pre-administration features and 6 mix-variables. The XGBoost established the best model based on the 10 pre-administration variables. The performances were accuracy 91.78%, sensitivity 90.70%, specificity 93.33%, AUC 97.00%, respectively. Similarly, the XGBoost developed a better model based on the 6 mix-variables, whose performances were accuracy 94.52%, sensitivity 95.35%, specificity 93.33%, AUC 99.00%, respectively.

Conclusion: Based on common EMR data, we developed two MTX response predictive models with excellent performance in JIA using machine learning. These models can predict the MTX efficacy early and accurately, which provides powerful decision support for doctors to make or adjust therapeutic scheme before or after treatment.

Keywords: methotrexate, juvenile idiopathic arthritis, prediction model, machine learning, clinical response

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INTRODUCTION

Methotrexate (MTX) is the first line treatment for the majority of patients with juvenile idiopathic arthritis (JIA). However, the efficacy of MTX varies greatly among individuals, with about 30 to 70% of JIA patients being effective (Ruperto et al., 2004; Foell et al., 2010). Patients who respond to MTX poorly are given biologicals alone or in co-treatment with MTX. Biologics can lead to more efficient disease control, but abuse of biologics can result in high costs and serious adverse reactions. Additionally, it usually takes 3–6 months before a decision is made as to MTX efficacy (Martini et al., 2019). Patients receiving "trial-and-error" therapy for such a long time may delay treatment, resulting in irreversible joint damage and even adverse reactions. Therefore, early identification of whether the patient is effective before starting MTX and then selection of appropriate therapy (MTX alone or combined with biologics) are of great significance for preventing disease progression. This means that it is very necessary to establish an efficacy prediction model before the onset of MTX in JIA.

Although MTX has been used to treat JIA for a long time, being able to predict who will respond to MTX is still very limited. To date, only Bulatovic et al. (2012) reported a predictive model for MTX response to JIA. However, the limitations of this model are as follows: the prediction accuracy was not high (the area under the curve, AUC, was only 72%); model variables contained controversial single nucleotide polymorphisms (SNPs), which required additional and expensive testing, thus limiting its widely available in clinical application. Moreover, this study only employed one traditional logistic regression algorithm, which is not applicable to the modeling of non-independent variables. In addition to this study, other studies on MTX response to JIA were only limited to discovering which indicators would affect the efficacy of MTX. But they did not provide a model for clinical application, so that it could not be easily applied in clinical practice (Hinks et al., 2011; Yanagimachi et al., 2011; Cobb et al., 2014; Zajc Avramovic et al., 2017).

Therefore, a simple, efficient and accurate MTX response prediction model is urgently needed to provide references for clinicians before treatment. In recent years, the predictive model developed by machine learning based on electronic medical record (EMR) data has played an excellent role in disease diagnosis, treatment, and prognosis. For example, in our previous work, we used a machine learning technique to acquire pediatric EMR and developed an auxiliary decision-making system for diseases diagnosis, which is comparable to that of human physicians (Liang et al., 2019); Machine learning is also used to predict the efficacy and prognosis of diseases in other diseases (Motwani et al., 2017; Browning et al., 2019). Similarly, in rheumatoid diseases, researchers used machine learning to establish disease diagnosis classifier, mortality prediction model and MTX related hepatotoxicity automatic recognizer basing on EMR data (Liao et al., 2010; Lin et al., 2015; Lezcano-Valverde et al., 2017). These results provide practical tools for the management of patients. However, currently, there are no reports about the prediction model of MTX response in JIA using machine learning only basing on EMRs.

The purpose of this study is to develop simple, efficient and accurate models using machine learning for early predicting the efficacy of MTX in JIA based on integrating temporal features before and after starting MTX within three months.

METHODS

Study Design and Population

We retrospectively collected the EMR data of children with JIA who visited Guangzhou Women and Children's Medical Center from January 2008 to October 2018. Inclusion criteria were: (1) patients were new-onset and met the International League of Associations for Rheumatology criteria for JIA (Petty et al., 2004; Martini et al., 2019). (2) The onset age was 1–16 years old. (3) Patients received monotherapy with MTX for at least 3 months. (4) Co-treatment with non-steroidal anti-inflammatory drugs or corticosteroids were allowed. Exclusion criteria were: (1) combined therapy with other interfering drugs (e.g. biologic agents, sulfasalazine, etc.) within 3 months. (2) MTX therapy did not reach 3 months. (3) Serious missing of medical records. A total of 674 JIA children using MTX were screened out, but 362 patients were eventually included for modeling and validating. Furthermore, we continued to collect 13 JIA patients from November 2018 to January 2019 for external verification.

The study was performed according to the Helsinki declaration. Ethical approval was obtained from the ethics committee of this center (no. 2016021645). This study was a part of a large clinical trial (NCT81603203). All data were anonymous and no identifiable personal data of patients were available for the analysis. No additional informed consent was required.

Assessment of MTX Clinical Response

Weekly MTX was given to all patients by either oral or subcutaneous route at 10–15 mg/m^2. Baseline disease activity was calculated before MTX treatment. Early response to MTX was evaluated at 3 months after using MTX. Since it is a retrospective study, it is difficult to collect subjective features such as patient/parent and physician's global assessment of disease. Therefore, JADAS or ACRpedi (see **Table 1** for the full name) scoring tools could not be applied to evaluate the response (Giannini et al., 1997; Consolaro et al., 2009). DAS44/ESR-3, a simplified standard related to the European League of Associations for Rheumatology criteria, was the most suitable choice for this retrospective study (Ranganath et al., 2007; Consolaro et al., 2009). The simplified formula of the disease activity is as follows: $y = 0.53938\sqrt{RAI} + 0.06465 * SJC44 + 0.33\ln\left(ESR\right) + 0.224$ (RAI, Ritchie articular index; SJC, Swollen joint count; ESR, erythrocyte sedimentation rate). The response was defined as a significant change of DAS44 scores from baseline to 3 months after starting MTX. Good response was defined as a significant decrease in DAS44 (>0.6), while a decrease of ≤0.6 was non-response.

Clinical Variables

All data were collected from EMRs before administration of MTX (baseline) and within 3 months after administration

TABLE 1 | The full name and abbreviation name of variables.

Full name of variables	Abbreviation name	Full name of variables	Abbreviation name
Age of methotrexate start	Age of MTX start	Hemoglobin	HGB
Age onset	Age onset	Indirect bilirubin	IBIL
Albumin	ALB	Immune globulin A	IgA
Alanine transaminase	ALT	Immune globulin E	IgE
Anti-cyclic citrullinated peptide	Anti-CCP	Immune globulin G	IgG
Active partial thrombin time	APTT	Immune globulin M	IgM
Aspartate aminotransferase	AST	JIA subtype	JIA subtype
Complement 3	C3	Lymphocyte	LYM
Complement 4	C4	Neutrophil	NEUT
CD16+CD56+	CD16+CD56+	Platelet	PLT
CD19+	CD19+	Prothrombin time	PT
CD3+Abs	CD3+Abs	Red blood cell	RBC
CD3+CD4+	CD3+CD4+	Rheumatoid factor-IgG	RF-IgG
CD3+CD8+	CD3+CD8+	Serum creatinine	SCr
C-reactive protein	CRP	Swollen joint count	SJC
Direct bilirubin	DBIL	Total bilirubin	TBIL
The first dose of MTX on the start	Dose0	Helper T cells/Suppressor T cells	Th/Ts
Erythrocyte sedimentation rate	ESR	Time interval	Time interval
Ferritin	FER	Tender joint count	TJC
Fibrinogen	FIB	Thrombin time	TT
Gender	Gender	Urea	Urea
Blood glucose	GLU	White blood cell	WBC
Hematocrit	HCT	Weight	Weight
Ritchie articular index	RAI	C-reactive protein near 3 months after administration	CRP/3m

CD, Cluster of differentiation cell; CD3+Abs, the absolute value of T cell with Cluster of differentiation 3; CD3+CD4+, the ratio of CD4+ divided by CD3+. Variables with the suffix "/3m" are those collected within 3 months after administration of MTX. For example, CRP/3m refers to the CRP variable collected within 3 months after administration.

(nearly 3 months). We collected a lot of variables, including: joint conditions [tender joint count (TJC), SJC, RAI, and JIA subtypes (oligoarticular, polyarticular, and other subtypes), joint imaging, etc.], the acute phase of inflammatory products [C-reactive protein (CRP), ESR], demographic data (age, gender, etc.), immune-related indicators [rheumatoid factor (RF), rheumatoid factor IgG (RF-IgG), antinuclear antibodies, anti-cyclic citrullinated peptide antibody, etc.], kidney function, liver function [total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), etc.], blood coagulation function [active partial thrombin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), etc.], blood routine testing, relevant lymphocytes (CD3+abs, CD3+CD4+, CD3+CD8+, etc.) and other data. See **Table 1** for a list of all variables. Because some variables were seriously missing, they were not be used for modeling. All variables used for modeling are shown in **Figure 1**.

Machine Learning

We collected two different sets of variables. Thus, two groups of models were established based on variables before the onset of MTX and mix-variables within 3 months after starting MTX respectively, for finding the best model. In the first group of models, referred to as pre-administration variables models (MTX-A), we included 46 variables (see the left part of **Figure 1**). In the second group of models, referred to as mix-variables models (MTX-B), we extended MTX-A by adding 32 new variables (see the right part of **Figure 1**). The main process can be divided into three steps: (1) data processing, (2) feature selection, (3) model generation and validation. Five-fold stratified cross-validation was used for assessing the performance and general error estimation of feature selection and model generation. **Figure 2** shows the flowchart of this work. Machine learning techniques were implemented in Python 3 (Python 3.6.5) using the package Scikit-learn (Scikit-learn 0.19.1).

Data Preprocessing

Some variables have been removed with >30% missing rates. In order to get a higher quality of the dataset, the missing values were filled with mean values of a group stratified by MTX response. For example, we used mean values of good response and non-response group to respectively fill the data of CRP in different outcome groups.

Feature Selection

Appropriate feature subsets were selected using ensemble models, including extremely randomized trees (ET), gradient boosting decision tree (GBDT), random forest (RF) and extreme gradient boosting (XGBoost). Firstly, data transformation was carried out on continuous variables to form four kinds of data: min-max normalization, z-score normalization, L2 normalization and original. Secondly, the above four algorithms were used to analyze the four forms of data and build 16 models using five-fold stratified cross-validation, so as to obtain the median importance ranking of variables in all models which is as the final features importance ranking. Finally, the next aim is to determine the feature set with the least variables but the highest

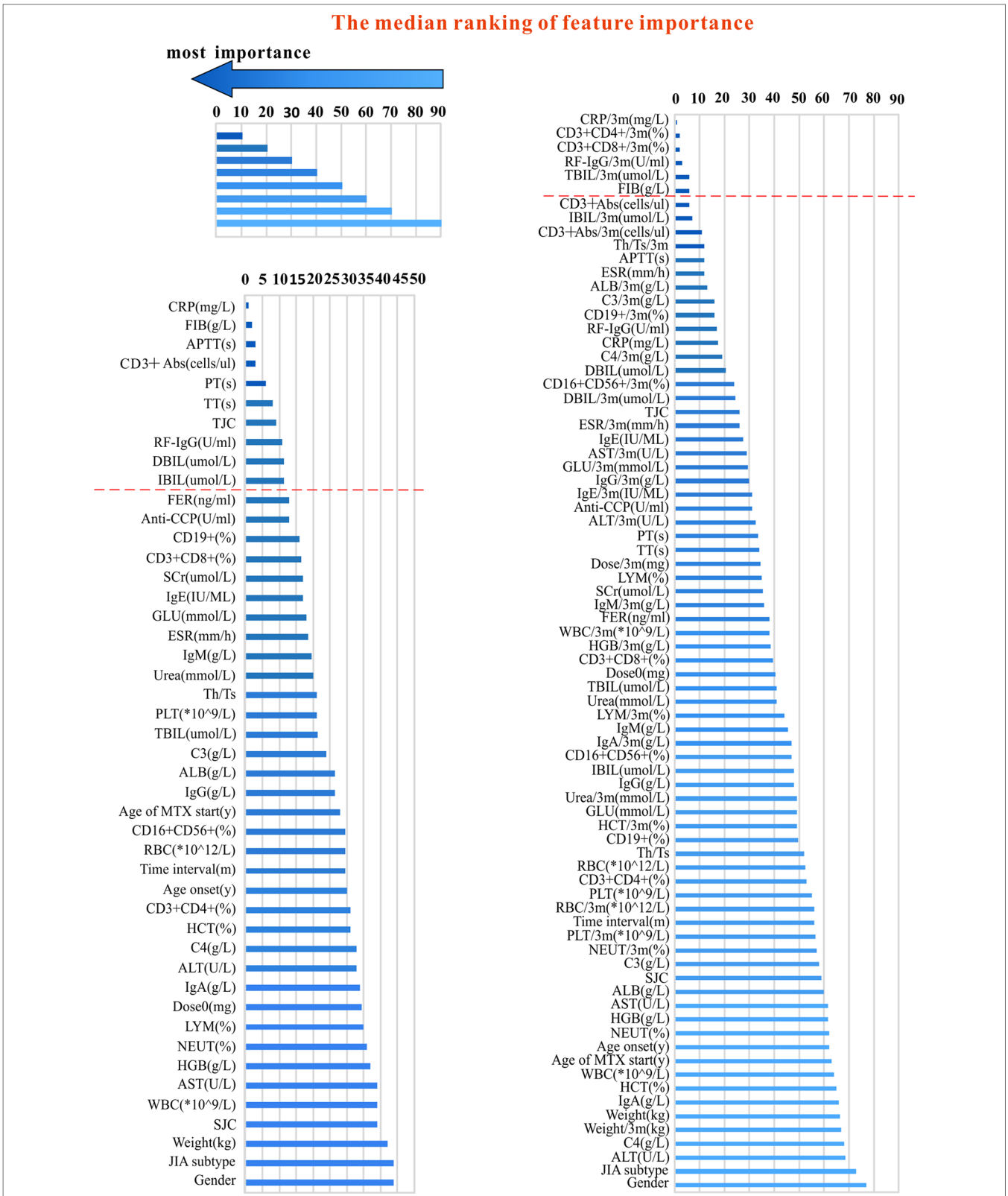

FIGURE 1 | The variables used for modeling and their importance ranking (in order of median importance). The left part of the figure shows the variables used in the pre-administration variables model. The right part of the figure shows a mixture of variables before and after administration (variables collected within 3 months after administration with MTX). The shorter the transverse column (i.e. the smaller the value), the greater importance of the median ranking of the variable (see the top and left part).

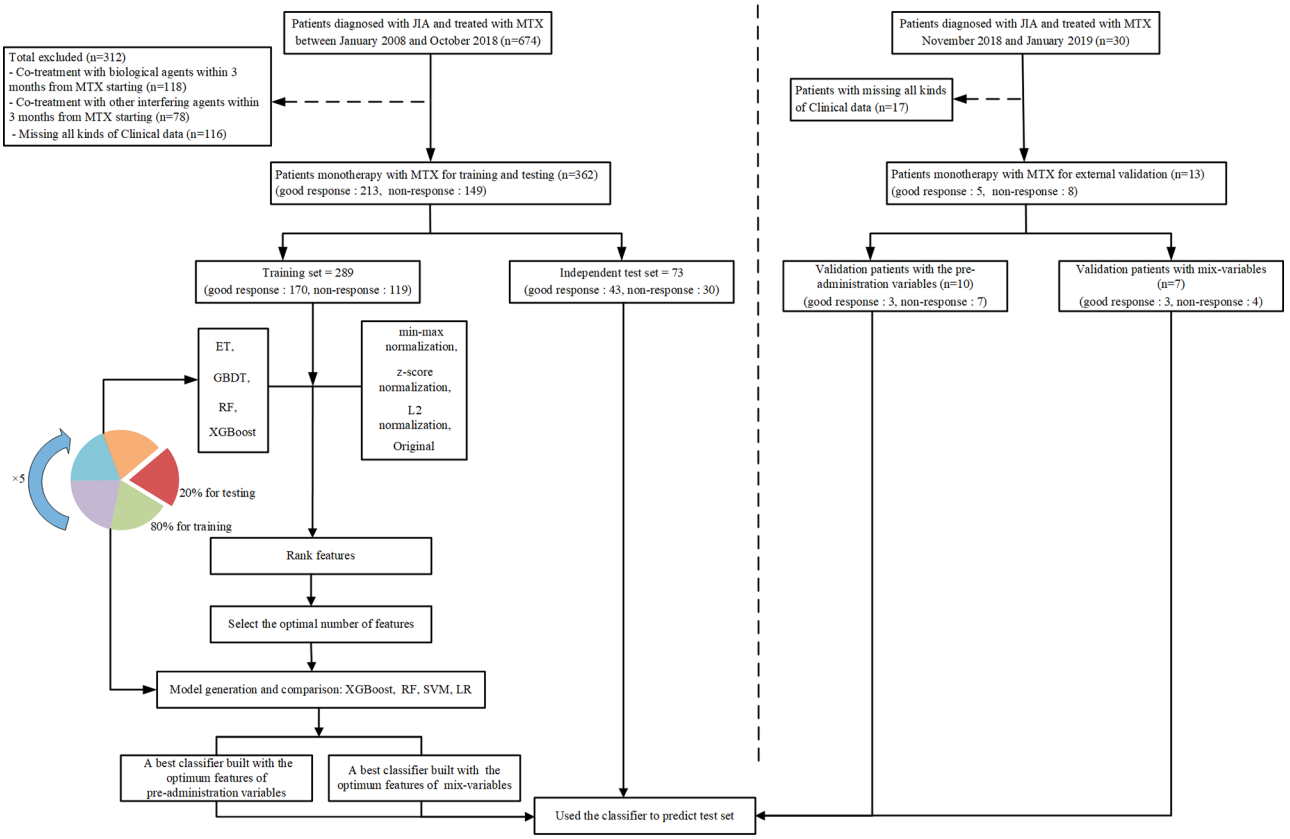

FIGURE 2 | The flowchart of model developing and validation. ET, extremely randomized trees; GBDT, gradient boosting decision tree; RF, random forest; XGBoost, extreme gradient boosting; SVM, support vector machine; LR, logistic regression.

predictive accuracy. The XGBoost algorithm was used to find the minimum-size list of features by forwarding feature selection, as follows: (1) beginning with the head of the ranked list of variables (the most important variable), XGBoost algorithm iteratively generates a new model by adding one variable at a time, and calculates its classification accuracy. (2) The list with the minimum size and optimum accuracy is therefore selected.

Model Generation and Validation

A cohort of 362 patients was randomly split into the training set and test set according to the ratio of 80:20. XGBoost, RF, support vector machine (SVM) and logistic regression (LR) algorithms were applied to develop classifiers respectively in our study. The classifiers were trained on the training set (n = 289), using the training set feature values (the minimum-size list of features) as input. Thus, each set of variables had 4 types of classifiers. Five-fold stratified cross-validation was used for internal validation. After training, classifiers were asked to predict the response of the test set (n = 73). For each set of variables, the four classifiers were compared with each other in terms of accuracy, and then the best classifier was selected as the final predictor. We further performed an external validation of the above classifiers with the subsequent collection of 13 patients.

RESULTS

Patient Characteristics

A total cohort of 362 patients with JIA was included in developing models, and 13 patients were subsequently collected to external validate the best model. **Table 2** describes the baseline characteristics of our study population. According to DAS44/

TABLE 2 | Baseline patient characteristics.

Characteristics	Data (n = 362)
Gender, n (male/female)	211/151
Age of MTX start, years, (mean ± SD)	6.7 ± 3.4
Age of disease onset, years, (mean ± SD)	6.3 ± 3.4
Time interval*, months, (mean ± SD)	5.6 ± 2.7
Polyarticular JIA, n	101
Oligoarticular JIA, n	186
Other types of JIA, n	75
Tender joint count, median (range)	3(0–36)
Swollen joint count, median (range)	4(0–36)
ESR, mm/h, (mean ± SD)	36.22 ± 33.34
CRP, mg/L, (mean ± SD)	24.81 ± 33.32
RF-IgG, U/ml, (mean ± SD)	23.68 ± 52.55
MTX dose at start, mg/m²/wk, median (range)	5.0(0.5–18.0)

**Time interval, the time from disease onset to initiation of MTX treatment.*

ESR-3 simplified standard, 213 patients were rated as good response and 149 patients as non-response.

Feature Selection

Median importance ranking of all variables before using MTX and mix-variables before and after administering with MTX were shown in **Figure 1** (left part and right part). The XGBoost algorithm was applied for selecting the minimum size and optimum accuracy features subset, and the results of this process were shown in **Figure 3**. We examined the predictive performance of the most prominent feature and identified the point at which there was no considerable gain in accuracy, sensitivity, and AUC, when adding the feature of the next highest ranking one to the model. The optimum values were obtained when these three measurements defined the most discriminative features. In the MTX-A predictors, the three measurements reached the optimum when 10 feature subsets were selected (see **Figure 3A**). The 10 selected significant variables are listed above the dotted red line in the left part of **Figure 1**. In the MTX-B predictors, the three measurements achieve maximum performance when 6 feature subsets were screened out (see **Figure 3B**). Variables above the dotted red line in the right part of **Figure 1** are these 6 features. The degree of contribution of all the above selected variables to response and the formulas behind modeling were described in detail in the **Supplementary**.

Model Performance and Comparison

Table 3 shows the classification accuracy results of the models which were evaluated using the test set. Of the MTX-A and MTX-B predictors, both the XGBoost models showed the best predictive performances. Therefore, the XGBoost models were selected as the final predictors. The performance of MTX-A XGBoost predictor was as follows: sensitivity 90.70% (95%CI: 82.0–99.4%), specificity 93.33% (95%CI: 84.4–100%), accuracy 91.78% (95%CI: 85.5–98.1%) and AUC 0.97. However, the MTX-B XGBoost predictor have a better performance, which

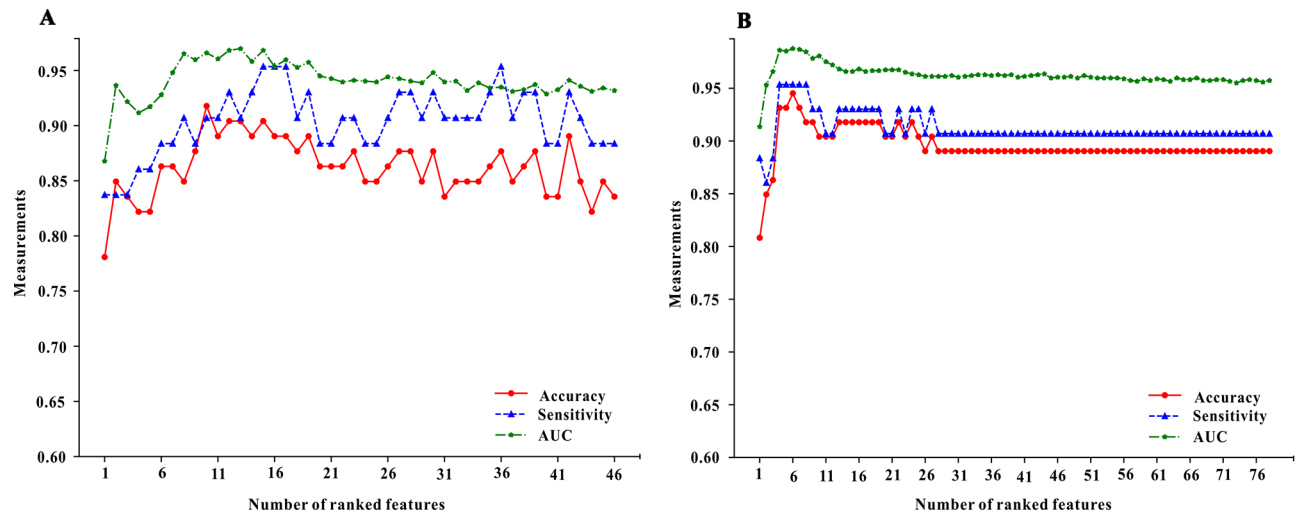

FIGURE 3 | The forward feature selection results for the model of pre-administration variables (the **A** part of figure 3) and the model of mix-variables before and after administration (the **B** part of figure 3). We examined the predictive performance of the most prominent feature and identified the point at which there was no considerable gain in accuracy, sensitivity, and area under the curve (AUC), when adding the feature of the next highest ranking one to the model. The optimum values were obtained when these three measurements defined the most discriminative features.

TABLE 3 | The classification performance results of the models.

Data set	Model	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	AUC
MTX-A	XGBoost	90.70	93.33	91.78	95.12	87.50	0.97
	RF	90.70	80.00	86.30	86.67	85.71	0.95
	SVM	79.07	83.33	80.82	87.18	73.53	0.87
	LR	65.12	73.33	68.49	77.78	59.46	0.80
MTX-B	XGBoost	95.35	93.33	94.52	95.35	93.33	0.99
	RF	95.35	93.33	94.52	95.35	93.33	0.98
	SVM	88.37	80.00	84.93	86.36	82.76	0.81
	LR	88.37	76.67	83.56	84.44	82.14	0.83

MTX-A, pre-administration variables prediction models; MTX-B, mix-variables models with features collected before and after administered with MTX within 3 months; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; XGBoost, extreme gradient boosting; RF, random forest; SVM, support vector machine; LR, logistic regression.

achieves sensitivity 95.35% (95%CI: 89.0–100%), specificity 93.33% (95%CI: 84.4–100%), accuracy 94.35% (95%CI: 89.3–99.7%) and AUC 0.99. **Figure 4** shows the mixed matrix results of each model in the MTX-A and MTX-B predictors of the test set.

External Verification and Clinical Application

Data of 13 patients newly collected were applied to externally validate the two XGBoost predictors. The performances of MTX-A XGBoost predictor were sensitivity 100.0%, specificity 86.1% and accuracy 90.0%, respectively. But the MTX-B XGBoost predictor got better performance results, with 100% sensitivity, specificity, and accuracy. Furthermore, we applied these predictors to two randomly selected patients to predict their outcomes. We input their clinical variables into the two predictors. Both models produced correct predictive outcomes (see **Table 4**).

DISCUSSION

We developed and validated two prediction models for MTX response in a large JIA cohort according to EMR data using machine learning. Models developed by XGBoost showed the best performance. CRP, CD3+Abs, RF-IgG, TJC, DBIL, IBIL, APTT, PT, TT, and FIB were variables screened out by the pre-administration variables model; and the mix-variables model filtered out features as follows: CRP/3m, CD3+CD4+/3m, CD3+CD8+/3m, RF-IgG/3m, TBIL/3m, and FIB, which were collected before and after administering MTX. The pre-administration variables model could accurately identify ninety-seven percent of patients whether responded to MTX (AUC 97%); 99% of patients were distinguished by the mix-variables model (AUC 99%).

To our knowledge, this is the first article to establish an accurate and easy-to-use predictive model for the efficacy of MTX in JIA based solely on EMRs using machine learning. Bulatovic et al. (2012) reported the first and so far the only one efficacy prediction model, which including variables of SNPs and ESR. The identified accuracy, sensitivity, specificity, positive predictive value, negative predictive value were 72, 78, 49, 83, and 41%, respectively. These results were all lower than ours. SNPs are important in precision medicine. However, a GWAS study found no direct correlation between SNPs related to the pathways of MTX and the efficacy in 759 JIA patients (Cobb et al., 2014). This suggests that SNPs may not necessary in revealing the outcome of JIA (Albers et al., 2009; Roszkiewicz and Smolewska, 2017). Moreover, the expression and activity of enzymes in children may be affected by growth, and the genotype may not directly reflect the phenotype (Zhao et al., 2010; Roszkiewicz and Smolewska, 2017). In addition, genotype detection increases the cost and time, which is not as convenient, efficient and cheap as conventional detection. These suggest that clinical phenotypic variables may be more important in influencing outcomes (Roszkiewicz and Smolewska, 2017). Our results verified the above view.

Of course, the low prediction accuracy of Bulatovic's study may be because of using only the traditional logistic regression, which may not be the optimal method. Machine learning is hot in recent years, which has gained remarkable achievements in biomedicine (Austin et al., 2013; Lin et al., 2015; Motwani et al., 2017; Browning et al., 2019; Liang et al., 2019). Specifically, machine-learning approaches may offer advantages over conventional techniques. The advantages of machine learning over traditional modeling methods are as follows: (1) machine learning can deal with more complex, high-dimensional and interactive variables, but the latter has limited ability to fix those problems. (2) Traditional modeling has poor generalization, though the former can model with strong generalization and better accuracy (Kruppa et al., 2012; Lee et al., 2018). Therefore, several advanced machine learning methods including SVM, RF, and XGBoost as well as LR were applied to model and compare the results in our study. Of our results, the performances of XGBoost models were the best, and the results of LR models were mostly poor. Further, the performances of those four modeling methods were all better than those of Bulatovic's study. These results also confirmed that XGBoost could effectively avoid overfitting and improve prediction performance. LR, as a kind of traditional analysis, may appear low-fitting (Lee et al., 2006). Therefore, when dealing with similar classification problems, it may be more appropriate to select advanced algorithms.

In addition to the above Bulatovic's study, other literature also explored which variables were associated with the efficacy of MTX (Yanagimachi et al., 2011; Cobb et al., 2014; Zajc Avramovic et al., 2017). But they did not establish a model, which could not be applied easily in clinical. For instance, some studies showed SNPs were associated with MTX efficacy (Hinks et al., 2011; de Rotte et al., 2012; Zajc Avramovic et al., 2017) clinical variables like TJC, ESR, CRP, etc. were also correlated with MTX response (Albers et al., 2009; Yanagimachi et al., 2011; Cobb et al., 2014; Franova et al., 2016). Variables considered in our study (n = 78) were more than and mostly different from those of the reported studies (Albers et al., 2009; Yanagimachi et al., 2011; Cobb et al., 2014; Franova et al., 2016). TJC, CRP and RF-IgG screened out by our study were reported to associate with disease activity, prognosis, efficacy of rheumatoid arthritis or JIA (Zborovskii et al., 1999; Cabral et al., 2005; Jaskowski et al., 2010). CD3+, CD4+, and CD8+ are the most important T lymphocytes, which are widely distributed in the joint synovial membrane and fluid and play an important role in the pathogenesis and classification of JIA (Finnegan et al., 2011; Hui et al., 2012; Van Nieuwenhove et al., 2019). One mechanism of MTX action is to regulate or inhibit T cell immunity to achieve the therapeutic effect (Johnston et al., 2005). In this study, these cells were contributed to MTX outcome, which is consistent with the MTX effect mechanism and similar to some reports (Isaacs, 2007; Bulatovic Calasan et al., 2015). Liver function variables (TBIL, DBIL, and IBIL) were also screened out. It is well-known that MTX is metabolized by the liver, so its pharmacokinetics will be affected by liver function, which will then affect pharmacodynamics. Coagulation markers (APTT, PT, TT, and FIB) also contributed to MTX efficacy in our study. We know ESR is an important variable in calculating disease activity and

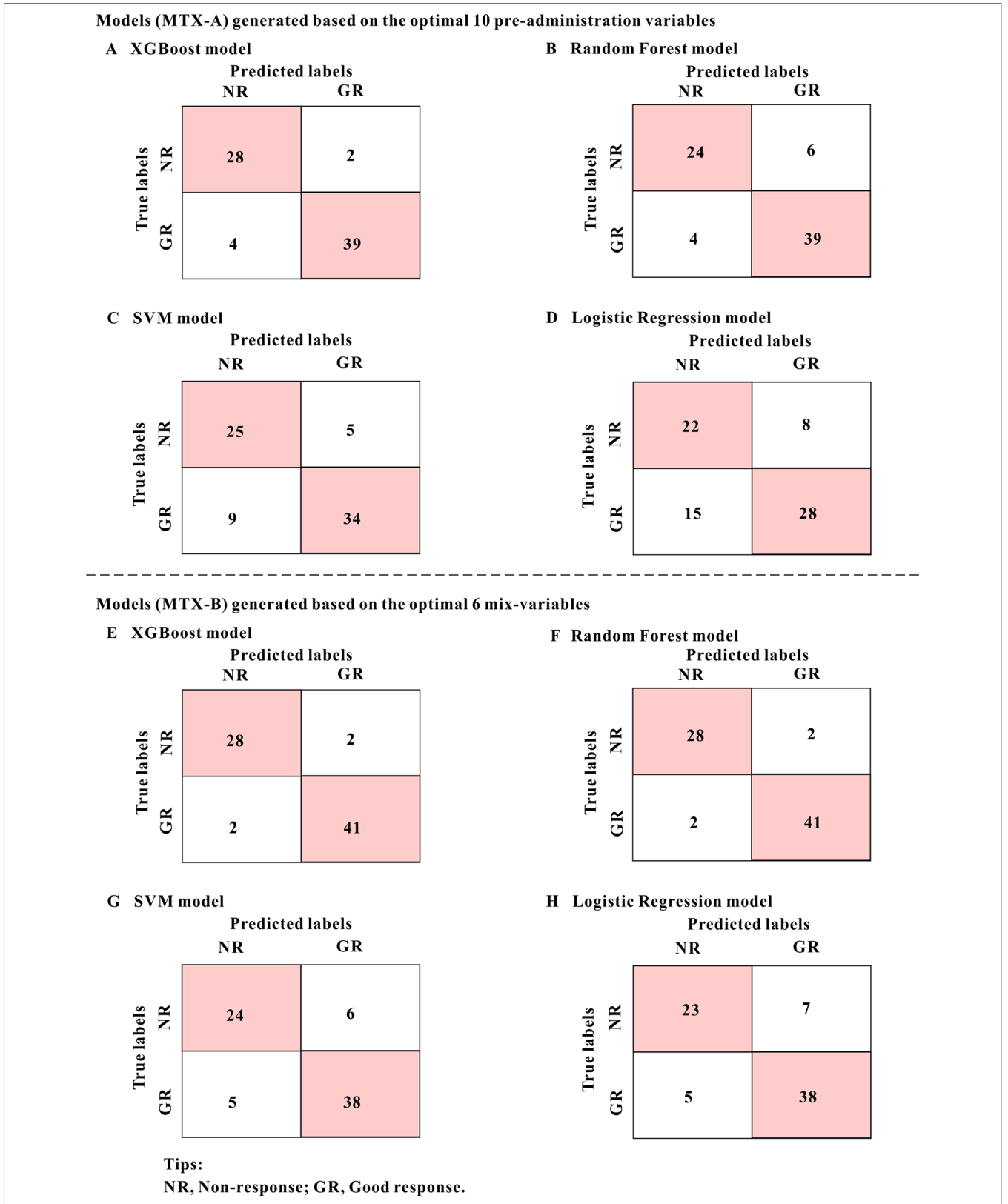

FIGURE 4 | The mixed matrix results of each model in the MTX-A and MTX-B predictors of the test set. For example, when the true lables were NR(non-response) and predicted lables were NR, it indicated that the number of NR was correctly predicted. However, when true lables were NR, and predicted labels were GR (good response), it indicated the number of NR incorrectly predicted to GR. As can be seen from the figure, the predicted values of model A (XGBoost model) and model E (XGBoost model) are the closest to the real values, indicating the best prediction performance. The numbers in the pink grids represent the number of cases that were accurately predicted.

TABLE 4 | The application of XGBoost predictors for clinical patients to predict their response.

MTX-A Predictor	Patient name	Input variables									Output
		CRP (mg/L)	FIB (g/L)	APTT (s)	CD3+Abs (cells/ul)	PT (s)	TT (s)	TJC	RF-IgG (U/ml)	DBIL (umol/L)	IBIL (umol/L)	
	AAA	35.30	2.77	34.20	2,454.46	12.00	10.90	1	21.50	1.30	4.80	Non-response
	BBB	150.00	4.71	26.22	3,309.00	14.40	10.90	2	5.90	2.60	2.70	Good response

MTX-B Predictor	Patient name	Input variables						Output
		CRP/3m (mg/L)	CD3+CD4+/3m(%)	CD3+CD8+/3m (%)	RF-IgG/3m(U/ml)	TBIL/3m (umol/L)	FIB(g/L)	
	AAA	49.60	38.00	50.00	11.70	6.17	2.77	Non-response
	BBB	32.30	34.44	26.77	3.90	7.60	4.71	Good response

In this table, variables were from clinical determination. The patient named AAA was responded to MTX in clinic practice, but the patient named BBB was not responded to MTX. We input their clinical variables into the two predictors. Both models produced correct predictive outcomes. Output means the prediction results of the MTX response.

MTX efficacy. While ESR depends mainly on plasma FIB (Bedell and Bush, 1985). Additionally, FIB affects the adhesion, spread, proliferation of endothelial cells, and the repair of joint synovial tissue. So FIB may have an indirect contribution to MTX treatment (Carney et al., 1992; Cid et al., 1993; Grober et al., 1993). As for the degree of contribution of all the above selected variables to the outcome (efficacy), we described details in the **Supplementary**. It can be seen from the **Supplementary** that CRP was the most significant variable for the pre-administration model, while for the mix-variables model, RF-IgG/3m was the most important.

Additionally, from our results, the mix-variables model was better than the pre-administration variables model. This indicated that temporal features after administration but before evaluating efficacy also had an important influence on the treatment outcome. Just as variables before and during pregnancy can both have an impact on pregnancy outcome. This suggests that in addition to considering the influence of pre-administration variables, the efficacy should be evaluated in combination with post-administration features. The limits of this study were the relatively small sample size, insufficient representativeness of externally verified samples and retrospective research. The next step is to conduct prospective studies to model and validate.

CONCLUSIONS

In summary, for the first time, based on EMR we used advanced machine learning to establish two early predictive models for the MTX efficacy in JIA, including pre-administration variables model and mix-variables model. The latter model performed better. The variables screened by the models were closely related to the pathogenesis of diseases, pharmacokinetics and pharmacodynamics of MTX, and could be fully explained. Interestingly, the coagulation indicators filtered out by our models may indicate the new pathogenesis of JIA and the unelucidated mechanism of MTX. This model is simple, efficient and accurate, and can be easily generalized by clinicians and pharmacists to make early treatment decisions to patients of different ethnic groups.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the Corresponding Author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by drug clinical research ethics committee of Guangzhou women and children's medical center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was Obtained From the Minor(S)' Legal Guardian/ Next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conceptualization: XM, YH, HL (16th author) and HZ. Data curation: XM, HWL, YC, FH, SZ and HL (13th author). Formal analysis: XC, HXL, LP, HL (16th author) and HZ. Funding acquisition: XM and MH. Investigation: XM, HWL, YC and FH. Methodology: XM, XC, JL, HXL and LP. Project administration: XM, MH, YH, HL (16th author) and HZ. Resources: HWL, SZ, PZ, YX and HZ. Software: XC and HXL. Supervision: XM. Validation:

PZ and YX. Writing — original draft: XM and XC. Writing — review and editing: XM, XC, HWL, JL, FZ, YH, HYL and HZ.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01155/full#supplementary-material

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Comparison of Healthcare Utilization and Costs Between RA Patients Receiving Biological and Conventional Synthetic DMARDs: A Nationwide Population-Based Cohort Study in Taiwan

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Background: The therapy with biological disease-modifying anti-rheumatic drugs (bDMARDs) has proven to rapidly reduce articular symptoms/signs, decrease morbidities, and improve health outcome in patients with rheumatoid arthritis (RA) and be cost-effective in Western countries. However, the difference in healthcare utilization and costs between conventional synthetic DMARDs (csDMARDs) and bDMARDs in the treatment of RA patients in Taiwan remains largely unexplored.

Methods: Two cohorts of RA patients and their matched controls were identified from the National Health Insurance Research database (NHIRD). The csDMARD cohort comprised of patients who submitted claims during 1997–2003 for cyclosporine≥50 mg/day with concomitant use of ≥2 csDMARDs for ≥28 days (n=1,569), whilst the bDMARD cohort comprised of patients who had ≥1 claim during 2003–2011 for bDMARD (n = 1,530). The per-patient per-year healthcare utilization and costs were estimated by bootstrapping method, with a comparison being undertaken between csDMARD and bDMARD.

Results: The incremental number of hospitalization days was reduced from 2.3 days for csDMARD to 0.58 day for bDMARD. When compared to csDMARD-treated patients, the incremental total costs and RA-related medication costs were significantly higher in bDMARD-treated patients (US$9,081 vs. US$2,481; US$8,992 vs. US$1,883). However, the combined incremental healthcare utilization costs and non-RA medication costs were significantly lower in bDMARDs-treated patients compared to csDMARD-treated patients (US$374.7 vs. US$1,156.2).

Conclusion: Although total costs increased as a result of introducing biologics in RA treatment, biologics have undoubtedly given rise to the benefits of reduced healthcare utilization. The increase in medication costs from biologics was offset by the lower costs of healthcare utilization. Our findings suggest that the medication costs of biologics may be alleviated by an improvement in clinical outcomes.

Keywords: rheumatoid arthritis, biologics, disease-modifying antirheumatic drugs, healthcare utilization and costs, Taiwan

INTRODUCTION

Rheumatoid arthritis (RA), a chronic autoimmune disease, has an annual incidence rate of about 0.4% in Taiwan, with females being affected more than males (female:male = 2:1 to 4:1) (Kuo et al., 2013). Dysregulation of immune system in RA results in chronic inflammation of the joints and extra-articular organs. Therefore, RA can lead to persistent inflammation of the affected joints, resulting in joint destruction/disability, a higher risk of cardiovascular disease (CVD), and increased mortality (Avina-Zubieta et al., 2012; Choy et al., 2014). Conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), such as methotrexate (MTX), can relieve the symptoms and delay the progression of RA. Therefore, csDMARDs are recommended as the first-line therapy for RA, either in succession or in a combination with other anti-inflammatory agents (Smolen et al., 2017). However, when there is a decline in treatment efficacy under these regimens, patients usually need alternative therapy; otherwise the disease can become more active and progressive.

Licensed biological agents, comprising of tumor necrosis factor (TNF)-α inhibitors, either monoclonal antibody or immunoglobulin fusion protein, which are grouped as biological DMARDs (bDMARDs), have proven to greatly enhance the effectiveness of RA treatment and improve the health outcomes, in terms of both preventing CVD (Barnabe et al., 2011; Solomon et al., 2013) and reducing mortality (Listing et al., 2015), when compared to those receiving csDMARDs (Smolen et al., 2007; Klareskog et al., 2009). These bDMARDs are available in Taiwan for the treatment of RA patients on whom received at least two csDMARDs (MTX and any one of hydroxychloroquine, sulfasalazine, d-penicillamine, azathioprine, leflunomide, and cyclosporine) according to the guidelines of the British Society for Rheumatology. (Ledingham and Deighton, 2005).

Although the health benefits achieved by the TNF inhibitors are notable, the high price of these agents precludes their widespread prescription and places a financial impact on the healthcare system in Taiwan; thus, csDMARDs, non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids continue to play primary roles in the treatment of RA in clinical practice, despite significant numbers of patients showed unsatisfactory responses or intolerance to these therapeutic agents and experienced recurrence of disease activity (Genovese et al., 2002; Voll and Kalden, 2005; Breedveld et al., 2006; Kievit et al., 2011). Among them, poor adherence/persistence or discontinuations are important contributors to treatment failure and disease progression; this, in turn, increases both healthcare utilization and expenditure (Grijalva et al., 2007).

Considering the high price of biologics, numerous studies have reported its cost-effectiveness for RA (Schoels et al., 2010); for example, whilst drug costs have increased among US-employed RA patients since bDMARDs were taken into use, overall medical costs have been reduced (Birnbaum et al., 2012). There is evidence also showing that biologics are associated with cost savings by offsetting the changes in employee utilization of drug and medical services through a reduction of the emergency visits and hospital days, and through an improvement of life quality (Birnbaum et al., 2012).

Similarly, in Taiwan, the annual expenditure on biologics in RA treatment has increased over time (NT$1.11 billion in 2009, NT$1.35 billion in 2010, and NT$1.65 billion in 2011) (National Health Insurance Administration, 2012). However, the overall cost-effectiveness in Taiwan have yet to be fully evaluated; also, there are limited studies estimating the resource utilization of RA patients using real-world data. Along with the first reimbursed bDMARD-etanercept in Taiwan in 2003, the study utilized National Health Insurance Research Database (NHIRD) with longitudinal claim data for the purpose of assessing the impact and cost-effectiveness of bDMARD in Taiwan by comparing the costs and healthcare utilization with csDMARD in the RA treatment.

METHODS

Study Design

This was a retrospective, epidemiological study aiming to assess the differences in healthcare utilization and costs between in RA patients treated with csDMARDs and bDMARDs, using NHIRD from January 1, 1996, to December 31, 2013. Because biologic was not available in the National Health Insurance (NHI) program until 2003, patients with pharmacy claims of csDMARD or bDMARD were identified from 1996 to 2003, and from 2003 to 2011, respectively. Since csDMARD and bDMARD chohorts were identified from different time period, the comparison between csDMARD and bDMARD was made indirectly to avoid cohort effects. Healthcare utilization and costs were evaluated for the specific categories of outpatient/emergency room visits, RA-related surgery, medication, and ward use.

Ethic Statements

The independent Ethics Committee/Institutional Review Board at Taipei Medical University approved this study (201209015). The study was conducted in accordance with the applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki. As all personal information was anonymized before analysis, patient consent was not deemed necessary by the Ethics Committee.

Data Source

The NHIRD is a comprehensive, population-based claims database compiled and maintained by the Taiwan National Health Research Institute. The Taiwan NHI program, launched in March 1995, is a mandatory social health insurance system which covers 99% of more than 23 million people. The dataset consisted of scrambled patient identification numbers, gender,

date of birth, primary and secondary diagnostic codes, and date, type (outpatient, inpatient, emergency visits), and fees charged of the services provided. The Longitudinal Health Insurance Database (LHID, 2010), which contain all the aforementioned claims records of a 1 million sample cohort representative of the Taiwanese beneficiaries in 2010, were used to identify a matched cohort of csDMARD and bDMARD, respectively.

Patient Selection

Patients with moderate to severe RA were enrolled during the retrospective study period (1996 to 2011).The diagnosis of RA (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9-CM codes 714.0) was made according to the 1987 American College of Rheumatology criteria (Arnett et al., 1988) and the Registry of Catastrophic Illness Patient Database (RCIPD) contained in the NHIRD. The including criteria for the two RA cohorts was as follows. First, for the csDMARD cohort, patients were included if they had medication claim for cyclosporine ≥ 50 mg/day with concomitant use of ≥2 csDMARDs for ≥ 28 days within 56 days from 1997 to 2003, as cyclosporine is recommended for the use in severe RA who have not responded adequately to methotrexate.(Cush et al., 1999). Concomitant csDMARDs considered for this analysis included methotrexate, sulfasalazine, hydroxychloroquine, d-penicillamine, azathioprine, and leflunomide. Second, for the bDMARD cohort, patients were selected if they had ≥ 1 claim for bDMARD from 2003 to 2011. The bDMARDs included etanercept, adalimumab, and rituximab (golimumab, tocilizumab, and abatacept were reimbursed after 2012). The date of the first claim when the patient met the inclusion criteria was defined as the index date.

A two-step approach was applied to ensure patients in csDMARD and bDMARD cohorts were mutually exclusive (**Figure 1**). First, patients who had received csDMARDs during 1997–2003 were selected. These patients were followed-up for at least two years until death, lost follow-up, or switching to bDMARDs, whichever came first. Second, patients who had received bDMARDs during 2003 to 2011 were selected and followed-up for at least two years until death or lost follow-up.

Population Matching

Because analytic cohorts were not formed by randomization, comparisons between the cohorts could be confounded by a selection bias. To adjust the potential cohort imbalances, csDMARD and bDMARD were matched 1:1 in the first place using age, gender, and RA severity according to RA duration, which was defined as the duration between the index date and the year when the patient had ≥3 claims with RA diagnoses were firstly occurred.

After comparable csDMARD and bDMARD cohorts were determined, propensity score matching (Rosenbaum and Rubin, 1984; D'Agostino, 1998) was performed on the csDMARD and bDMARD cohorts and their respective controls at a ratio of 1:4. The score measures the similarity between RA cases and their controls in terms of a vector of observable characteristics, namely, age, gender, region, and comorbidity profile. Region was defined by the branches of the NHI Administration in which the subjects were enrolled. The comorbidity profile was evaluated using the Charlson Comorbidity Index (CCI), (Romano et al., 1993), a weighted summary measure of important concomitant diseases within one year before the index date, with RA being excluded.

A two-step approach to find the respective matched controls for csDMARD and bDMARD was employed (**Figure 1**). After excluding subjects who had previously been diagnosed with RA (ICD-9-CM code: 714.xx) during 1996 to 2013 in the LHID, 2010 sample cohort files, the matched controls of csDMARD was established first, followed by that of bDMARD, to ensure two control cohorts were mutually exclusive. The index date of the csDMARD and bDMARD was assigned to their respective matched controls.

Study Measures and Outcomes

Baseline Characteristics

Patient characteristics were measured on the index date, with the data including demographic characteristics (age, gender, region, and index year) and RA duration.

Healthcare Utilization

All-cause annual healthcare utilization was calculated, including outpatient (OPD) visits, emergency room (ER) visits, number of hospitalizations, and number of hospitalization days.

Healthcare Costs

All-cause annual healthcare costs were summarized for the total costs, pharmacy costs, and sub-total costs under various healthcare settings (including OPD, ER, hospitalization, and RA-related surgery). Pharmacy costs were then further divided into RA and non-RA related costs, where RA-related drug costs were those costs associated with csDMARDs or bDMARDs.

Statistical Analyses

Given that cost data are positive values which follow a non-normal distribution and can also often have zero values, the normality assumption is likely to be invalid due to the skewness of the cost data. We therefore used the non-parametric bootstrapping procedure (Jiang and Zhou, 2004) to carry out the statistical inferences and determine the 95% confidence interval (CI) for per-patient per-year (PPPY) healthcare utilization and costs, with 1,000 non-parametric replications being drawn from the source cohorts. This method estimates the empirical distributional function of the data without imposing any probability density function.

We calculated the PPPY utilization/costs as the sum of the utilization costs for each patient divided by the sum of the total number of days in the observation period for each patient, multiplied by 365 days. All of the costs were adjusted to 2013 US dollars. SAS version 9.4 (SAS Institute Inc., Cary, NC) was used for all of the statistical analyses carried out in this study,

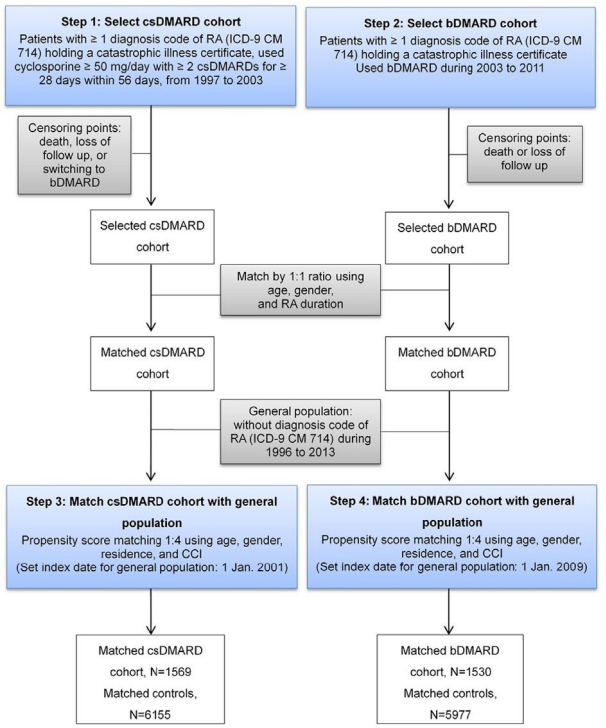

FIGURE 1 | Flowchart of sample selection and population matching.

with a two-sided alpha level of 0.05 being used to determine the statistical significance in all of the comparisons.

RESULTS

Patient Characteristics

After applying the study eligibility criteria and population matching, as shown in **Table 1**, we identified a total of 1,569 patients in the csDMARD cohort versus 6,155 in the csDMARD control cohort, and 1,530 patients in the bDMARD cohort versus 5,977 in the bDMARD control cohort. No significant differences in age, gender, and region were found between csDMARD cohort and csDMARD control, or between bDMARD cohort and bDMARD control.

All-Cause Healthcare Utilization

A summary of the annual all-cause healthcare utilization following the application of bootstrapping is provided in **Table 2**. RA patients were found to have significantly higher numbers of OPD visits and hospitalizations than the general population, although the incremental numbers were comparable between cSDMARD and bDMARD treatment, as compared to the general population.

As shown in **Table 2**, the number of incremental hospitalization days was reduced from 2.3 days for csDMARD to 0.58 day for bDMARD. The length of hospitalization stay was comparable between RA patients using bDMARD and the general population, but significantly longer for RA patients on csDMARD than that for the general population (csControl).

All-Cause Healthcare Costs

As illustrated in **Table 3**, total PPPY costs of the healthcare resources greatly increased for RA patients, compared to the general population, particularly for the bDMARD cohort (total incremental costs: bDMARD vs. csDMARD = US$9,081 vs. US$2,481). The major difference was found in the medication costs, which accounted for 75.9% (US$1,883) of the total incremental costs for csDMARD, and 99.0% (US$8,992) of the total incremental costs for bDMARD. As regards the total RA patient costs, bDMARD costs accounted for a high share up to 79.4% (US$8,712), whereas csDMARD costs accounted for 35.3% (US$1,327). Moreover, the incremental hospitalization costs fell by 53.5%, from US$457.5 for csDMARD to US$212.6 for bDMARD. Increments in RA-related surgery costs fell by

TABLE 1 | Clinical and demographic characteristics.

Variables	csDMARD Comparison		bDMARD Comparison	
	csDMARD N = 1569	csControl N = 6155	bDMARD N = 1530	bControl N = 5977
Age (years), mean ± SD	51.5 ± 12.8	51.8 ± 13.1	51.4 ± 12.9	51.5 ± 13.4
Gender, No. (%)				
Male	354 (22.6)	1385 (22.5)	342 (22.4)	1350 (22.6)
Female	1215 (77.4)	4770 (77.5)	1188 (77.6)	4627 (77.4)
Region, No. (%)				
Northern	451 (28.7)	1826 (29.7)	741 (48.4)	2867 (48.0)
Central	667 (42.5)	2531 (41.1)	379 (24.8)	1466 (24.5)
Southern	419 (26.7)	1670 (27.1)	377 (24.6)	1511 (25.3)
Eastern	32 (2.0)	128 (2.1)	33 (2.2)	133 (2.2)
RA duration (years), mean ± SD	1.9 ± 1.5	–	2.1 ± 1.4	–
Index period (years), mean ± SD	7.4 ± 3.1	9.9 ± 0.5	4.9 ± 2.3	5.0 ± 2.3

RA, rheumatoid arthritis; csDMARD, conventional synthetic disease-modifying antirheumatic drug; bDMARD, biologic disease-modifying antirheumatic drug; SD, standard deviation; CCI, Charlson Comorbidity Index.

TABLE 2 | All-cause annual healthcare utilization per person per year comparison among DMARD and non-RA control using bootstrapping.

Category	csDMARD Comparison, mean (patient-year)			bDMARD Comparison, mean (patient-year)		
	csDMARD (95% CI*)	csControl (95% CI*)	Difference	bDMARD (95% CI*)	bControl (95% CI*)	Difference
Outpatient visits	31.9 (30.9-32.8)	22.3 (22.0-22.7)	9.50	32.5 (31.5-33.4)	23.1 (22.7-23.6)	9.30
Emergency room visits	0.22 (0.2-0.2)	0.16 (0.2-0.2)	0.06	0.31 (0.3-0.3)	0.29 (0.3-0.3)	0.02
Admissions	0.44 (0.4-0.5)	0.18 (0.2-0.2)	0.26	0.48 (0.4-0.5)	0.25 (0.2-0.3)	0.23
Hospitalization days	3.9 (3.5-4.3)	1.6 (1.3-1.8)	2.30	3.2 (2.8-3.5)	2.6 (2.1-3.0)	0.58
RA-related surgery	0.039 (0.03-0.04)	0.007 (0.006-0.007)	0.03	0.031 (0.03-0.04)	0.007 (0.006-0.008)	0.02

**indicates empirical-bootstrapping confidence interval.*

TABLE 3 | All-cause healthcare costs per patient per-year comparison among DMARD and non-RA control using bootstrapping.

Category	csDMARD Comparison, mean ± SD (US$/patient-year)			bDMARD Comparison, mean ± SD (US$/patient-year)		
	csDMARD (95% CI*)	csControl (95% CI*)	Difference	bDMARD (95% CI*)	bControl (95% CI*)	Difference
Total costs	3,757 ± 69.0 (3,623-3,897)	1,276 ± 31.1 (1,216-1,336)	2,481	10,975 ± 119.2 (10,746-11,200)	1,894 ± 50.8 (1,793-1,993)	9,081
Outpatient visits	784.7 ± 25.2 (733.2-828.1)	644.5 ± 23.6 (598.1-691.3)	140.2	830.0 ± 25.1 (777.7-877.2)	953.9 ± 35.0 (883.3-1,021)	-124
Admissions	723.1 ± 34.2 (659.3-793.2)	265.6 ± 10.7 (242.7-285.2)	457.5	632.9 ± 36.2 (562.2-701.0)	420.3 ± 17.4 (385.6-454.0)	212.6
Emergency room visits	22.8 ± 1.5 (19.6-25.5)	16.5 ± 0.6 (15.3-17.5)	6.3	33.8 ± 2.0 (29.6-37.5)	33.9 ± 1.3 (31.1-36.4)	-0.07
RA-related surgery	162.2 ± 10.2 (142.9-182.4)	23.0 ± 1.5 (19.8-25.6)	139.2	115.5 ± 11.1 (92.5-135.2)	23.5 ± 2.3 18.7-27.6)	91.9
Total medication costs	2,249 ± 39.6 (2,172-2,322)	366.7 ± 8.7 (349.5-384.2)	1,883	9,514 ± 107.8 (9,305-9,725)	521.6 ± 17.7 (485.7-554.9)	8,992
RA medication	1,327 ± 30.5 (1,265-1,380)	3.2 ± 1.0 (0.9-4.8)	1,324	8,712 ± 107.8 (8,510-8,921)	5.6 ± 1.6 (2.2-8.2)	8,707

**indicates empirical-bootstrapping confidence interval.*

34%, from US$139.2 for csDMARD to US$91.9 for bDMARD (**Table 3**).

As shown in **Figure 2**, the outpatient costs were increased with csDMARD but reduced with bDMARD (188% reduction, from US$140.2 to −US$124). The incremental non-RA medication costs were reduced by 48.8%, from US$558.5 for csDMARD to US$286 for bDMARD. Finally, despite the increased medication used costs in bDMARD, total healthcare utilization in combination with non-RA medication costs were reduced by 67.6%, from US$1,156.2 for csDMARD to US$374.7 for bDMARD.

DISCUSSION

This nationwide study, using a cross-sectional database from the NHI program, firstly described the clinical outcomes and patterns of direct medical costs in RA patients using DMARDs (traditional or biological) as compared to the general population with non-RA over a long-term observation period, running from 1996 to 2013 in Taiwan. We demonstrated that the use of bDMARD reduced healthcare resources by shortening the length of hospitalization stay (1.72 days shorter) and a reduction of the healthcare utilization costs when compared to csDMARD, and resulted in the reduction of the incremental costs by 67.6% (csDMARD vs. bDMARD: US$1,156.2 vs. US$374.7).

Various types of biologics have been introduced over the past decade to effectively treat RA patients; however, whilst this treatment regimen has clearly reduced health care utilization, it continues to represent a financial impact on the NHI program. Our analysis shows that bDMARDs have substantially increased the total costs of RA patients through a three-fold increase in total costs; bDMARD medication costs accounted for almost 80% of the total costs, as compared to csDMARDs, which accounted for only 35.3% of the total costs. The financial impacts of bDMARD adoption were also reported in other countries; for example, a French observational study reported that annual medical costs had increased almost three-fold after the introduction of etanercept (Juillard-Condat et al., 2008), whilst another study reported a three-fold increase over the prior year in healthcare costs after the introduction of biologics, due to the increased drug costs (Johansson et al., 2015).

Despite the increased medication costs, some studies reveal that bDMARDs have been proven to have cost-saving effects on healthcare. Consistent with our analyses, a retrospective

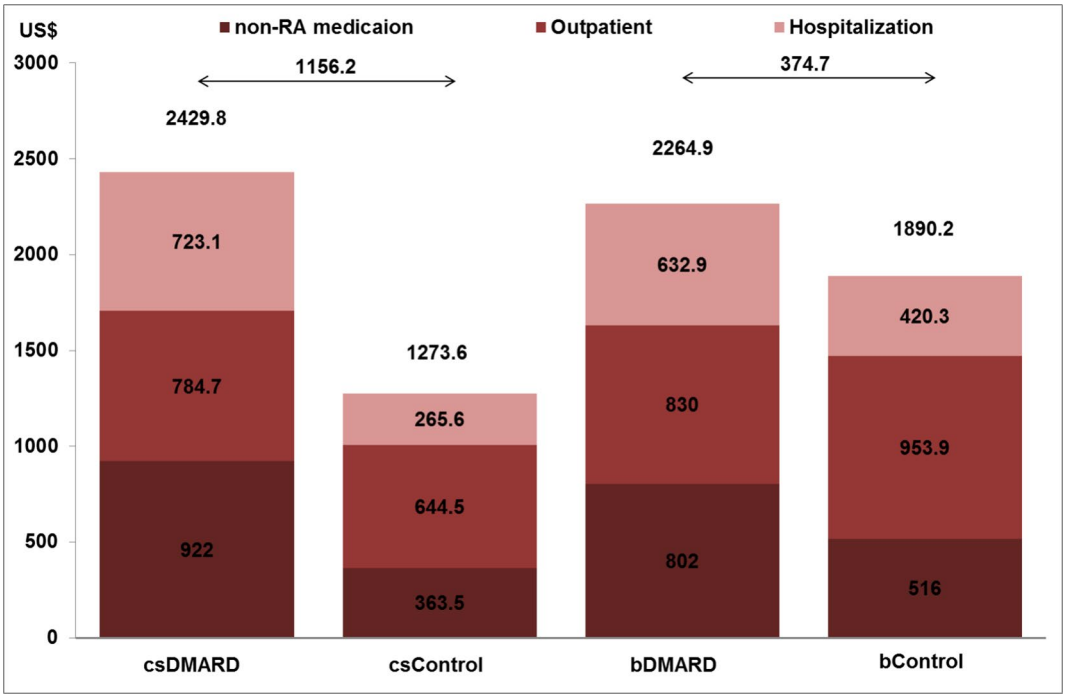

FIGURE 2 | Healthcare system and non-RA medication costs and total medication costs per patient year for RA and non-RA patients.

analysis of a large US claims database found that all-cause healthcare costs were higher in patients receiving csDMARDs prior to the introduction of bDMARDs (Betts et al., 2016). The difference was US$772 for patients using 1 vs. 2 csDMARDs and US$2,390 for patients using 2 vs. 3 csDMARDs. An overview of the differences in the healthcare utilization and costs for RA patients between 1997 and 2006 also concluded that biologics may be associated with cost savings by offsetting the changes in drug expenditure; the specific cost savings identified were reductions in medical services, including hospital days and emergency visits (Birnbaum et al., 2012). When compared to 1997, annual drug costs had increased by US$633 per patient by 2006, but medical costs had fallen by US$618 per patient. The cost-saving effects of bDMARD were also reflected in clinical outcomes such as reduced incidences of CVD, comorbidity, and mortality rates (Pappas et al., ; Barnabe et al., 2011; Solomon et al., 2013). Along with the reduced healthcare utilization in our analysis, the clinical benefits suggest a possible association with an improved control of RA and its comorbidities under bDMARD therapy.

Conversely, csDMARDs-treated patients were found to incur higher healthcare costs. These patients might have suboptimal responses, which may cause additional clinical and economic burdens (Kotak et al., 2013). These burdens were not only limited to direct medical costs but also the indirect costs incurred by society as a result of lost productivity and reduced patient and family incomes, since it has been reported that patients with moderate disease activity were more likely to be unemployed due to disability (Kotak et al., 2013).

Furthermore, RA frequently leads to presenteeism, the cost of which is usually higher than medical costs (Olsson et al., 2004), whilst the use of multiple csDMARDs has been reported to be associated with joint damage due to inadequate therapeutic response and some significant side effects (O'Dell et al., 2013). This implies that the increased healthcare utilization incurred by csDMARDs, which in turn, may be associated with a higher disease burden, resulting in reduced productivity at work and an increase in indirect social costs. On the other hand, the improved clinical outcomes such as articular symptoms/signs, the duration of morning stiffness, and fatigability under bDMARDs treatment may well offset these indirect social costs.

The strength of our study is in the utilization of the NHIRD from the Taiwan reimbursement system, which provides universal healthcare coverage for 99% of the Taiwan population. Based on its distinguishing comprehensive data and long observational period, the NHIRD is an ideal data source for epidemiologic research. Furthermore, our research represents not only a cross-sectional study, but also a longitudinal study, since it spans lengthy study periods of up to 10 years. The longitudinal data provide an opportunity to detect changes or developments in the characteristics of the target population as well as the long-term influence of RA management during the DMARD transition period, from traditional treatment to new biologics, thereby identifying sequences of events.

However, a few limitations of this study should be addressed. Firstly, with data from a different era, it was difficult to directly compare healthcare utilization under

traditional treatment with that under biologics due to differences in patient populations and reimbursement policies, improvements in medical care, and currency inflation. We therefore used an indirect means of comparing RA patients over these different periods by comparing the samples with their matched non-RA population over the same sample period. Even so, given that the impact of the above factors may, to some extent, have influenced our results, it cannot be ignored. Secondly, by using claims data, we were unable to evaluate the indirect social costs attributable to different RA management. To achieve this, we would need to be able to investigate productivity losses from employer perspectives to see whether RA and its comorbidities can lead to a reduction in work productivity, and whether substantial medication expenses after the introduction of bDMARD may have eased such reduced productivity. Finally, the comorbidities were identified using ICD-9-CMcodes that are used for administration purposes; however, certain comorbidities may be underestimated. Furthermore, the surgery codes were not validated, so it is possible that some patients underwent RA-related surgery for other diseases.

In conclusion, although total costs have been increased with the introduction of biologics in RA treatment, bDMARDs potentially resulted in the benefit of reduced healthcare utilization; as such, the increase in medication costs from biologics seems to have been offset by the reduced costs of healthcare utilization, which suggests that the medication costs of biologics may well be offset by an improvement of clinical outcomes.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

As a requirement of publication, the authors have provided the publisher with a signed confirmation of compliance with legal and ethical obligations, including, but not limited to, the following: authorship and contribution, conflicts of interest, privacy and confidentiality, and where applicable, protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors also confirm that this article is unique and not under consideration or published in any other publication and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section.

AUTHOR CONTRIBUTIONS

The contributions made by the individual authors are as follows. D-YC: study design and results interpretation. C-HT: study design, data collection, and data analysis. FY: data analysis. L-WT: data analysis. All authors have participated in manuscript writing and editing.

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The Off-Label Use of Antineoplastics in Oncology Is Limited But Has Notable Scientific Support in a University Hospital Setting

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Purpose: The off-label (OL) use of antineoplastic drugs for the treatment of various types of tumors in patients of different disease stages is becoming a common occurrence. The objective of this study was to analyze these patterns by quantification and characterization of the OL use of antineoplastic drugs and their level of scientific evidence in a medium/high-complexity Spanish general university hospital.

Method: All oncology patients who underwent OL treatment with one or several antineoplastics during the 10 years from 2002 to 2012 were retrospectively selected. The use of these drugs was considered OL if they were used for indications, stages, lines of treatment, or chemotherapy schemes not reflected in the summary of product characteristics published by the European Medicines Agency at the time of prescription. To calculate the prevalence of patients who received one or more OL treatments during the study period, all patients whose primary or secondary diagnosis had been coded with the diagnoses included in the study were selected through the minimum basic data set (MBDS). This database was cross-referenced with that of the Farmatools® program (Dominion®), which collects information on all patients receiving chemotherapy to obtain the total number of patients who received chemotherapy in the hospital during this period.

Results: In total, 684 patients and 866 OL treatments were included. The prevalence of patients undergoing OL treatment with antineoplastics was 6%. OL treatments were used mainly for breast, gynecological, lung, and gastric tumors. The most often-used antineoplastic was paclitaxel, followed by gemcitabine, carboplatin, vinorelbine, and capecitabine, which were used mainly in monotherapy and with palliative intent. A total of 56.1% of the OL schemes used had a level of evidence of 2A according to the National Comprehensive Cancer Network, and 55.3% had a level of evidence of 2B according to Micromedex®.

Conclusion: The OL use of antineoplastics in oncology patients is limited; their use is mainly focused in a small group of tumors and at advanced stages of disease. OL use of antineoplastics occurs under palliative therapeutic strategies with a limited number of

drugs, preferably off-patent drugs. In addition, these OL treatments have high levels of clinical evidence.

Keywords: off-label use, antineoplastic agents, evidence, prevalence, oncology

INTRODUCTION

Cancer is one of the major health problems in countries with developed healthcare systems and is currently the leading cause of death worldwide. Advances and improvements in diagnoses and therapies are contributing to the control and reduction of the death rate from this disease in the United States (USA) and Europe (de Angelis et al., 2014; Ferlay et al., 2015; Organización Mundial de la Salud_OMS, 2018).

Increasing patient survival and quality of life in turn increases the likelihood that patients will receive additional lines of treatment (Hillner and Smith, 2009; Schickedanz, 2010). However, the guidelines and lines of chemotherapy approved by the regulatory agencies are not sufficient to treat the different stages and clinical forms of disease among affected patients. This limitation of the approved therapeutic offerings causes physicians to resort to the use of antineoplastic drugs for conditions that are different from those specified in the product's technical sheet, which is known as off-label (OL) use.

In clinical practice, OL use in the treatment of various types of tumors and progressing stages of disease is a frequent and relevant reality for patients, prescribing physicians, and the economic cost of the healthcare system. Despite its importance, to date, analyses of the efficacy and efficiency of the OL use of drugs in oncology have been limited. Most of the data available are estimates based on a survey conducted in 1991 by the General Accounting Office (GAO) (Laetz and Silberman, 1991; United States General Accounting Office, 1991) of the USA among oncologist members of the American Society of Clinical Oncology (ASCO). According to the results, more than half of the patients (56%) underwent OL treatment with at least one drug, and 33% of all drugs administered were under conditions other than those specified on the data sheet. In 2007, the ASCO and the European Society for Medical Oncology (ESMO) reported that approximately 50% of the use of antineoplastics was for indications that were not reflected on the data sheet (American Society of Clinical Oncology, 2006; Casali and Executive Committe of ESMO, 2007). According to the National Comprehensive Cancer Network (NCCN®) estimate, in the USA, 50%–75% of all uses of antineoplastics in oncology are OL (Benson and Brown, 2008; Cohen et al., 2009).

Several descriptive studies of OL use in oncology have been performed, such as a study conducted by Levêque et al. (2005) in France, which estimated an annual prevalence of 6.7% by analyzing OL prescriptions of 10 antineoplastic drugs for 10 tumor types. In Switzerland, Joerger et al. (2014) determined that 27.2% of antineoplastic administrations were OL, and a study by Mellor et al. (2009) in Australia reported that 35% of the prescriptions were OL and that the prevalence increased from 22% in 2001 to 35% in 2008.

The present study analyzes the OL use of any antineoplastic agent for patients treated over 10 years. We attempted to minimize the transitory impact of periods related to the upcoming approval of a specific drug by analyzing a long period of time. This approach also allowed us to evaluate OL use in all tumors without limiting the analysis to neoplasms. The study was conducted in an oncology service in the setting of a medium/high-complexity general university hospital. The pattern of OL use was analyzed by quantification and characterization according to the tumor type and stage and the progressive clinical phase of the patient. We also investigated the level of scientific evidence that supports OL use in oncology.

MATERIALS AND METHODS

This observational and retrospective study was conducted at the Prince of Asturias University Hospital located in Alcalá de Henares (Madrid, Spain) with 500 beds, specialized diagnosis and treatment units, and more than 10 highly differentiated clinical specialties, which offers healthcare coverage to a population of approximately 400,000 inhabitants. This study analyzed the period between January 1, 2002, and December 31, 2012.

All patients with OL use of one or several antineoplastic drugs, either as a single agent or in combination, during the study period were included. The use of these drugs was considered OL if they were used in indications, stages, lines of treatment, or chemotherapy schemes that were not reflected in the summary of product characteristics published by the European Medicines Agency at the time of prescription. We excluded patients who received treatments with experimental drugs or drugs unauthorized in Spain and those uses that were considered OL due to their use in posologies or routes of administration other than those authorized. Each patient was counted only once; in the cases of patients with more than one OL use of a drug, each treatment was counted separately.

The clinical histories and the dispensing data of the selected patients were reviewed. Information on each patient's age, sex, Eastern Cooperative Oncology Group (ECOG) status, diagnosis, tumor stage, metastasis, line of treatment, OL drug, type of OL use (monotherapy or combination), patent status and route of administration of the drug, and whether the treatment had curative or palliative intent was collected. To evaluate the status of the patent, we defined a drug "not protected by the patent" if it had expired before or during the study period. We examined the level of evidence of the chemotherapy schemes with OL use (CSOLs) according to the NCCN v1.2015 and Micromedex® 2015 compendia.

Abbreviations: OL, off-label; SmPC, summary of product characteristics; CSOLs, chemotherapy schemes with off-label use; MBDS, minimum basic data set; AEMPS, Spanish Agency for Medicines and Health Products.

The diagnoses were classified according to the ICD.9.10.MC (Ministerio de Sanidad and Servicios Sociales e Igualdad, 2014) classification and were grouped into 14 primary tumor locations or types. A miscellaneous group was created for OL use in rare tumors and those with an absolute frequency lower than 5. A "*patent-protected drug*" was defined as a drug that, at the end of the study (December 31, 2012), was still under a valid patent.

The types of OL use that were classified as monotherapy were additionally classified as "*not indicated for the tumor*" if the drug did not have any indication for that tumor type or in any of the stages of the tumor or as "*indicated in combination*" if the drug had an indication for a certain tumor type in combination with other agents. Likewise, for OL uses classified as combination therapy, those "*with other indications*" were distinguished if they were indicated for that tumor type as monotherapy in another line of treatment or in combination with other agents and as "*without indication*" when at least one drug in the combination had no indication for that type of tumor.

To calculate the prevalence of patients who received one or more OL treatments during the study period, all patients whose *primary or secondary diagnosis* had been coded with the diagnoses included in the study were selected through the minimum basic data set. This database was cross-referenced with that of the Farmatools® program (Dominion®), which collects information on all patients receiving chemotherapy to obtain the total number of patients who received chemotherapy in the hospital during this period.

Statistical Analysis

A descriptive statistical analysis of the data was conducted. Conformity tests of the categorical variables related to the characteristics of the patients and the OL use of antineoplastic agents (tumor location, drug used, type of OL, intention of treatment, and patent status of the drug) were performed by comparing the proportion of the sample using a chi-square test. A value of $p \leq 0.05$ was considered significant. All statistical analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

The study was approved by the Ethics Committee for Scientific Research at Prince of Asturias University Hospital and was classified by the Spanish Agency for Medicines and Health Products as post-authorization study with designs other than prospective follow-up (EPA-OD).

RESULTS

Demographic and Clinical Characteristics of the Sample With Oncological Disease

This study included 794 patients with oncological disease and 980 OL treatments during the 10 years of the study. After the exclusion criteria were applied to this sample, 684 patients and 866 OL treatments were included in the analysis (**Figure 1**).

The study showed a significantly higher amount of OL use in women with an average age of less than 65 years and good general condition ($p < 0.001$). OL use was predominant in patients with stage IV disease and the presence of metastasis ($p < 0.001$) (**Table 1**).

OL treatment Is Preferably Used in Oncology for a Small Group of Tumor Types and With Limited Use of Agents

Among all cancer patients treated with chemotherapy (n = 11,385), the prevalence of those receiving OL use of antineoplastic drugs was 6% (684/11,385).

OL treatments were used mainly in four locations or tumor types: breast cancer (25.2%), gynecological tumors (16.1%), lung cancer (11.8%), and gastric cancer (10.2%). The OL use in these tumor types was significantly greater than that in all other tumor types (63.3% vs 36.7%, respectively; $p < 0.001$) (**Table 2**).

We found an OL use of 27 antineoplastic agents, and the most commonly used was paclitaxel (19.2%), followed by gemcitabine (10.9%), carboplatin (9.6%), vinorelbine (8.8%), and capecitabine (8.7%). The OL use of these five drugs was significantly more frequent than that of the other 22 drugs (57.2% vs 42.8%, respectively; $p < 0.001$) and was essentially focused on the four

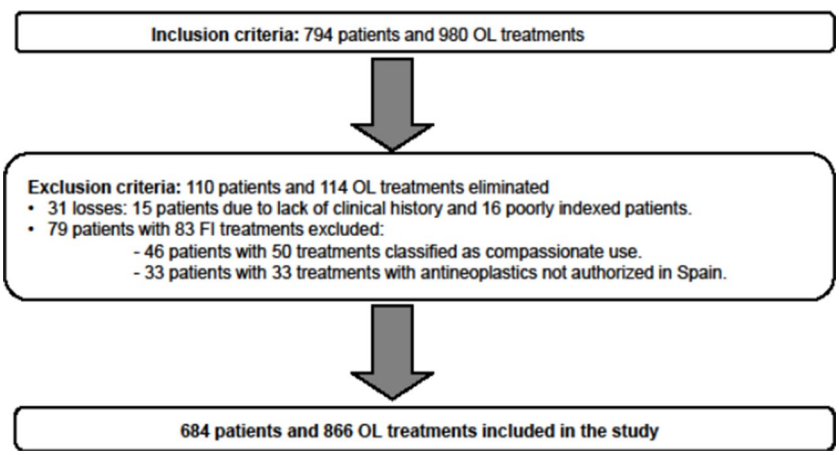

FIGURE 1 | Inclusion or exclusion of patients from the study according to the selection criteria.

TABLE 1 | General characteristics of patients who received off-label treatments.

Patient characteristics	N = 866	%
Gender*		
Male	370	42.7
Female	496	57.3
Age*		
Mean age of patients (years)	58.21 ± 11.57	
Age <65	601	69.4
Age ≥65	265	30,6
Performance status (ECOG)*		
0	402	46.4
1	308	35.6
2	156	18
Presence of metastatic disease*		
Yes	684	79
No	182	21
Disease stage*		
I	39	4.5
II	59	6.8
III	84	9.7
IV	684	79
Previous antineoplastic treatment line		
0-3	704	81.3
3-6	142	16.4
6-9	19	2.2
>9	1	0.1

*$p < 0.001$.

TABLE 2 | Primary location of tumors involved in off-label use of antineoplastic drugs.

Tumor location	Treatment (n = 866)	%
Breast	218	25.2
Gynecological	139	16.1
Lung	102	11.8
Gastric	88	10.2
Head and neck	69	8
Biliopancreatic	64	7.4
Colorectal	53	6.1
Soft tissue sarcoma	29	3.3
Bladder-urothelial	25	2.9
Prostate and testicle	24	2.8
Melanoma	22	2.5
Esophagus	15	1.7
Miscellaneous	12	1.4
Liver	6	0.7

TABLE 3 | Characteristics of treatments with off-label use of antineoplastics.

Drug	Treatment (n = 866)	%
Paclitaxel	195	19.2
Gemcitabine	111	10.9
Carboplatin	98	9.6
Vinorelbine	89	8.8
Capecitabine	88	8.7
Irinotecan	77	7.6
Trastuzumab	74	7.3
Oxaliplatin	71	7
Docetaxel	54	5.3
Bevacizumab	36	3.5
Temozolomide	22	2.2
Liposomal doxorubicin	19	1.9
Cetuximab	16	1.6
Etoposide	13	1.3
Lapatinib	11	1.1
Cisplatin	8	0.8
Topotecan	8	0.8
IL-2	5	0.5
Pemetrexed	5	0.5
Adriamycin	4	0.4
Albumin-paclitaxel	4	0.4
Pegylated liposomal doxorubicin	3	0.3
Nilotinib	2	0.2
Cyclophosphamide	1	0.1
Imatinib	1	0.1
Sorafenib	1	0.1
Sunitinib	1	0.1
Type of off-label use		
Monotherapy	609	70.3
Not indicated for the tumor	458	52.9
Indicated in combination	151	17.4
Combinations	257	29.7
With other indications	126	14.6
Without indication	131	15.1
Intent of off-label treatment		
Curative	182	21
Palliative	684	79
Patent status		
Patent protected	235	27.1
Off-patent	631	72.9
Line of off-label treatment		
1ª line	150	17.3
2ª line	243	28.1
3ª line	197	22.7
4ª line	114	13.2
Successive lines	162	18.7

most frequent tumor types. The OL use of drugs as palliative treatment predominated over their use as curative treatment (79% vs 21%, $p < 0.001$) (**Table 3**).

The wide OL use of paclitaxel was due to its use in monotherapy in nonsmall cell lung cancer; cancers of the cervix, bladder, and esophagus; and head and neck tumors. The OL use of gemcitabine was notable as a single agent in breast and ovarian tumors, for which it has an indication when combined with other agents. OL use of carboplatin occurred for breast cancer, both as monotherapy and combined with other agents. The OL use of vinorelbine was due to its use as a single agent for the treatment of gynecological and prostate tumors; the OL use of capecitabine mainly included its use in a neoadjuvant form in rectal cancer and as an adjuvant in high-risk stage II colon cancer and with palliative intent in pancreatic cancer.

Most of the drugs used were not protected by patents (73% vs 27%; $p < 0.001$). The most commonly used route of administration was intravenous. Regarding the line of treatment for OL use of antineoplastics, no significant differences were observed between the first four lines used. However, 50.8% of OL uses were as second- and third-line treatments.

The OL treatments were mainly used in monotherapy (70.3% vs 29.7%; $p < 0.001$), with a notable use of drugs without any type of indication approved for the tumor on which they were

used (52.9%). **Table 4** indicates the type of OL use for each tumor site.

OL Treatments Are Mostly Supported by Clinical Evidence

The analysis of the degree of evidence of the 114 CSOLs included in the study showed the following: the NCCN® indicated a 2A level of evidence for 56.1% of the schemes, while the Micromedex® indicated that practically the same percentage (55.3%) of schemes had a 2B level of evidence. The same analysis was performed on the 866 OL treatments included in the study; in this case, 64% of the treatments used had a 2A level of evidence according to the NCCN® and a 2B level of evidence according to Micromedex®.

The percentages of CSOLs that were not reflected in either of the two compendia were 22.8% in NCCN® and 34.2% in Micromedex® however, these CSOLs accounted for only 11.6% and 19.4% of 866 OL treatments analyzed, respectively (**Table 5**).

DISCUSSION

The results of this study showed that in the setting of a general university hospital, the OL use of antineoplastic drugs in patients with tumor pathology is limited and is focused on a small group of tumors (breast, gynecological, lung, and gastric) in patients under 65 years old with good general condition and advanced-disease stages. The OL use of antineoplastic drugs mainly occurs in a palliative therapeutic strategy with a limited number of drugs, preferably off-patent drugs. In addition, these OL treatments attain high levels of clinical evidence.

TABLE 4 | Type of off-label use for each location/tumor type.

Location/Tumor type	Type of Label Treatment							
	Monotherapy				Combination			
	Not indicated for the tumor		Indicated in combination		Without indication		With other indications	
	Patients (n)	(%)	Patients (n)	(%)	Patients (n)	(%)	Patients (n)	(%)
Biliopancreatic								
Pancreas	46	86.8	0	0	7	13.2	0	0
Biliary	10	90.9	0	0	1	9.1	0	0
Bladder-urothelial	24	96	0	0	1	4	0	0
Breast	27	12.39	41	18.81	46	21.1	104	47.7
Colorectal								
Colon	17	77.3	0	0	5	22.7	0	0
Rectal	30	96.8	0	0	1	3.2	0	0
Esophagus	15	100	0	0	0	0	0	0
Gastric	70	79.6	17	19.3	1	1.1	0	0
Gynecological								
Cervix	22	66.7	0	0	11	33.3	0	0
Endometrium	5	23.8	0	0	16	76.2	0	0
Ovary	43	50.57	32	37.63	10	11.8	0	0
Head and Neck	37	73.9	14	20.3	18	26.1	0	0
Liver	6	100	0	0	0	0	0	0
Lung								
Microcytic	28	87.5	4	12.5	0	0	0	0
Not microcytic	5	7.15	43	61.45	0	0	22	31.4
Melanoma	17	77.3	0	0	5	22.7	0	0
Miscellaneous								
GIST (gastrointestinal stromal tumor)	2	100	0	0	0	0	0	0
Neuroendocrine	0	0	0	0	2	100	0	0
Osteosarcoma	0	0	0	0	1	100	0	0
Kidney	1	100	0	0	0	0	0	0
Adrenal	1	100	0	0	0	0	0	0
Thymoma	2	66.7	0	0	1	33.3	0	0
TOD (tumor of unknown origin)	2	100	0	0	0	0	0	0
Prostate and Testicle								
Prostate	11	100	0	0	0	0	0	0
Testicle	12	100	0	0	0	0	0	0
Soft tissue sarcoma	24	82.8	0	0	5	17.2	0	0

TABLE 5 | Evidence for off-label treatment.

	Off-label chemotherapy regimens n = 114	%	Treatments n = 866	%
NCCN®				
1	6	5.3	113	13
2A	64	56.1	554	64
2B	16	14	104	12
3	2	1.8	3	0.3
N/A	26	22.8	92	10.6
***MICROMEDEX*®**				
1	1	0.9	30	3.5
2A	8	7	101	11.7
2B	63	55.3	555	64.1
3	3	2.6	6	0.7
N/A	39	34.2	174	20.1

N/A (Not applicable): chemotherapy regimens for which evidence is not included in the compendium that was evaluated.

The OL use of antineoplastic drugs in oncology is a therapeutic practice that is used in healthcare in a highly variable manner according to the published studies. Such studies have specifically analyzed the prescriptions for patients with specific tumors without specifying whether they are different patients or successive lines used in the same patient. This fact can explain the variability observed in the reflected prevalence of OL use—between 6% and 35% (Leveque et al., 2005; Mellor et al., 2009; Joerger et al., 2014). Our results of the analysis of all patients who received OL antineoplastic treatment show that the frequency of use of this therapeutic strategy is limited and is focused on 6% of patients. These data contrast with those obtained in the GAO survey conducted on a sample of North American medical oncologists (Laetz and Silberman, 1991; United States General Accounting Office, 1991). These differences can be explained, in addition to the methodological reasons previously described, by other reasons, including accessibility and control of the OL use of antineoplastic drugs in the setting in which this study was conducted: a hospital of a national universal healthcare system for the population served by the center with free access by the ill. However, until 2009, the management of requests and approval for the OL use of antineoplastic drugs in our national healthcare system was centralized in a review committee external to the hospital; thus, this management strategy for OL drug use could have influenced the low comparative prevalence found in our study.

To assess the OL treatments used in routine clinical practice, the epidemiology of the tumor and advances in the clinical and therapeutic management of the disease must be taken into account (Hanahan and Weinberg, 2000; Kocs and Fendrick, 2003; Newell, 2005; Hillner and Smith, 2009; Schickedanz, 2010). Likewise, improvements in supportive care have also increased the number of candidates for additional chemotherapy lines (Kocs and Fendrick, 2003). Our study shows that the OL use of antineoplastic drugs is significantly greater in palliative oncological treatment strategies, in metastatic stages of the disease, and especially as second- and third-line treatments. These findings are consistent with those obtained in the USA by the GAO (Laetz and Silberman, 1991; United States General Accounting Office, 1991; Eastman, 2005) on the OL use of antineoplastic agents and those of other studies with smaller sample sizes (Kocs and Fendrick, 2003; Peppercorn et al., 2008; Roila et al., 2009; Cioffi et al., 2012; Cohen et al., 2013; Joerger et al., 2014).

This work indicates that the OL use of antineoplastic drugs is mainly focused on breast cancer, gynecological tumors, lung cancer, and gastric cancer. These four neoplasms share the characteristics of high prevalence and/or mortality (Ferlay et al., 2013; de Angelis et al., 2014; Ferlay et al., 2015; Organización Mundial de la Salud_OMS, 2018; SEOM, 2019). In previous studies, such as that of Joerger et al. (2014), which included 10 tumor types, the OL use of antineoplastics was more prevalent for gastrointestinal, breast, lung, and gynecological tumors. In the study conducted by Leveque et al. (2005), of 10 tumor types, prostate, breast, bladder, and ovarian cancers had the most OL prescriptions.

Our findings indicate that the results obtained by other authors who performed selective analysis of some tumor types included those more prevalent in a general analysis of all tumors (Leveque et al., 2005; Mellor et al., 2009; Joerger et al., 2014).

In our study, OL use was mainly focused on the use of 5 of 27 drugs, including paclitaxel, gemcitabine, carboplatin, vinorelbine, and capecitabine. All five drugs are cytotoxic agents with recognized activity in different tumors, and their use is supported by more and less extensive studies and for advanced pathologies. We found that paclitaxel had the most OL use; in contrast, OL use of docetaxel was limited. These data could be explained by the introduction of generic drugs and the changes observed in toxicity patterns, which could have changed the attitudes toward medical prescription. This possibility could affect the discrepancy between the OL use of docetaxel observed in our study and that found by other authors (Leveque et al., 2005; Joerger et al., 2014). Joerger et al. (2014) examined the OL use of 10 antineoplastics and 437 prescriptions in their analysis and found that OL use was more frequent with paclitaxel, followed by carboplatin and docetaxel. In the study by Levêque et al. (2005), the drug most commonly prescribed for OL use was docetaxel, followed by oxaliplatin, fludarabine, carboplatin, gemcitabine, paclitaxel, and irinotecan. The authors explained that the wide use of docetaxel was mainly due to its use in prostate cancer; in 2002, docetaxel still did not have an indication for this type of tumor in France.

The evaluation of our results on the OL use of antineoplastics in relation to other studies requires consideration of some relevant aspects of oncological treatment. Among them, the analyzed period is notable due to the advances made in the management of neoplasms and the scope of the study, which includes the healthcare system in which the study is conducted, the country, and the hospital level. From a general perspective, the distribution of the OL use of antineoplastics according to the type or tumor location reflected in our study shows patterns of indication and use similar to those described in two previous

studies. It should be noted that these three studies were conducted in European healthcare settings.

Notably, the OL use of antineoplastic drugs is more common in monotherapy than in combination therapies, with a predominant use in groups of drugs without any authorized indication for the tumor for which they were used. This finding can be related to the palliative intent of treatment and the use of these drugs as second- and third-line treatments. In this type of clinical situation, an increase in patient survival is sought *via* good control of the disease, with quality of life prevailing over other factors. In addition, the previous lines of treatment will limit the available options according to the health results obtained with the use of these drugs and any toxicities developed during the treatment.

In our analysis, a majority of the drugs used for OL treatments were not protected by patents. This finding is consistent with that of other publications that establish that an increase in OL use may occur among antineoplastic drugs after the patent has expired. This loss of patent could lead to a lack of interest on the part of the pharmaceutical industry to obtain new indications (Radley et al., 2006; Casali and Executive Committe of ESMO, 2007; Roila et al., 2009; Gota and Patial, 2011; Lerose et al., 2012; Wittich et al., 2012; Conti et al., 2013). Likewise, other drugs are not authorized for all indications for which they could be effective, mainly due to the high economic cost and time required to obtain a new indication (Boos, 2003; Gupta and Nayak, 2014).

Currently, several authors have established that in clinical practice, a chemotherapy scheme is supported by evidence and therefore is "*medically accepted*" if it is considered category 1 or 2A by the NCCN compendium or class 1, 2A, or 2B by Micromedex® (American Society of Clinical Oncology, 2006; Benson and Brown, 2008; Mehr, 2012). Independent clinical investigations, despite being considered of lower scientific quality than clinical trials, generate experience and provide data on the efficacy and safety of OL use of antineoplastics (Gazarian et al., 2006; Casali and Executive Committe ESMO, 2007; Mellor et al., 2009; Feliu and Espinosa, 2013; Gupta and Nayak, 2014).

Despite the debate that exists regarding the evidence supporting the OL use of antineoplastic drugs (Gazarian et al., 2006; Abernethy et al., 2009; Irwin et al., 2012; Pfister, 2012), our results indicate that most of these OL uses are performed with significant scientific support, such as that collected in the North American therapeutic compendia. This coincides with studies previously published by Gota and Patial (2011) and Mellor et al. (2012) that used the NCCN to assess the degree of evidence of the OL schemes analyzed. Regarding the OL use of antineoplastic drugs that were not included in these compendia, drugs or regimens used in marginal cases were included, in which the justification for their use is based on results published in scientific journals without having reached the levels of so-called recognized therapeutic clinical evidence. It should also be noted that the compendia used originated from the USA, while the usual clinical practice was performed in Europe, and the approved indications for each drug are different between the two continents.

Our analysis presents some important limitations such as a one-center study, and therefore, the results focus on the type of population that our hospital serves, and epidemiologically infrequent or "rare" tumors do not reach a relevant qualitative significance. Multicentric studies should be carried out to confirm our results and to analyze the use of OL antineoplastics in less frequent tumors.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ Supplementary Files.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comite Etico de Investigacion Clinica del Hospital Universitario Principe de Asturias. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

MF designed the study, performed the experiments, analyzed the data, and wrote the manuscript. RV designed the study, performed the experiments, and wrote the manuscript. MY performed the experiments. FE performed the experiments. JG performed the experiments. MI performed the experiments. MA designed the study and wrote the manuscript.

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REVIEW
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Review of Statistical Methodologies for Detecting Drug–Drug Interactions Using Spontaneous Reporting Systems

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Concomitant use of multiple drugs for therapeutic purposes is known as "polypharmacy situations," which has been recognized as an important social problem recently. In polypharmacy situations, each drug not only induces adverse events (AEs) but also increases the risk of AEs due to drug–drug interactions (DDIs). The proportion of AEs caused by DDIs is estimated to be around 30% of unexpected AEs. The randomized clinical trials in pre-marketing typically focus emphasis on the verification of single drug safety and efficacy rather than the surveys of DDI, and therefore, patients on multiple drugs are usually excluded. However, unlike pre-marketing randomized clinical trials, in clinical practice (= post marketing), many patients use multiple drugs. The spontaneous reporting system is one of the significant sources drug safety surveillance in post-marketing. Commonly, signals of potential drug-induced AEs detected from this source are validated in real-world settings. Recently, not only methodological studies on signal detection of "single" drug, but also on several methodological studies on signal detection of DDIs have been conducted. On the other hand, there are few articles that systematically summarize the statistical methodology for signal detection of DDIs. Therefore, this article reviews the studies on the latest statistical methodologies from classical methodologies for signal detection of DDIs using spontaneous reporting system. This article describes how to calculate for each detection method and the major findings from the published literatures about DDIs. Finally, this article presented several limitations related to the currently used methodologies for signal detection of DDIs and suggestions for further studies.

Keywords: pharmacovigilance, statistical methodology, signal detection, spontaneous reporting systems, drug–drug interaction

INTRODUCTION

For safety surveillance of a drug, several data-mining algorithms are used to detect quantitative signals from spontaneous reporting systems. The data-mining algorithms include the frequency statistical models are the *proportional reporting ratio* (*PRR*) (Evans et al., 2001) and the *reporting odds ratio* (*ROR*) (van, Puijenbroek et al. 2002), and the Bayesian statistical models are [the *information component* (*IC*) as the *Bayesian Confidence Propagation Neural Network* (*BCPNN*) (Bate et al., 1998) and the *gamma-Poisson shrinker* (*GPS*) (Szarfman et al., 2002) used as the empirical Bayes geometric mean (*EBGM*)].

Although, the recent extension of the *IC* and the *GPS* can accommodate signals of high-order interactions (Almenoff et al., 2003; Yang and Fram, 2004; Norén et al., 2006; DuMouchel and Harpaz, 2012), generally, the *PRR* and the *ROR* are exploited for early signal detection of unknown "single" drug-induced adverse events (AEs). And these detection models might detect potential drug-induced AEs that could not be found clinical trials of pre-marketing using spontaneous reporting systems including post-marketing data.

The randomized clinical trials in pre-marketing typically focus emphasis on the verification of single drug safety and efficacy rather than the surveys of drug–drug interactions (DDIs), and therefore patients on multiple drugs are usually excluded from the clinical trial. However, unlike pre-marketing randomized clinical trials, in clinical practice (= post marketing), many patients use multiple drugs, as in polypharmacy situations.

Concomitant use of multiple drugs can affect the biological action of the related drugs. The main types of DDIs include pharmacokinetic and pharmacodynamic interactions (Aronson, 2004). Of them, the pharmacokinetic interactions might affect the metabolism of drug that determine bioavailability. On the other hand, there is no change in blood levels of drugs in the pharmacodynamic interactions, which can occur either competitively or non-competitively at the pharmacological receptor level.

In concomitant use of multiple drugs, each drug not only induces AEs but also increases the risk of AEs due to DDIs. The proportion of AEs caused by DDI has been estimated to be around 30% of unexpected AEs (Pirmohamed and Orme, 1998).

Adverse events caused by DDIs can also be prevented if discovered early like single drug-induced AEs, and it is practically difficult to examine the interactions of all drug combinations in the pre-marketing stage (Banda et al., 2016). Therefore, post-marketing surveys will help early detection of unknown AEs not only caused by single drug but also DDIs.

Recently, several methodological studies on signal detection of DDIs have been conducted. Herein, we review studies on the statistical methodologies for signal detection of DDIs using spontaneous reporting systems.

STATISTICAL METHODOLOGY

Logistic Regression Model

van Puijenbroek et al. proposed a statistical method using the *logistic regression model* for detecting signals of DDIs from a spontaneous reporting system (van, Puijenbroek et al. 1999; van, Puijenbroek et al. 2002).

The *ROR* is a statistical model similar to odds ratio (van, Puijenbroek et al. 2002), and using the *logistic regression model* shown in Eq. 1, the *ROR* adjusted for age, gender, and concomitant drugs (*drug* D_1 and *drug* D_2) is used as the *adjusted ROR*.

$$log\left(odds\right) = \beta_0 + \beta_1\ a + \beta_2\ G + \beta_3\ x_1 + \beta_4\ x_2 + \beta_5\ x_1\ x_2 \qquad (1)$$

where, a = age, G = gender, x_1 = *drug* D_1, x_2 = *drug* D_2, and x_1 x_2 = the concomitant use of *drug* D_1 and *drug* D_2.

In their first study, the authors showed that concomitant use of oral contraceptives and the antifungal itraconazole resulted in the occurrence of withdrawal bleeding. In the second study, the authors showed that the efficacy of diuretics decreased with the concomitant use of diuretics and non-steroidal anti-inflammatory drugs, resulting in worsening of congestive heart failure (van, Puijenbroek et al., 1999).

Signal detection using the *logistic regression model* has some limitations (e.g., ignoring dependencies/associations between AEs and regression analysis of more than 10,000 drugs as included in a spontaneous reporting system).

To overcome the limitations of the *classical logistic regression model*, a new statistical model; the *Bayesian logistic regression model*, which extended the logistic regression model corresponding to data of very large dimensions, was proposed. The *Bayesian logistic regression model* can perform regression analysis using millions of predictors contained in a spontaneous reporting system. (Genkin et al., 2007).

Using the *Bayesian logistic regression model*, Caster et al. also addressed masking effect (*cf. Limitation*) that affects background reporting of AEs (Wang et al., 2010) and confounding caused by the concomitant use of multiple drugs (Caster et al., 2010).

Extended Gamma-Poisson Shrinker Model

Multi-Item Gamma-Poisson Shrinker Model

The *multi-item gamma-Poisson shrinker* (*MGPS*) *model* is currently used by the US Food and Drug Administration (FDA) and is a statistical model that extended the *GPS* model for detecting signals of potential DDIs (Almenoff et al., 2003; Yang and Fram, 2004).

The *MGPS model* can calculate the score of "*Drug–Drug–Event*" or "*Drug–Event–Event*" (including that of with higher-order interactions such as "*Drug–Drug–Drug–Event*" or "*Drug–Drug–Event–Event*"). Moreover, the *MGPS model* can be applied to the itemsets of size 3 or more, but as the number of items increases, the calculation amount explosively increases.

In the *MGPS model*, *Excess2* is used an indicator value. The signal detection threshold value is not set, and as the value of *Excess2* is relatively large, the influence of interaction caused by concomitant drugs is predominantly suspected.

For an arbitrary itemset, it is desirable to estimate the expectation $\lambda = E\ [N/E]$. Where, N is the observed frequency of the itemset (= number of reports) and E is the count predicted from an assumption that items are independent, that is, the baseline count.

The observed frequency of itemset is defined by $i, j, k,\ldots$, as $N_i, N_j, N_k\ldots, E$ and other variables are defined as subscript letters as well as N. For example, E_{ij} is the baseline prediction for the number of involving items i and j.

As a common model, baseline counts are calculated based on the assumption of within-stratum independence. E calculated under this assumption is often expressed as $E0$.

If all reports are assigned to the strata denoted by $s = 1, 2,\ldots,$ S, the proportion of reports in stratum s that contain the item i is expressed by P_i^s, and the total number of reports in stratum s is expressed by n_s.

Here, the frequency of baseline for triple itemset (*i*, *j*, and *k*) is defined under independence as:

$$E0_{ijk} = \sum n_s P_i^s P_j^s P_k^s \tag{2}$$

For itemsets of size 3 or more, an "all-2-factor" loglinear model can be defined as the frequency *E*2 for the itemsets that match all the estimated pairwise two-way marginal frequencies but contain no high-order dependencies.

For itemsets of size 3 (e.g., DDI: *drug* D_1 and *drug* D_2, and AE), the estimated frequency of the all-2-factor loglinear model can be defined as the frequency *E*2 prediction by simple subtraction is compared.

For example:

$$Excess2_{ijk} = \lambda_{ijk} E0_{ijk} - E2_{ijk} \tag{3}$$

The parameter λ is estimated by the geometric means, denoted as *EBGM*, of their empirical Bayes posterior distributions.

Detecting the signals of DDIs using the *MGPS model* is based on the *EBGM* value of the two drugs and the lower of the 90% confidence interval (CI) being larger than the upper of the 90% CI estimates for each of the two drugs.

Example, in one of the reports the signals of potential DDIs detected using the *MGPS model* is the AE profile of verapamil (the calcium channel blocker) and the combination of three classes of cardiovascular drugs (Almenoff et al., 2003).

This result revealed that the *MGPS model* for disproportionality measure is a promising statistical model for detecting signals of potential DDIs in polypharmacy situations.

Regression-Adjusted Gamma-Poisson Shrinkage Model

The *GPS model* proposed by DuMouchel is worse than the *logistic regression model* (Harpaz et al., 2013). However, unlike the *GPS model*, signal detection using *t*-tests in *logistic regression models* is not suitable for small samples such as rare AEs (DuMouchel and Pregibon, 2001).

DuMouchel et al. proposed the *Regression-adjusted gamma-Poisson shrinkage* (*RGPS*) model, which integrated the *GPS model* and the *logistic regression model* into a hybrid detection model with the advantages of both, to overcome the disadvantages of the *GPS* model (DuMouchel and Harpaz, 2012).

The *RGPS model* is similar to the *MGPS model* (cf. *Multi-item Gamma-Poisson Shrinker Model*) in that the relative reporting rate (*RRR*) is entered into the *Bayesian gamma-Poisson shrinking algorithm*, and a reliable estimate rate and CI are obtained.

On the contrary, the major difference between the *RGPS model* and the *MGPS model* is that the *MGPS model* do not consider the effects for polypharmacy, and thus may lead to the underestimation of disproportionality estimate for the drug of interest. In addition, the *RGPS model* can handle this question.

Additionally, the values of the adjusted expected value (E) in *the RGPS model* is not calculated by standard logistic regression but instead the extended logistic regression.

It is recommended to replace *EBGM* as the posterior geometric mean with the *empirical Bayes relative reporting ratio* (*EBRRR*) as the posterior mean in the *RGPS model*.

For each response, the (N_j, E_j) pairs from the previous step are input into a gamma-Poisson shrinkage algorithm. The prior distributions are assumed to be simple gamma distributions rather than a mixture of two gamma distributions as is done in the *MGPS model*. Specifically, a two-parameter gamma Poisson model is used to produce shrinkage estimates, where the prior distribution of the relative reporting ratios is assumed to be Gamma (γ, δ) and where the (N_j, E_j) pairs are used to estimate the hyperparameters γ and δ. The posterior mean of a drug relative reporting ratio is then $EBRRR_j = (N_j + \gamma)/(E_j + \delta)$, and *RRR*05 and *RRR*95 are computed using the appropriate gamma distribution Gamma($N_j + \gamma$, $E_j + \delta$) (DuMouchel and Harpaz, 2012).

In the *RGPS model* of DDIs, n_{jk} is defined as the number of reports including both $drug_j$ and $drug_k$, and N_{jk} is defined as the number of reports related to expected AEs. Then, $EBRRR_j$ and $EBRRR_k$ are defined as the corresponding disproportionality estimates for the two drugs in report *i*.

$$p_i = P_\alpha\left(\mu_i = \beta_{0_{g(i)}} + \sum X_{ij}\beta_j\right) \tag{4}$$

where P_α is the function that links the linear predictor μ_i to the probability scale and β_j and $\beta_{0g(i)}$ are the estimated coefficients for the drugs and intercepts, where the intercept depends on which grouped-stratum $g(i)$ report *i* belong to. Additionally, Let $X_{ij} = 1$ if drug *j* is included in report *i*, $X_{ij} = 0$ otherwise, and let N_j be the number of events reported with drug *j*.

E_{jk} is defined as the expected value (*E*) of N_{jk} under the null hypothesis that both $drug_j$ and $drug_k$ have no effect of the *RRR*.

$$E_{jk} = \sum X_{ij} X_{ik} P_\alpha\left(\mu_i - \beta_j - \beta_k\right)\left(1 \le j < k \le J_{\text{int}}\right) \tag{5}$$

where, β_j or β_k is considered as 0 if the suspected drug was not in the *logistic regression model*.

"No interaction" indicates that the disproportionality measure for both the drugs (= N_{jk}/E_{jk}) is expected to be higher for the $EBRRR_j$ and $EBRRR_k$. Therefore, the no-interaction expected count is defined as follows:

$$E_{jk}^* = E_{jk}^* \max\left(EBRRR_j, EBRRR_k\right) \tag{6}$$

There will be J_{int} ($J_{\text{int}} - 1$)/2 raw interaction ratio ($INTRR_{jk}$) of the form:

$$INTRR_{jk} = \frac{N_{jk}}{E_{jk}^*} \tag{7}$$

DuMouchel et al. proposed a method to use one-parameter prior gamma distribution (γ_1, γ_1), of mean 1, as a model for the

mean of $INTRR_{jk}$, and estimate γ_1 by inputting the set $(N_{jk,}\ E^*_{jk})$ into the empirical Bayes estimation.

As a result, the posterior mean of the interaction ratio is expressed as follows:

$$INTEB_{jk} = \frac{N_{jk} + \gamma_1}{E^*_{jk} + \gamma_1} \tag{8}$$

The posterior 5% limit ($INTEB_{05jk}$) and posterior 95% limit ($INTEB_{95jk}$) are the corresponding quantiles of the gamma distribution $(N_{jk} + \gamma_1, E^*_{jk} + \gamma_1)$.

The proposed *RGPS model* only presents interaction estimates if $INTEB_{05} > INTEB_{05\min}$ or $INTEB_{95} < INTEB_{95\max}$ with the default values $INTEB_{05\min} = 1$ and $INTEB_{95\max} = 1/3$ by DuMouchel and Harpaz (2012).

If the *INTEB* is very low, it has not yet been completely verified whether it are the signals of potential DDIs. However, because such results are often obtained, the further verification will be necessary.

Extended Information Component Model

The *IC* is a measure of association of pairs of drug and AEs only, but there is often an interest in high-order interactions as DDIs (= itemsets of size 3).

Although, an extension of the *IC* to 3rd-order associations including 3 itemset as DDIs was proposed by Orre et al. (2000), the proposed method did not compensate for pairwise associations. Therefore, Norén et al. (2006) proposed the following definition for the *extended IC model*:

$$IC_{xyz} = IC_{xy|z} - IC_{xy} = IC_{yz|x} - IC_{yz} \tag{9}$$

where,

$$IC_{xy|z} = log_2 \frac{P(xy\,|\,z)}{P(x\,|\,z)P(y\,|\,z)} \tag{10}$$

As with simple algebraic operations, IC_{xyz} can be re-expressed as follows:

$$IC_{xyz} = log_2 \frac{P(yz\,|\,x)}{P(y\,|\,x)P(z\,|\,x)} - log_2 \frac{P(y)P(z)}{P(y,z)} = log_2 \frac{P(x,y,z)P(x)P(y)P(z)}{P(x,y)P(x,z)P(y,z)} \tag{11}$$

Although arbitrarily accurate estimates for the posterior mean of *IC* distribution can be used (Koski and Orre, 1998), the maximum *a posteriori* (m.à.p.) estimates can be used for central estimates instead, because *IC* distribution is generally unimodal.

There are three main advantages of the m.à.p. estimate.

First, it is well suited for use in stratified *IC*. Second, it has the intuitive property of being equal to 0 when the estimated joint probability is equal to the product of the estimated marginal probabilities. Third, the concept of most likely value for an unknown parameter is perhaps more natural than that of the expected value.

These are important aspects in drug safety applications, and the results must be interpretable not only by statisticians but also by non-statisticians such as medical professionals.

Norén et al. (2006) proposed the following m.à.p. estimate. Most of the theory developed for the pairwise *IC* model ($IC_{\text{m.à.p.}}$ model) holds approximately the *IC model* for higher order.

In one of the reports, the signals of potential DDIs detected using the *extended IC model* was terfenadine and ketoconazole-induced ventricular fibrillation. There were five reports of ventricular fibrillation due to the combination of terfenadine and ketoconazole in the VigiBase® as a spontaneous reporting system, and the extended *IC* ($IC_{xyz\ m.à.p.}$) value is 2.40 with the lower of the 95% CI of 1.08 (Norén et al., 2006).

Ω Shrinkage Measure Model

The *Ω shrinkage measure model* was proposed to calculate the observed-to-expected ratio as a spontaneous reporting system for detecting the signals of potential DDIs (Norén et al., 2008).

Norén et al. criticized the *logistic regression model* in missing out on several signals that strongly suggestive of potential DDIs, additionally, they demonstrated that after conducting comparative studies using the World Health Organization database, the *Ω shrinkage measure model* is a refined method compared to the *logistic regression model*.

For the *Ω shrinkage measure model*, the observed reporting ratio was f_{11} of AE caused by concomitant use of 2 drugs: *drug* D_1 and *drug* D_2, in addition, its expected value was $E[f_{11}]$.

$$f_{00} = \frac{n_{001}}{n_{00+}},\ f_{10} = \frac{n_{101}}{n_{10+}},\ f_{01} = \frac{n_{011}}{n_{01+}},\ f_{11} = \frac{n_{111}}{n_{11+}} \tag{12}$$

where, n is the number of reports shown in **Figure 1**. For example, n_{111} is the number of reported target AEs caused by *drug* D_1 and *drug* D_2.

$E[f_{11}]$ is unknown. However, f_{11} can be compared with the estimator g_{11} of $E[f_{11}]$, g_{11} is given as follows:

$$g_{11} = 1 - \frac{1}{\max\left(\frac{f_{00}}{1-f_{00}}, \frac{f_{10}}{1-f_{10}}\right) + \max\left(\frac{f_{00}}{1-f_{00}}, \frac{f_{01}}{1-f_{01}}\right) - \frac{f_{00}}{1-f_{00}} + 1} \tag{13}$$

When $f_{10} < f_{00}$ (which denote no risk of AE caused by *drug* D_1), the most sensible estimator $g11 = \max(f_{00}, f_{01})$ is yielded and the *vice versa* when $f_{01} < f_{00}$.

Norén et al. defined a non-shrinkage measure for detecting AEs caused by *drug* D_1 and *drug* D_2 as follows:

$$\Omega_0 = log_2 \frac{f_{11}}{g_{11}} \tag{14}$$

	Target AE	All other AEs	Total
drug D_1 and *drug* D_2	n_{111}	n_{110}	n_{11+}
Only *drug* D_1	n_{101}	n_{100}	n_{10+}
Only *drug* D_2	n_{011}	n_{010}	n_{01+}
Neither *drug* D_1 or *drug* D_2	n_{001}	n_{000}	n_{00+}
Total	n_{++1}	n_{++0}	n_{+++}

AE: adverse events; *n*: the number of reports (*e.g.* n_{+++}: the number of all reports, n_{111}: the number of *drug* D_1 and *drug* D_2 induced target AE reports).

FIGURE 1 | Four-by-two contingency table for the evaluation of drug–drug interaction.

	drug D_2	Not *drug* D_2
drug D_1	p_{11} $(= n_{111}/n_{11+})$	p_{10} $(= n_{101}/n_{10+})$
Not *drug* D_1	p_{01} $(= n_{011}/n_{01+})$	p_{00} $(= n_{001}/n_{00+})$

AE: adverse events, *n*: the number of reports (e.g. n_{111}: the number of *drug* D_1 and *drug* D_2 induced target AE reports), *p*: the proportion of reports.

FIGURE 2 | Two-by-two contingency table for the evaluation of drug–drug interaction.

However, since the occurrence of AE is rare, g_{11} might show very small, and therefore, Ω_0 is sensitive to spurious relationship and tends to falsely detect a signal.

This is a well-known phenomenon in screening pairwise drug-AE excessive reporting rates in a spontaneous reporting system, and shrinkage has been proven to be an effective approach in reducing the sensitivity to random fluctuations in disproportionality measures based on rare cases. The models such as the *BCPNN* and *EBGM* also used pairwise measures of disproportionality as shrinkage measures.

To construct a similar shrinkage measure from Eq. 14, Norén et al. re-expressed the observed and expected *RRR* f_{11} and g_{11} in terms of the observed number of reports n_{111} and expected numbers of reports $E_{111} = g_{11} \times n_{11+}$, respectively:

$$\frac{f_{11}}{g_{11}} = \frac{n_{111}/n_{11+}}{E_{111}/n_{11+}} = \frac{n_{111}}{E_{111}} \tag{15}$$

and proposed the Ω shrinkage measure:

$$\Omega = log_2 \frac{n_{111}+\alpha}{E_{111}+\alpha} \tag{16}$$

α is the tuning parameter that determines the shrinkage strength. When α = 0, Ω = Ω_0. The effect of α is equivalent to that of α additional expected reports, and exactly matches the increase in the observed number.

Shrinkage regression can be set as the value of tuning parameter based on cross-validation estimates for classifier performance. However, in a disproportionality analysis, there is no objective basis for selecting a particular value for α. Therefore, in the *Ω shrinkage measure model*, α = 0.5 was set to provide sufficient shrinkage for avoiding disproportional highlighting based on rare reports.

In the frequentist method, Ω differs slightly from Ω_0 for large n_{111} and E_{111}, and the variance of Ω_0 is given as follows:

$$\mathrm{Var}\left(\Omega_0\right) = \mathrm{Var}\left(log_2 \frac{n_{111}}{E_{111}} \right) \approx \frac{1}{n_{111} log\left(2\right)^2} \tag{17}$$

Using Eq. 17, the lower of the 95% CI for Ω can be estimated using the following equation:

$$\Omega_{025} = \Omega - \frac{\phi\left(0.975\right)}{log\left(2\right)\sqrt{n_{111}}} \tag{18}$$

where, $\phi(0.975)$ is 97.5% of the standard normal distribution.

On the contrary, in Bayesian method, the exact CI for μ can be obtained as solutions to the following equation, for appropriate posterior quantiles μ_q:

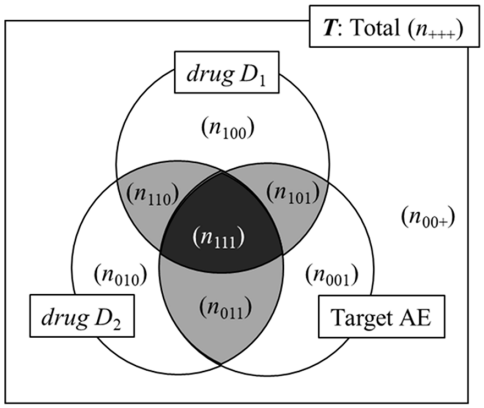
$$\int_0^{\mu_q} \frac{\left(E_{111}+\alpha\right)^{n_{111}+\alpha}}{\Gamma\left(n_{111}+\alpha\right)} u^{n_{111}+\alpha-1} e^{-\left(n_{111}+\alpha\right)u} du = q \tag{19}$$

where, α is the tuning parameter. n_{111} and E_{111} are the number of reported target AEs caused by *drug* D_1 and *drug* D_2 and their expected values.

Here, the logarithm of the solution to Eq. 19 for q = 0.025 and 0.975 provides Ω_{025} (the lower limits of 95% CI) and Ω_{075} (the upper limits of 95% CI), respectively.

In both frequentist and Bayesian methods, $\Omega_{025} > 0$ is used as a threshold for detecting the signals of the concomitant use with *drug* D_1 and *drug* D_2.

Qian et al. built a computerized system in which data acquisition and placement are automated. The signals of potential DDIs were then detected using this system. (Qian et al., 2010). This study detected the signals of potential DDIs using three different models; the *Ω shrinkage measure model*, the *logistic regression model* (cf. *Logistic Regression Model*), and the *additive model and multiplicative models*



AE: adverse events, ***T***: total of report (all reports), n: the number of reports (n_{+++}: the number of all reports, n_{111}: the number of *drug* D_1 and *drug* D_2 induced target AE reports).

FIGURE 3 | Venn diagram for the evaluation of drug–drug interaction.

(cf. *Additive and Multiplicative Models*). A comparison of signals detected using the three models revealed that the signals of potential DDIs detected on average by at least two models could reflect the fact that the 3 models are highly correlated (Qian et al., 2010).

Additive and Multiplicative Models

Thakrar et al. (2007) proposed the *additive model* and *multiplicative model* for detecting the signals of potential DDIs. For two models, Thakrar et al. (2007) conducted the retrospective study for detecting the signals of known DDIs using the FDA Adverse Event Reporting System.

The *additive model* assumes that drug related risks increase additively, on the contrary, the *multiplicative model* assumes that drug related risks increase synergistically. *Additive Model* and *Multiplicative Model* provide the details of each model using **Figure 2**.

Additive Model

In the additive model, if the risk associated with *drug* D_1 without *drug* D_2 is the same as the risk associated with *drug* D_1 and *drug* D_2 together, then there is no signal of DDI. In other words, there are potential DDIs if the combination risk is high compared to what is expected based on the individual drug:

$$p_{11} - p_{00} = \left(p_{10} - p_{00}\right) + \left(p_{01} - p_{00}\right) \tag{20}$$

This equality implies (*RD*: risk difference):

$$RD_{drug\ D_1 \cap drug\ D_2} = RD_{only\ drug\ D_1} + RD_{only\ drug\ D_2} \tag{21}$$

That is, when $RD_{drug\ D1 \cap drug\ D2} - RD_{only\ drug\ D1} + RD_{only\ drug\ D2} > 0$ ($p_{11} - p_{10} - p_{01} + p_{00} > 0$), the signal of the *additive model* is detected.

The formal statistical test for DDIs is performed within the framework of binomial distribution linear regression:

$$risk\ of\ event = \alpha_0 + \alpha_1\ x_1 + \alpha_2\ x_2 + \alpha_{12}\ x_1\ x_2 + \varepsilon$$
$$\left(\alpha_{12} = p_{11} - p_{10} - p_{01} + p_{00}\right) \tag{22}$$

Multiplicative Model

In the *multiplicative model*, under the assumption that the null hypothesis is true (i.e., no interaction), the proportion of an AE associated with the concomitant use of *drug* D_1 and *drug* D_2 is the same as the proportional risks of individual drugs in the absence of either *drug* D_1 or *drug* D_2.

$$\frac{p_{11}}{p_{00}} = \frac{p_{10}}{p_{00}} \times \frac{p_{01}}{p_{00}} \tag{23}$$

or

$$\frac{p_{11}\ /\left(1-p_{11}\right)}{p_{00}\ /\left(1-p_{00}\right)} = \frac{p_{10}\ /\left(1-p_{10}\right)}{p_{00}\ /\left(1-p_{00}\right)} \cdot \frac{p_{01}\ /\left(1-p_{01}\right)}{p_{00}\ /\left(1-p_{00}\right)} \tag{24}$$

This equality implies:

$$PRR_{drug\ D_1 \cap\ drug\ D_2} = PRR_{only\ drug\ D_1} \times PRR_{only\ drug\ D_2} \tag{25}$$

or

$$ROR_{drug\ D_1 \cap\ drug\ D_2} = ROR_{only\ drug\ D_1} \times ROR_{only\ drug\ D_2} \tag{26}$$

Therefore, if the measure shown in Eq. 27 or Eq. 28 exceeds 1 it can be determined that the signals of potential DDIs are detected. In modeling terminology, the following multiplicative model (Eqs. 25 and 26) can be applied for *log-linear regressions* and *logistic regressions*:

log-linear regressions

$$log\left(risk\ of\ event\right) = \beta_0 + \beta_1\ x_1 + \beta_2\ x_2 + \beta_{12}\ x_1\ x_2 + \varepsilon$$
$$\left(e^{\beta_{12}} = \frac{PRR_{drug\ D_1 \cap\ drug\ D_2}}{PRR_{only\ drug\ D_1} \times PRR_{only\ drug\ D_2}}\right) \tag{27}$$

logistic regressions

$$logit\left(risk\ of\ event\right) = \gamma_0 + \gamma_1\ x_1 + \gamma_2\ x_2 + \gamma_{12}\ x_1\ x_2 + \varepsilon$$
$$\left(e^{\gamma_{12}} = \frac{ROR_{drug\ D_1 \cap\ drug\ D_2}}{ROR_{only\ drug\ D_1} \times ROR_{only\ drug\ D_2}}\right) \tag{28}$$

where, x_1 = *drug* D_1, x_2 = *drug* D_2, x_1 x_2 = the concomitant use of *drug* D_1 and *drug* D_2.

Thakrar et al. (2007) showed that the *additive model* has higher sensitivity than that of the *multiplicative model* in detecting the signals of potential DDIs. Therefore, Noguchi et al. compared the power of the *additive model* with that of the *multiplicative model* for the *combined risk ratio model* (cf. *Combination Risk Ratio Model*). Similar to the result of Takagi et al., the *additive model* presented higher detection power than that of the *multiplicative model* (sensitivity: 95.62 vs. 65.46%, specificity: 96.92 vs. 98.78%, Youden's index: 0.925 vs. 0.642, positive predictive value: 89.47% vs. 93.64%, negative predictive value: 98.78 vs. 91.26% *F*-score: 0.924 vs. 0.771) (Noguchi et al., 2018a).

Combination Risk Ratio Model

To estimate the degree of potential safety risk in combination, Susuta and Takahashi (2014) proposed a risk assessment method for combined use of drugs at a frequency where two or more drugs are reported simultaneously, assuming that the possibility of drug interaction is a combined risk in the occurrence of AEs.

The concomitant use risk was determined when the ratio between the concomitant use indicator and the indicator (e.g., *PRR*, *ROR*) obtained separately for both agents exceeded 2. The following is an expression using the *PRR* as the indicator.

$$Combination\ risk\ ratio = \frac{PRR_{drug\ D_1 \cap\ drug\ D_2}}{\max\left(PRR_{drug\ D_1}, PRR_{drug\ D_2}\right)} \quad (29)$$

When $n_{111} \geq 3$, $PRR_{drug\ D1\ \cap\ drugD2} > 2$, $\chi^2_{\ drug\ D1\ \cap\ drugD2} > 4$, *Combination risk ratio* > 2, it was a signal of DDIs.

The formula for calculating *PRR* and χ^2 is as follows:

$$PRR = \frac{\left(N_{11} / N_{1+}\right)}{\left(N_{01} / N_{0+}\right)} \quad (30)$$

$$\chi^2 = \frac{n_{+++} \times \left(\left|N_{11} \times N_{00} - N_{10} \times N_{01}\right| - n_{+++} / 2\right)^2}{N_{1+} \times N_{+1} \times N_{0+} \times N_{+0}} \quad (31)$$

Additionally, to calculate the *PRR* and the χ^2 of *drug* $D_1 \cap$ *drug*D_2, *drug* D_1 and *drug* D_2, replace it as follows.

drug $D_1 \cap$ *drug*D_2: $N_{11} = n_{111}$, $N_{00} = n_{000} + n_{010} + n_{100}$, $N_{10} = n_{110}$, $N_{01} = n_{001} + n_{011} + n_{101}$, $N_{1+} = n_{11+}$, $N_{+1} = n_{++1}$, $N_{0+} = n_{00+} + n_{01+} + n_{10+}$, $N_{+0} = n_{++0}$.

drug D_1: $N_{11} = n_{111} + n_{101}$, $N_{00} = n_{000} + n_{010}$, $N_{10} = n_{110} + n_{100}$, $N_{01} = n_{001} + n_{011}$, $N_{1+} = n_{11+} + n_{10+}$, $N_{+1} = n_{++1}$, $N_{0+} = n_{00+} + n_{01+}$, $N_{+0} = n_{++0}$.

drug D_2: $N_{11} = n_{111} + n_{011}$, $N_{00} = n_{000} + n_{100}$, $N_{10} = n_{110} + n_{010}$, $N_{01} = n_{001} + n_{101}$, $N_{1+} = n_{11+} + n_{01+}$, $N_{+1} = n_{++1}$, $N_{0+} = n_{00+} + n_{10+}$, $N_{+0} = n_{++0}$.

To check the validity of the *combination risk ratio model*, the reports of Stevens–Jonson syndrome (SJS) or toxic epidermal necrolysis caused by the DDIs were analyzed using the Japanese Adverse Drug Event Report database.

As for the concomitant use of suspected drugs, which fulfill the situations of concomitant use risk, SJS: 10 candidates out of 159 combinations and toxic epidermal necrolysis: 22 candidates out of 111 combinations were detected.

In addition, this method proposed by Susuta et al. has been used to search for the DDIs related to the concomitant use of angiotensin receptor blockers and thiazide diuretics combination therapy by Noguchi et al. (2015) and for detecting signals of the concomitant use of deferasirox with other drugs by Mizuno et al. (2016) in Japan.

Chi-Square Statistics Model

Gosho et al. (2017) proposed the *chi-square statistics model* for detecting the signals of potential DDIs.

First, they developed the following measure (χ_0) to estimate the discrepancy between the observed and expected numbers of AEs with drug combinations:

$$\chi_0 = \frac{n_{111} - E_{111}}{\sqrt{E_{111}}} \quad (32)$$

The expected number of AEs (E_{111}) can be estimated using $E_{111} = g_{11} \cdot n_{11\cdot}$, presented in *Ω Shrinkage Measure Model*. The measure χ_N, which is the square root of the chi-square test statistic, is based on the normal approximation of the *Poisson* model, and therefore, χ_N is not suitable for the evaluation of rare events. Thus, when evaluating rare events, it is generally considered more appropriate to use the chi-square test with Yate's correction than the standard chi-square test (Yates, 1934), hence, χ was also corrected with the correction term "0.5" based on the chi-square test with Yate's correction:

$$\chi = \frac{n_{111} - E_{111} - 0.5}{\sqrt{E_{111}}} \quad (33)$$

Gosho et al. (2017) set $\chi > 2$ and $\chi > 2.6$ as thresholds for detecting the signals of AEs caused by DDIs in a simulation study. These cutoff values are specified based on 95% and 99% of chi-square distribution with one degree of freedom. According to this simulation study, with the criterion: $\chi > 2$, false positives are controlled within acceptable ranges, additionally the *chi-square statistics model* showed higher sensitivity and AUC than those of both frequentist and Bayesian methods of the *Ω shrinkage measure model* (Gosho et al., 2017).

Similar to the *Ω shrinkage measure model*, the detection of signal using the *chi-square statistics model* is designed to focus on the detection of synergistic rather than antagonism among some DDIs.

Gosho (2018) used the *chi-square statistics model* and he *Ω shrinkage measure model* to examine the clinical drug–drug interactions that cause hypoglycemia and rhabdomyolysis (Gosho, 2019).

Association Rule Mining Model

To comprehensively search for the signals of potential DDIs, if a calculation using the conventional methods that simply create combinations from a large database such as a spontaneous reporting system is used, the considered number of the concomitant use would be enormous. Therefore, it would be difficult to detect the signals of potential DDIs at an early stage.

Contrarily, the *association rule mining model* is frequently used to find interesting combinations hidden in large databases, and not just medical databases. In the *association rule mining model*, the "*a priori algorithm*" can be used to reduce the number of calculations (Agrawal et al., 1993; Agrawal and Srikant,1994).

If the *association rule mining model* was used, it is unnecessary to calculate indicators for all combinations of the concomitant use, as the previous models.

An indicator of a general association rule model is shown below.

Among the transaction T as a set of items, an association rule can be expressed as the antecedent of rule $X \rightarrow$ the consequent of rule Y; where, X and Y are mutually exclusive sets of items.

There are several indicators of the *association rule mining model*. First, the *support* is defined as the percentage of all items in both X and Y to transaction T in the data. That is, how frequently the rules ($X \rightarrow Y$) occur within transaction T. The *support* is as follows:

$$support\left(X \rightarrow Y\right) = \frac{\left\{X \rightarrow Y\right\}}{\left\{T\right\}} \tag{34}$$

Second, the *confidence* is the conditional probability $P(Y|X)$, and measures the reliability of the interference made by the rules ($X \rightarrow Y$). The *confidence* is as follows:

$$confidence\left(X \rightarrow Y\right) = \frac{support\left(X \rightarrow Y\right)}{support\left(X\right)} \tag{35}$$

Third, the *lift* of an association rule represents the ratio of probability. It is the ratio between the *confidence* of the rule and the *support* of the itemset in the consequent of the rule. The *lift* is as follows:

$$lift\left(X \rightarrow Y\right) = \frac{confidence\left(X \rightarrow Y\right)}{support\left(Y\right)} = \frac{support\left(X \rightarrow Y\right)}{support\left(X\right) \times support\left(Y\right)} \tag{36}$$

If the *lift* is > 1, it shows the degree to which those two occurrences depend on each other. Therefore, the *lift* is often used frequently to assess the interest of a rule.

Finally, the *conviction* of an association rule can be interpreted as the ratio of the expected frequency that X occurs without Y if X and Y are independent and divided by the observed frequency of incorrect predictions. The *conviction* is as follows:

$$conviction\left(X \rightarrow Y\right) = \frac{1 - support\left(Y\right)}{1 - confidence\left(X \rightarrow Y\right)} \tag{37}$$

In the *lift*, even if X and Y are interchanged, the value of the indicator is the same. On the contrary, in the *conviction*, when X and Y are interchanged, the value of the indicator is different. This indicates that the *lift* cannot be evaluated correctly if Y is actually the antecedent of rule and X is actually the consequent of rule, and the *conviction* can be also evaluated correctly in such a situation.

So far, we have introduced four indicators that are particularly commonly used in the *association rule mining model*. Next, three search models of the signals of potential DDIs using these indicators are shown using **Figure 3**.

Shirakuni's Method of Association Rule Mining Model

Shirakuni et al. (2009) examined the combined use and discrete use of 2 drugs using *association rule mining model*.

In the combined use of two drugs model, the antecedent of rule X was defined as *drugs* D_1 and D_2, and the consequent of rule Y was defined as the target AE (AE).

$$support\left(drug\ D_1\ and\ drug\ D_2 \rightarrow AE\right) = \frac{\left\{drug\ D_1\ and\ drug\ D_2 \rightarrow AE\right\}}{\left\{T\right\}} \tag{38}$$

$$confidence(drug D_1 and drug D_2 \rightarrow AE) = \frac{support(drug D_1 and drug D_2 \rightarrow AE)}{support(drug D_1 and drug D_2)} \tag{39}$$

In the discrete use of 2 drugs model, the antecedent of rule X was defined as *drugs* $D_{1\ (or\ 2)}$-induced AE, and the consequent of rule Y was defined as *drugs* $D_{2\ (or\ 1)}$. In this rule, both hypotheses and conclusions are relevant to the AE, and therefore, signals can be detected from *drugs* D_1 and *drugs* D_2 individually.

The *support* and *confidence* of each drug is calculated for both *drugs* D_1 and *drugs* D_2 based on the cases of patients presenting AEs included in the dataset.

$$support\left(drug\ D_{1(or\ 2)}\ induced\ AE \rightarrow drug\ D_{2(or\ 1)}\right) = \frac{\left\{drug\ D_{1(or\ 2)}\ induced\ AE \rightarrow drug\ D_{2(or\ 1)}\right\}}{\left\{T\right\}} \tag{40}$$

$$confidence(drug D_1\ and drug D_2 \rightarrow AE) = \frac{support(drug D_{1(or2)} induced AE \rightarrow drug D_{2or1})}{support(drug D_{1(or2)} induced AE)} \tag{41}$$

Kubota purposed that because the *PRR* show the generation ratio of AEs, the result is evaluated regardless of sample size and

χ^2 is important when examining the total sample size (Kubota, 2001). Therefore, the drugs with high *log PRR* and *log* χ^2 values are considered to have a strong signal.

To evaluate *Shirakuni's method*, the *signal score* obtained by adding the *log PRR* and *log* χ^2 was used as the strength of the signal. This *signal score* is also used to compare signals for sex and age differences (Noguchi et al., 2018b; Noguchi et al., 2018c).

$$Signal\ score = log\ PRR + log\ \chi^2 \tag{42}$$

The FDA Adverse Event Reporting System dataset had sufficient information to apply the *association rule mining model*. In the *association rule mining model*, high indicators of the *support* and *confidence* are generally evaluated as a strong relationship. Next, Shirakuni et al. (2009) compared each *signal score* of the SJS caused by DDIs with the results of the association rule mining model to evaluate the performance of the proposed model.

In this result, the correlation between "discrete use of 2 drugs" and the *signal score* was weaker than that of "combined use of 2 drugs." Therefore, it was concluded that, among the two methods of the *association rule mining mode*l proposed by Shirakuni et al. (2009), the method focused on "combined use of 2 drugs" detected such important signals at an early stage.

Harpaz's Method of Association Rule Mining Model

In *Harpaz's method* (Harpaz et al., 2010), like the combined use of two drugs model suggested by Shirakuni et al. (Shirakuni et al., 2009), the antecedent of rule *X* was defined as drugs D_1 and D_2, and the consequent of rule *Y* was defined as the AE.

However, in the *association rule mining model*, it is sometimes inappropriate to evaluate using the *confidence* value. For example, frequently reported AEs (e.g., nausea) produce large confidence values regardless of the drug associated with AEs. Whereas, rarely reported AEs may produce small confidence values, although AEs are strongly associated with certain drugs.

Therefore, in *Harpaz's method*, the *RRR* was used instead of *confidence* as the second parameter to qualify the worthiness or strength of an association rule (Harpaz et al., 2010).

The *RRR* is defined as the ratio of the observation frequency of the rule to the prediction frequency of the baseline, and is shown as Eq. 43.

The other disproportionality analysis methods are based on the *RRR*, namely the *BCPNN* and the *EBGM* in the signal detection of a single drug.

$$RRR = \frac{Observed}{Expected} = confidence\left(drug\ D_1\ and\ drug\ D_2 \rightarrow AE\right) \times N \tag{43}$$

N is the total number of records in the data.

Extrapolating from Harpaz's evaluation sample, the full set of potential DDIs identified by the method can be described by the taxonomy and proportions shown below.

Drugs are divided into the following three categories; (1) drugs known to be administered together or treat the same indication: 57%; (2) drugs with the same active ingredient: 2%; and (3) supposedly unrelated drugs: 41%.

AEs are divided into the following four categories: (1) one of the drugs is known to cause effect: 22%; (2) all drugs are known to cause effect: 21%; (3) none of the drugs is known to cause effect: 27%; and (4) confounded association, where drugs are administered to treat the AE: 30%.

The DDIs are divided into the following two categories: (1) known drug interaction: 35% and (2) unknown drug interaction: 65%.

In evaluations using *Harpaz's method*, the results demonstrate that a significant number of DDIs can be identified. Additionally, the very low *p*-value indicates that it is extremely unlikely that Harpaz's method detected them just by chance, and thus is a valid statistical model for signal detection.

Noguchi's Method of Association Rule Mining Model

We proposed *Noguchi's method* using the *association rule mining model* (Noguchi et al., 2018a). In *Noguchi's method*, the antecedent of rule *X* was defined as *drug* $D_{2\ (or\ 1)}$ and the consequent of rule *Y* was defined as *drug* $D_{1\ (or\ 2)}$-induced AE. That is, *Noguchi's method* focuses on how much additional *drug* $D_{2\ (or\ 1)}$ contributes to *drug* $D_{1\ (or\ 2)}$-induced AE.

$$\begin{aligned} &lift\left(drug\ D_{2(or1)} \rightarrow drug\ D_{1(or2)} induced\ AE\right) \\ &= \frac{confidence\left(drug\ D_{2(or1)} \rightarrow drug\ D_{1(or2)} induced\ AE\right)}{support\left(drug\ D_{1(or2)} induced\ AE\right)} \end{aligned} \tag{44}$$

The *lift* according to this model indicates that the presence of *drug* $D_{2\ (or\ 1)}$ influences the probability of *drug* $D_{1\ (or\ 2)}$-induced AE. Furthermore, in this method, it was confirmed by *conviction* that the DDIs obtained are not a false prediction.

$$\begin{aligned} &conviction\left(drug\ D_{2(or1)} \rightarrow drug\ D_{1(or2)} induced\ AE\right) \\ &= \frac{1 - support\left(drug\ D_{1(or2)} induced\ AE\right)}{1 - confidence\left(drug\ D_{2(or1)} \rightarrow drug\ D_{1(or2)} induced\ AE\right)} \end{aligned} \tag{45}$$

In the study by Noguchi et al., *lift* of >1 and *conviction* of >1 were used as the criterion for detection using the *association rule mining model*. As the risk data for verification was created by the *combination risk ratio model* presented in *Section 2.6*, there is no combination of n < 3 in the risk data for verification. Therefore, in the verification, the combination of $n_{111} < 3$ was excluded from the signal and $n_{111} \geq 3$ was added to the criterion for detection.

Noguchi's method has high detection power (sensitivity: 99.05%, specificity: 92.60%, Youden's index: 0.917, positive predictive value: 78.57%, negative predictive value: 99.72% *F*-score: 0.876) like the *additive model* and *multiplicative model* (Noguchi et al., 2018a).

In *Noguchi's method*, to compare the detection power, all combinations of DDIs were calculated using the *association rule mining model*. Therefore, it has not been determined how much computation time could be reduced compared to the previous methods using the *a priori algorithm*.

However, given the number of drugs registered in the spontaneous reporting systems, there are several potential combinations of DDIs. As *Noguchi's method* simplifies the computation, it is expected that the time for signal detection will be reduced as well as statistical models using other *association rule mining model* in actual search.

The *association rule mining model* is easy to extend to higher-order interactions. However, among the three methods presented in this review, the gold-standard has not been determined.

The chi-squared statistics is useful to determine the statistical significance level. Alvarez showed that chi-square statistics can be calculated directly using *confidence*, *support*, and *lift* with Eq. 46 (Alvarez, 2003).

$$\chi^2 = T \times \left(lift\right)^2 \times \frac{support \times confidence}{\left(confidence - support\right) \times \left(lift - confidence\right)} \tag{46}$$

The chi-squared statistics make it easy to validate combinations obtained using the standard *association rule mining model* (e.g., *Shirakuni's method* and *Noguchi's method*), and can identify statistically significant signals of DDIs that might be false positives.

Causal Association Rule Discovery Model

As described in *Association Rule Mining Model*, the *association rule mining model* is often used to discover the signals of potential DDIs in the spontaneous reporting system. However, the main limitation of the traditional *association rule mining model* is that the strength of signals is measured based on correlation, not causality.

Several studies have been reported on the concept of causality, such as inductive causality models (Pearl, 2000), causal Bayesian network based methods (Spirtes et al., 2001), an additive noise model (Hoyer et al., 2008), and a hybrid approach (Cai et al., 2013), however causal discovery on high-order and sparse data of DDIs is still unsolved.

To solve this problem, instead of reconstructing a causal Bayesian network, Cai et al. proposed the *causal association rule discovery* (*CARD*) *model* with the aim to detect the true causal relationship between the concomitant use of two drugs and AEs (Cai et al., 2017).

For the rule $X \rightarrow Y$ with $X \geq 3$, any sub-rules containing two antecedents must also form the V-structure with the AE: *drugs* $D_1 \rightarrow$ AE $\leftarrow$ *drugs* D_2 (e.g., aspirin $\rightarrow$ Bleeding $\leftarrow$ warfarin).

Because the interesting of rule X (*drugs* D_1, *drugs* D_2) $\rightarrow Y$ (AE) is dependent on the weakness of its sub-rules, and the *causal association interesting measure* (*CAIM*) is defined as follows:

$$CAIM\left(X \rightarrow Y\right) = \min_{\left\{X_{i1},\, X_{i2}\right\} \subset Y} CAIM\left(X_{i1}X_{i2} \rightarrow Y\right) \tag{47}$$

The dominance of the *CARD model* was determined by physician assessment of 100 randomly selected higher-order associations detected using the *CARD model* and *Harpaz's method of association rule mining model* (cf. *Harpaz's Method of Association Rule Mining Model*) (Harpaz et al., 2010). In the identification of known DDI, the *CARD model* was more accurate than *Harpaz's method*: *CARD model* (20%) vs. *Harpaz's method* (10%). Furthermore, in the *CARD model*, the detection of unknown combinations is less than *Harpaz's method*: *CARD model* (50%) vs. *Harpaz's method* (79%) (Cai et al., 2017).

LIMITATION

The spontaneous reporting systems used in these studies are based on clinical trials and post-marketing spontaneous reports, so only AEs observed are registered, and their causal relationship is unclear. Therefore, the cases may be underreported. Furthermore, the number of reports and signal values are influenced by various factors. Although not necessarily apparent, the number of cases increases in the first 2 years post-marketing and then begins to decrease. This is known as the Weber effect (Weber, 1984; Hartnell and Wilson, 2004).

The number and score of signals also possibly fluctuate during several years after launching (Hochberg et al., 2009). After drug-induced AE is highlighted, the number of reports may generally be accelerated. This is known as the notoriety effect (Pariente et al., 2007).

Additionally, the reports of drugs in the same class to those reported may also be accelerated. This is known as the ripple effect (Pariente et al., 2007).

The signal may be underestimated by numerous reports and that the same AE is associated with other drugs. This is called the masking effect or cloaking effect (Wang et al., 2010).

Matsuda et al. (2015) clarified that factors related to drug-induced AEs reporting attitudes in Japan may be different from those in other countries due to the involvement of medical representatives early post-marketing phase vigilance as a part of Japanese unique system of surveillance and the voluntary reporting process.

Thus, the spontaneous reporting systems are affected by several reporting biases and the state of the country's survey. Furthermore, the report rates of AEs vary from year to year, and the value of the signal can easily vary with the timing of the survey.

In addition to the general limitations of study using the spontaneous reporting systems, the research of DDIs has some unique limitations.

In the surveillance for of DDIs, the lack of information about one of the two drugs will overestimate the *RRR* of drug-induced AEs, when either drug is used alone (Norén et al., 2008).

This is a serious problem in evaluating the AEs of DDIs, because it leads to under-reporting of n_{111} and over-reporting of n_{101} or n_{011} (**Figure 1**). Furthermore, some of these statistical models do not apply to interactions with three or more drugs.

Finally, these statistical models are designed to focus on the detection of synergism rather than antagonism among some interaction of DDIs.

CONCLUSIONS AND PERSPECTIVES

In this review, we have discussed statistical methodologies for signal detection of DDIs in spontaneous reporting systems. To

the best of our knowledge, this is the latest review including recently proposed statistical methodologies.

The bivariate disproportionality analysis (e.g., single drug-induced AE) represents the bulk of daily routine of PhV. However, as the use of multiple drugs becomes more common, the problems of AEs due to DDIs cannot be ignored. Therefore, in the future operations of PhV, it is important to detect signals of unknown DDIs at an early stage.

In the bivariate disproportionality analysis, the frequentist methods generally have the following advantages and limitations compared with Bayesian methods. Several comparative studies of detection trends of these detection approaches have been reported (van, Puijenbroek et al., 2002; Kubota et al., 2004; Li et al., 2008; Bonneterre et al., 2012; Ang et al., 2016; Pham et al., 2019).

The advantages of the frequentist methods are generally as follows: 1. early signal detection, 2. sensitive, 3. easily applicable, and 4. easy to understand. While the limitations are 1. detection of false positive signals and 2. low specificity.

Although these advantages and limitations are considered to show a similar tendency in the signal detection models of DDIs, at this stage, the verification is not sufficient. Furthermore, the statistical models introduced in *Statistical Methodology* are not sufficiently clarified the difference in detection power. Therefore, in the future, it is necessary to examine the similarity and specificity of the signal detection tendency of each statistical model introduced.

As mentioned in *Limitation*, there are various biases (Weber, 1984; Hartnell and Wilson, 2004; Pariente et al., 2007; Hochberg et al., 2009; Wang et al., 2010; Matsuda et al., 2015) as these signals are calculated using the spontaneous reporting system. So the signal obtained is only a hypothesis. This does not change whether it is signals of single drug or DDIs. Therefore, considerable attention must also be paid to the interpretation of results in signal research of DDIs.

As indicated so far, most studies have focused on the analysis of AEs caused by the concomitant use of two drugs. However, in polypharmacy patients, the occurrence of AEs by interaction of multiple drugs (e.g., fourth order drug interaction: *drug* D_1*–drug* D_2*–drug* D_3*–AE*) is a concern. Therefore, in the future, establishment of a signal detection method for this higher order drug interaction will be more important.

This review has introduced only statistical methodologies for detecting DDIs based on the number of AEs reported.

In recent years, the method for detecting the signals that use time-to-onset instead of the number of reports have been studied (van Holle et al., 2012; van Holle et al., 2014; Scholl and Van Puijenbroek, 2016), but there are no examples of using them for DDIs. Since it may be possible to detect the signals that cannot be obtained with the statistical models introduced in this review, further studies are expected.

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YN and HT wrote the manuscript. TT also contributed with the paper organization. All the authors contributed with the bibliographic research.

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Search for Therapeutic Agents for Cardiac Arrest Using a Drug Discovery Tool and Large-Scale Medical Information Database

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The survival rate of cardiac arrest patients is less than 10%; therefore, development of a therapeutic strategy that improves their prognosis is necessary. Herein, we searched data collected from medical facilities throughout Japan for drugs that improve the survival rate of cardiac arrest patients. Candidate drugs, which could improve the prognosis of cardiac arrest patients, were extracted using "TargetMine," a drug discovery tool. We investigated whether the candidate drugs were among the drugs administered within 1 month after cardiac arrest in data of cardiac arrest cases obtained from the Japan Medical Data Center. Logistic regression analysis was performed, with the explanatory variables being the presence or absence of the administration of those candidate drugs that were administered to ≥10 patients and the objective variable being the "survival discharge." Adjusted odds ratios for survival discharge were calculated using propensity scores for drugs that significantly improved the proportion of survival discharge; the influence of covariates, such as patient background, medical history, and treatment factors, was excluded by the inverse probability-of-treatment weighted method. Using the search strategy, we extracted 165 drugs with vasodilator activity as candidate drugs. Drugs not approved in Japan, oral medicines, and external medicines were excluded. Then, we investigated whether the candidate drugs were administered to the 2,227 cardiac arrest patients included in this study. The results of the logistic regression analysis

showed that three (isosorbide dinitrate, nitroglycerin, and nicardipine) of seven drugs that were administered to ≥10 patients showed significant association with improvement in the proportion of survival discharge. Further analyses using propensity scores revealed that the adjusted odds ratios for survival discharge for patients administered isosorbide dinitrate, nitroglycerin, and nicardipine were 3.35, 5.44, and 4.58, respectively. Thus, it can be suggested that isosorbide dinitrate, nitroglycerin, and nicardipine could be novel therapeutic agents for improving the prognosis of cardiac arrest patients.

Keywords: cardiac arrest, drug repositioning, claims database, drug discovery tool, vasodilator

INTRODUCTION

Despite advances in treatment, cardiac arrest still results in a high mortality rate. In the United States alone, more than 550,000 cardiac arrest cases are reported annually, with the survival discharge rate being only 12% and 24.8% for out-of-hospital and in-hospital patients, respectively (The American Heart Association, 2013). Considering the increase in aging population, the number of cardiac arrest patients is also expected to increase; hence, there is an urgent need to develop a treatment that improves the prognosis of patients suffering cardiac arrest.

Cardiac arrest damages heart functions and those of other organs. Particularly, it results in myocardial dysfunction at the early stages after resuscitation; moreover, circulation becomes very unstable (Jentzer et al., 2015). Myocardial dysfunction is associated with early death, and if improvement in cardiac function cannot be achieved within 24 h, conditions such as multiple organ failure, which have poor prognosis, are known to occur (Laurent et al., 2002). Therefore, normalization of hemodynamics is very important for improving the prognosis after cardiac arrest.

In recent years, drug repositioning (DR) has attracted attention as a strategy of drug development. In the DR approach, new pharmacological effects of already approved drugs with known safety for humans are identified. These drugs are then used as new therapeutic agents for other diseases according to the identified effect (Ashburn and Thor, 2004; Zamami et al., 2017). The number of new drugs introduced in the market is decreasing yearly because the development of new drugs is expensive and time-consuming (Reuters, 2011). The main advantage of using the DR approach is that it can reduce drug development time and cost. Moreover, it reduces the risk of development failure due to unintended adverse effects and pharmacokinetic problems in the clinical trial stage. Various types of big data are now available, and utilization of these data can be highly useful for the DR approach. We anticipate that DR can contribute to the development of therapeutic agents that improve prognosis of cardiac arrest patients.

One such big data in the field of life science is the drug discovery science database. It includes a wide variety of information such as chemical structures, physical properties, pharmacological actions, and genes related to diseases. TargetMine has been developed by the National Institutes of Biomedical Innovation, Health and Nutrition (Japan). It integrates more than 30 databases on bioinformatics worldwide (Chen et al., 2016). It can be used to identify related diseases and drugs based on a specific gene. Similarly, a representative medical information big data for the medical sciences is Japan Medical Data Center (JMDC) Claims Database. It is a database of 5.6 million cases collected from Japan's health insurance association, which includes information on the diagnosis of diseases and prescription medicines and reflects the actual clinical practices. Using these databases, it is possible to evaluate the efficacy and safety of drugs in the real-world setting (Sugiyama et al., 2016; Yokoyama et al., 2018).

It is difficult to develop an experimental drug for cardiac arrest due to the urgency of treatment and ethical problems. However, we can use the DR approach by utilizing drug discovery science databases and medical information databases to identify candidate drugs expected to be effective in humans. Therefore, in this study, we aimed to search for drugs that can improve the survival rate of cardiac arrest patients using drug discovery tools and large-scale medical databases through the DR approach.

METHODS

Extraction of Candidate Drugs by Targetmine

TargetMine internally integrates and combines various data from around the world. It is possible to use it for drug development by simultaneously specifying conditions such as target genes, proteins, and pharmacological actions, and thus simplifying complicated tasks. Compared with other data warehousing tools, TargetMine has simpler operation, and users can collect and prioritize information quickly and efficiently without the need for special programming skills (Chen et al., 2011; Chen et al., 2016). The occurrence of cardiac arrest causes systemic circulatory failure and hypoxia. Therefore, we focused our literature search on vasodilators because of their use in maintaining circulation after resumption of heart functioning (Yuuki et al., 1991). In this study, we used Anatomical Therapeutic Chemical Classification System (ATC) codes (widely used as a method to classify medicines according to efficacy, site of action, target organ, and chemical characteristics) as an extraction method of candidate drugs. We searched for pharmaceutical ingredients with an ATC code associated with vasodilator effect (**Supplementary Table 1**). TargetMine is a comprehensive tool and is suitable for this study because it can be used to extract information on drugs

with pharmacological actions that correspond to specific ATC classifications.

Analysis of Large-Scale Medical Information

The claims database used in analysis was provided by the Japan Medical Data Center (JMDC), which includes approximately 3 million cases (as of November 2015) of receipt information. Information from medical hospital receipts, Diagnosis Procedure Combination (DPC) receipts, medical out-of-hospital receipts, and dispensing receipts was integrated. It was possible to obtain information, including disease name, medical treatment, and the dispensed items, from the insurance documents (**Supplementary Table 2–8**). Moreover, since a unique ID is given to each participant, if one patient visits multiple medical institutions, it is possible to trace a series of processes from the occurrence of a disease to its convergence. Nevertheless, since all data are highly encrypted unlinkable anonymized information, it is not possible to identify an individual. The receipt dataset used in this study is provided in seven files: "patient information," "receipt information," "facility information," "doctor information," "injury information," "pharmaceutical information," and "medical treatment information." The items contained in each file and their contents are shown in **Supplementary Tables 2, 3, 4, 5, 6, 7,** and **8**. It should be noted that the diagnostic names in this dataset are used by the WHO for referring to causes of death and disease. The classification was based on the 10th edition of "International Statistical Classification of Diseases and Related Health Problems" (ICD-10). Drugs were classified according to the Anatomical Therapeutic Chemical Classification System (ATC classification) developed by the European Pharmaceutical Market Research Association (EphMRA). From January 2005 to May 2014, patients with the following (ICD-10) codes were classified as cardiac arrest patients (n = 2,546): I469 for cardiac arrest, I472 for pulseless ventricular tachycardia, I490 for transient ventricular fibrillation; and Japan specific medical action codes: J046 for non-thoracotomy heart massage, J047 for counter shock. Among these patients, those with traumatic cardiac arrest or <18 years of age or unconfirmed diagnosis or having missing data were excluded. Finally, 2,227 patients were included in the study. Cardiac arrest date was defined as the date at which the diagnosis of cardiac arrest was started; if the diagnosis date was unknown, the date on which the practice for cardiac arrest was performed was used. If multiple cardiac arrests occurred during the study period, the first date was taken as the patient's cardiac arrest date. We used the presence or absence of "the fee for providing treatment information at discharge" in claim data to define survival discharge. Patients who were charged this fee within 1 year from the day of cardiac arrest were defined as survival discharge patients. The data were processed using Microsoft Access 2013, and the information contained in the seven files described above was linked based on the patient ID. Because data about "birth date," "join date," "JMDC data start date," and "medical treatment date" could not be obtained, these dates were all set to the first day of that month. In addition, "the withdrawal date" and "JMDC data end date" were set to the last day of that month. In case the "medical care start date," the "prescription date of medicine," or the "treatment date" were blank, the "consultation date" was used instead.

Narrowing Down Candidate Drugs

First, the drugs that were not currently approved in Japan were excluded. Parenteral administration suitable for the the patients with cardiac arrest because of they suffer from sever condition and are unconscious. Therefore, the drugs for which no injectable form was available were excluded. Subsequently, information of drugs administered within 1 month after cardiac arrest was extracted from 2,227 cardiac arrest cases identified as target patients. Of these, drugs administered in ≤10 patients were excluded as statistically reliable results could not be obtained when the number of patients was very small. The correlation between the administration of the candidate drugs and the survival discharge within 1 year was analyzed by logistic regression analysis. The administration of each candidate drug (diltiazem, disopyramide, flecainide, verapamil, isosorbide nitrate, nicardipine, and nitroglycerin) within 1 month from the cardiac arrest date was set as the explanatory variable, and the survival discharge within 1 year from the cardiac arrest date was set as the objective variable (**Figure 1**). The adjusted odds ratios (ORs) were calculated for the drugs showing a significant relationship with survival discharge in simple logistic regression analysis. The covariates used for the adjustment are shown n **Supplementary Table 9**.

Statistical Analysis

Continuous variables are presented as the mean ± standard deviation (SD) and categorical variables by using frequencies and percentages. To compare the effects of administration of each drug on survival discharge, simple logistic regression was performed with administration of diltiazem, disopyramide, flecainide, verapamil, isosorbide dinitrate, nicardipine, and nitroglycerin as covariates (**Table 3**). Isosorbide dinitrate, nitroglycerin, and nicardipine were divided into two groups based on the presence or absence of administration. Standardized mean differences (**Tables 4**, **5**, **6**) were calculated after adjusting each covariate using the inverse probability of treatment weighting (IPTW) method, and the variation of the covariates in both groups was examined. Using the IPTW method, adjusted ORs were calculated for comparing the rates of survival discharge between the two groups (**Table 7**). Analyses were performed using R statistical software version 3.1.2., and statistical significance was defined as a $p < 0.05$.

Ethics Statement

This study was carried out in accordance with the recommendations of the Ethical Guidelines for Epidemiological Research by the Ministry of Health, Labour and Welfare. The protocol was approved by the ethics committees of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital (no. 105056). One cannot identify individuals from JMDC's claims database because all personal data including names are deleted and converted to an ID that cannot be linked with

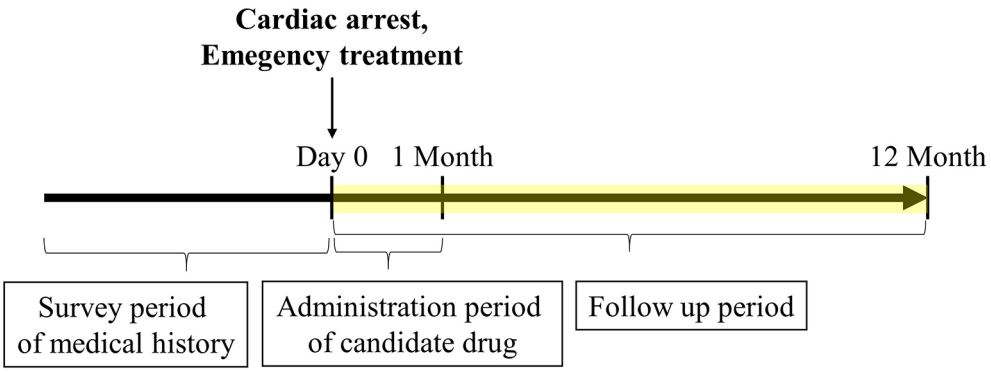

FIGURE 1 | Timeline of claims data analysis. The cardiac arrest date was set as day 0. The frequency of survival discharge within 1 year after cardiac arrest was determined. Only administration of candidate drugs limited to 1 month or less after cardiac arrest was included. The medical history and emergency treatment recorded before cardiac arrest and on the day of cardiac arrest were included, respectively.

any personal information. Since this study was an observational study with anonymized information, no treatment intervention, and no collection of human samples, the requirement of obtaining informed consent was waived by the ethics committee.

RESULTS

Candidate Drug Data Extraction Using Targetmine

Of the drugs shortlisted using TargetMine, drugs with ATC code "C01B" included 51 drugs, "C01DA," "C02CA," and "C02CC" included 10 drugs each, "C04" included 52 drugs, and "C08" included 44 drugs. The total number of drugs excluding duplicates was 165 (**Table 1**).

Of these 165 candidate drugs, 39 were excluded, including those not either approved in Japan or those for which no injectable form was available. After further shortlisting for the drugs administered within 1 month from the day of cardiac arrest, 11 drugs were obtained. Finally, after exclusion of drugs that were used by ≤10 patients and those included in statistical analysis as covariates, seven drugs were shortlisted as the drug candidates for this study. The names and frequencies of drug administration of each drug are shown in **Table 2**, and the process of data extraction for drugs is shown in **Figure 2**.

Relationship Between Drug Use and Survival Discharge

Logistic regression analysis indicated significant positive correlation between the administration of candidate drugs (the explanatory variable) and survival discharge within 1 year (the objective variable) for isosorbide dinitrate (OR = 3.80, 95% CI = 2.52–5.66), nitroglycerin (OR = 2.79, 95% CI = 1.59–4.74), and nicardipine (OR = 2.27, 95% CI = 1.25-3.99) (**Table 3**).

Propensity score matching was performed to minimize patient background bias in the treatment and non-treatment groups. Standardized effects of each covariate before and after adjustment using the IPTW method are shown in **Tables 4**, **5**, **6**. The values of standardized effect decreased after adjustment compared with the values before adjustment, and the patient background bias was uniform. The adjusted odds ratios after adjustment with the IPTW method were as follows: isosorbide dinitrate, 3.35 (95% CI = 1.79–6.26); nitroglycerin, 5.44 (95% CI = 3.06–9.68); and nicardipine, 4.58 (95% CI = 2.53–8. 28) (**Table 7**). These findings suggest that the survival discharge rate was significantly high in all three treatment groups.

DISCUSSION

Even after matching on covariates using propensity scores, the odds ratios for survival discharge within 1 year after cardiac arrest were significantly high in each of the isosorbide dinitrate, nitroglycerin, and nicardipine administration groups. This indicated that all three drugs improved the survival rate on hospital discharge.

To date, no large-scale clinical studies demonstrating the efficacy of nitroglycerin, isosorbide dinitrate, and nicardipine in cardiac arrest patients have been reported. However, some case reports were published reporting resuscitation being achieved by the administration of nitroglycerin in refractory cardiac arrest (Ward and Reid, 1984; Osada et al., 2000; Stefaniotou et al., 2014). Moreover, it was reported that acute coronary syndrome (ACS), which occurs after resuscitation from cardiac arrest, and cardiac arrest caused by ACS were improved by the administration of isosorbide dinitrate (Heming et al., 2010; Manzo-Silberman et al., 2010). In animal experiments using the heart of a paralyzed rat, nicardipine administration significantly improved blood flow in ischemic state (Tachibana, 1990; Mitchell et al., 1992).

Nitrate drugs, such as nitroglycerin and isosorbide dinitrate, stimulate guanylate cyclase in vascular smooth muscle cells *via* nitrogen oxide. At low doses, nitrates dilate the vessels in the venous system and at high doses dilate the arterial system vessels to reduce resistance. They provide stress relief (by pulmonary capillary pressure reduction) and afterload relief (by mild elevation of cardiac output and decrease in peripheral vascular resistance). Moreover, nitrates are widely used in heart failure caused by ischemic heart disease for their coronary artery dilation effect.

TABLE 1 | Names of shortlisted candidate drugs extracted using TargetMine (excluding duplicates).

Drug names (165)			
Ajmaline	Doxazosin	Lidocaine hydrochloride	Propafenone
Amiodarone	Doxazosin mesylate	Lidocaine hydrochloride monohydrate	Propafenone hydrochloride
Amiodarone hydrochloride	Dronedarone	Lidoflazine	Propyl nitrate
Amlodipine	Dronedarone hydrochloride	Lorajmine hydrochloride	Quinidine
Amlodipine besylate	Encainide	Lorcainide	Quinidine gluconate
Amlodipine maleate	Encainide hydrochloride	Lorcainide hydrochloride	Quinidine phenylethyl barbiturate
Aprindine	Ergoloid mesylates	Manidipine	Quinidine polygalacturonate
Aprindine hydrochloride	Erythrityl tetranitrate	Manidipine hydrochloride	Quinidine sulfate
Azapetine	Ethacizine	Methyl nicotinate	Sparteine
Bamethan	Fasudil	Mexiletine	Sparteine sulfate
Bamethan sulfate	Fasudil hydrochloride	Mexiletine hydrochloride	Suloctidil
Barnidipine	Fasudil hydrochloride hydrate	Mibefradil	Tedisamil
Barnidipine hydrochloride	Felodipine	Mibefradil dihydrochloride	Tedisamil sesquifumarate
Bencyclane	Fendiline	Moricizine	Tocainide
Bencyclane fumarate	Fendiline hydrochloride	Moricizine hydrochloride	Tocainide hydrochloride
Benidipine	Flecainide	Moxisylyte	Tolazoline
Benidipine hydrochloride	Flecainide acetate	Moxisylyte hydrochloride	Tolazoline hydrochloride
Benzyl nicotinate	Gallopamil	Nafronyl oxalate	Trimazosin
Bepridil	Gallopamil hydrochloride	Naftidrofuryl	Trimazosin hydrochloride
Bepridil hydrochloride	Guanazodine	Niacin	Trolnitrate phosphate
Bethanidine sulfate	Guanethidine	Nicardipine	Urapidil
Bretylium tosylate	Guanethidine sulfate	Nicardipine hydrochloride	Urapidil hydrochloride
Buflomedil	Guanoclor sulfate	Nicergoline	Verapamil
Buflomedil hydrochloride	Guanoxabenz	Nicergoline tartrate	Verapamil hydrochloride
Bunaftine	Guanoxan sulfate	Nicotinyl alcohol	Vernakalant hydrochloride
Buphenine	Hydroquinidine	Nifedipine	Vinburnine
Butalamine	Hydroquinidine hydrochloride	Nilvadipine	Vincamine
Cetiedil citrate	Ibutilide	Nimodipine	Vincamine hydrochloride
Ciclonicate	Ibutilide fumarate	Nisoldipine	Visnadine
Cifenline	Ifenprodil	Nitrendipine	Xanthinol niacinate
Cifenline succinate	Ifenprodil tartrate	Nitroglycerin	
Cilnidipine	Indoramin	Nylidrin hydrochloride	
Cinepazide maleate	Indoramin hydrochloride	Pentaerythritol tetranitrate	
Clevidipine	Inositol niacinate	Pentifylline	
Cyclandelate	Isosorbide dinitrate	Pentoxifylline	
Debrisoquin	Isosorbide mononitrate	Perhexiline	
Debrisoquin sulfate	Isoxsuprine	Perhexiline maleate	
Dihydroergocristine	Isoxsuprine hydrochloride	Phenoxybenzamine	
Dihydroergocristine mesilate	Isoxsuprine lactate	Phenoxybenzamine hydrochloride	
Diltiazem	Isradipine	Phentolamine	
Diltiazem hydrochloride	Kallidinogenase	Phentolamine mesylate	
Diltiazem maleate	Lacidipine	Prazosin	
Disopyramide	Lercanidipine	Prazosin hydrochloride	
Disopyramide phosphate	Lercanidipine hydrochloride	Procainamide	
Dofetilide	Lidocaine	Procainamide hydrochloride	

TABLE 2 | Number of cardiac arrest patients administered each drug.

Drug name	Number of patients administered	Drug name	Number of patients administered	Drug name	Number of patients administered
Ajimarin	0	Diltiazem	45	Fassille	5
Apringin	9	Cilnidipine	0	Felodipine	0
Amiodarone	119	Doxazosin	0	Phentolamine	0
Amlodipine	0	Trazoline	0	Prazosin	0
Isoxsuprine	0	Niacin	0	Flecainide	28
Inositol	0	Nicardipine	98	Procainamide	10
Ifenprodil	0	Nicergoline	0	Propafenone	0
Urapidil	0	Nisoldipine	0	Benidipine	0
Kallidinogenase	0	Nitrangepin	0	Bepridil	0
Quinidine	0	Nitroglycerin	103	Verapamil	134
Guanethidine	0	Nifedipine	0	Mexiletine	2
Disopyramide	32	Nilvadipine	0	Lidocaine	559
Dihydroergocristine	0	Barnidipine	0	Isosorbide nitrate	164

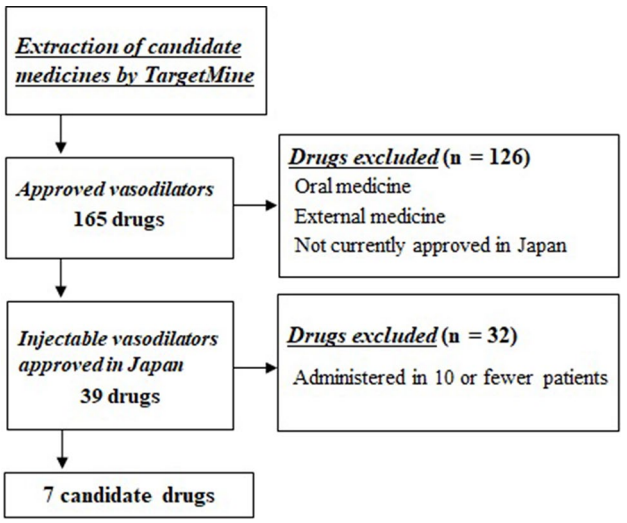

FIGURE 2 | Selection flowchart of candidate drug. First, 165 approved drugs having vasodilator activity were extracted using the drug discovery tool TargetMine. Next, drugs that were not approved in Japan, oral drugs, and external drugs were excluded, and seven drugs were selected that were administered to ≥10 patients within 1 month of cardiac arrest.

Dihydropyridine (DHP) calcium antagonists, such as nicardipine, relax vascular smooth muscles by blocking the membrane voltage-dependent L-type calcium channels involved in the influx of extracellular calcium ions. They are used to treat hypertension by relaxing muscles and reducing peripheral vascular resistance. Their main pharmacological actions include coronary and peripheral vasodilator activity, cardiac contractility, and suppression of impulse conduction system. However, cardiac suppression is hardly seen at clinical doses. Nicardipine has excellent organ blood flow maintenance effect and is used in cases of organ failure due to hypertension. Among DHP calcium antagonists, nicardipine has a short onset time and half-life. It is used for hypertensive emergencies and is highly specific for cerebral blood vessels (Takenaka and Handa, 1979).

One common pharmacological effect of the three selected agents (isosorbide dinitrate, nitroglycerine, and nicardipine) is dilation of the coronary artery, which we suggest contributed to the improvement in survival rate after cardiac arrest in this study. Treatment of patients with cardiac arrest involves management of blood pressure; vasoconstrictors such as adrenaline and

TABLE 3 | Relationship between each drug and survival discharge.

Explanatory variable	*p* value	Odds ratio (95% CI)
Diltiazem	0.573	0.76 (0.27–1.87)
Disopyramide	0.199	1.92 (0.64–4.82)
Flecainide	0.272	1.79 (0.57–4.74)
Verapamil	0.181	1.45 (0.82–2.43)
Isosorbide nitrate	<0.001	3.80 (2.52–5.66)
Nicardipine	0.006	2.27 (1.25–3.99)
Nitroglycerin	<0.001	2.79 (1.59–4.74)

TABLE 4 | Comparison of isosorbide dinitrate covariates.

Background factor	(number of patients) [SD]		Standardized effect values	
	Isosorbide nitrate administration group (*n* = 164)	Non-administration group (*n* = 2,063)	Before adjustment	After adjustment
Male	83.5% (137)	71.8% (1482)	0.26	0.07
Age	56.1 [10.5]	55.0 [13.3]	0.09	0.08
Medical history				
Ischemic heart disease	26.8% (44)	18.7% (385)	0.21	0.01
Cerebrovascular disease	9.1% (45)	12.2% (252)	0.09	0.02
Kidney disease	6.7% (11)	8.8% (181)	0.07	0.08
Liver disease	15.9% (26)	19.0% (392)	0.08	0.12
Chronic lung disease	22.0% (36)	24.6% (507)	0.06	0.1
Heart failure	19.5% (32)	24.5% (506)	0.12	0.11
Diabetes mellitus	34.1% (56)	23.8% (491)	0.24	0.04
High blood pressure	42.7% (70)	39.1% (806)	0.07	0.11
Hyperlipidemia	28.7% (47)	20.2% (416)	0.21	0.1
Malignant neoplasm	10.4% (17)	20.2% (417)	0.25	0.12
Emergency treatment factor				
Out-of-hospital cardiac arrest	25.6% (42)	18.1% (373)	0.19	0.13
Average number of instances of defibrillation	0.40 [0.49]	0.29 [0.49]	0.23	0.17
Tracheal intubation	27.4% (45)	20.5% (422)	0.17	0.04
Artificial respiration	32.9% (54)	27.5% (567)	0.12	0.06
Hypothermia	9.1% (15)	1.0% (21)	0.64	0.03
Average number of adrenaline doses	0.64 [1.78]	1.38 [3.55]	0.21	0.04
Vasopressin	0.61% (1)	0.5% (11)	0.01	0.05
Amiodarone	20.1% (33)	2.4% (49)	0.94	0.01
Lidocaine	17.7% (29)	2.9% (60)	0.75	0.01
Nifekalant	2.4% (4)	0.5% (10)	0.25	0.23

TABLE 5 | Comparison of nitroglycerin covariates.

Background factor	(number of people) [SD]		Standardized effect values	
	Nitroglycerin administration group (*n* = 103)	Non-administration group (*n* = 2,124)	Before adjustment	After adjustment
Male	79.6% (82)	72.4% (1537)	0.16	0.01
Age	56.4 [12.6]	55.0 [13.2]	0.11	0.08
Medical history				
Ischemic heart disease	30.1% (31)	18.7% (398)	0.29	0.12
Cerebrovascular disease	14.6% (15)	11.9% (252)	0.08	0.09
Kidney disease	12.6% (13)	8.4% (179)	0.15	0.05
Liver disease	13.6% (14)	19.0% (404)	0.14	0.01
Chronic lung disease	24.3% (25)	24.4% (518)	0	0
Heart failure	24.3% (25)	24.2% (513)	0	0.06
Diabetes mellitus	26.2% (27)	24.5% (520)	0.04	0.01
High blood pressure	49.5% (51)	38.8% (825)	0.22	0.05
Hyperlipidemia	22.3% (23)	20.7% (440)	0.04	0
Malignant neoplasm	12.6% (13)	19.8% (421)	0.18	0.03
Emergency treatment factor				
Out-of-hospital cardiac arrest	23.3% (24)	18.4% (391)	0.13	0.05
Average number of instances of defibrillation	0.42 [0.85]	0.29 [0.46]	0.26	0.06
Tracheal intubation	27.2% (28)	20.7% (439)	0.16	0.03
Artificial respiration	41.7% (43)	27.2% (578)	0.32	0.04
Hypothermia	7.8% (8)	1.3% (28)	0.51	0.03
Average number of adrenaline doses	1.12 [2.88]	1.34 [3.48]	0.06	0.06
Vasopressin	0.0% (0)	0.6% (12)	0.08	0.08
Amiodarone	13.6% (14)	3.2% (68)	0.55	0.11
Lidocaine	27.2% (28)	2.9% (61)	1.24	0.08
Nifekalant	1.9% (2)	0.6% (12)	0.17	0.04

TABLE 6 | Comparison of nicardipine covariates.

Background factor	(number of people) [SD]		Standardized effect values	
	Nicardipine administration group (*n* = 98)	Non-administration group (*n* = 2,129)	Before adjustment	After adjustment
Male	76.5% (75)	72.5% (1544)	0.09	0.05
Age	58.0 [10.9]	54.9 [13.2]	0.23	0
Medical history				
Ischemic heart disease	28.6% (28)	18.8% (401)	0.25	0.07
Cerebrovascular disease	18.4% (18)	11.7% (249)	0.21	0.03
Kidney disease	8.2% (8)	8.6% (184)	0.02	0.01
Liver disease	17.3% (17)	18.8% (401)	0.04	0
Chronic lung disease	24.5% (24)	24.4% (519)	0	0.05
Heart failure	26.5% (26)	24.0% (512)	0.06	0.09
Diabetes mellitus	28.6% (28)	24.4% (519)	0.1	0.15
High blood pressure	53.1% (52)	38.7% (824)	0.29	0.05
Hyperlipidemia	27.6% (27)	20.5% (436)	0.17	0.03
Malignant neoplasm	18.4% (18)	19.5% (416)	0.03	0.1
Emergency treatment factor				
Out-of-hospital cardiac arrest	28.6% (28)	18.2% (387)	0.27	0.06
Average number of instances of defibrillation	0.32 [0.49]	0.28 [0.49]	0.04	0.03
Tracheal intubation	35.7% (35)	20.3% (432)	0.38	0.04
Artificial respiration	53.1% (52)	26.7% (569)	0.59	0.11
Hypothermia	8.2% (8)	1.3% (28)	0.54	0.02
Average number of adrenaline doses	1.78 [3.72]	1.31 [3.44]	0.14	0.01
Vasopressin	0.0% (0)	0.6% (12)	0.08	0.08
Amiodarone	9.2% (9)	3.4% (73)	0.31	0.03
Lidocaine	27.6% (27)	2.9% (62)	1.26	0.01
Nifekalant	3.1% (3)	0.5% (11)	0.32	0.05

TABLE 7 | Covariate adjusted odds ratios.

Drug name	Adjusted odds ratio (95% CI)	*p* value
Isosorbide nitrate	3.35 (1.79–6.26)	<0.001
Nitroglycerin	5.44 (3.06–9.68)	<0.001
Nicardipine	4.58 (2.53–8.28)	<0.001

noradrenaline are commonly administered for this purpose. However, no sufficient evidence is available to support the effect of vasoconstrictors on the survival and discharge rate of adult patients after resumption of heart rate; excessive administration may lead to decreased blood flow. The prognosis improvement could have resulted from increased oxygen supply to the heart through the dilation of the coronary artery, relief in the heart afterload, and the increase in whole organ blood flow to the heart.

In addition, nicardipine has been shown to directly dilate cerebral blood vessels (Takenaka and Handa, 1979) and nitroglycerin has been reported to improve neurologic prognosis after cardiac arrest in animal studies (Chen et al., 2011). Irreversible damage to cranial nerves in post-cardiac arrest syndrome (PCAS), reported in most patients with cardiac arrest, is a very important risk factor for long-term poor prognosis of cardiac arrest patients. Thus, it can be suggested that increased cerebral blood flow, which suppresses irreversible damage to the cranial nerves associated with circulatory failure, also contributed to the improvement of long-term outcomes.

This study has several limitations. First, we could not evaluate the effect of drugs not approved in Japan. Because the JMDC claims database contains only Japanese claim data, we could only include drugs approved in Japan in the analysis. Thus, we may have missed promising drug candidates, which should be considered in future studies. Second, we defined each comorbidity based on the description in the claim data. However, the diagnostic methods and diagnosis criteria could be inconsistent, and, thus, the quality of diagnosis might be inconsistent. Third, the JMDC claims database does not provide some information, such as the cause of cardiac arrest, the quality of cardiopulmonary resuscitation, medication adherence, the dose and duration of drug administration, and clinical laboratory test results. In the studies using propensity scores, all factors affecting treatment allocation should be investigated and included as covariates. However, propensity score matching cannot be used as a substitute to the randomized comparison trials, which can balance out unknown covariates. In future, prospective observational studies should be conducted and information on background factors related to cardiac arrest should be collected.

CONCLUSION

Three drugs (isosorbide dinitrate, nitroglycerine, and nicardipine) were identified in this study as candidate novel therapeutic agents to improve the prognosis of cardiac arrest patients. These results could prove to be valuable in therapeutic drug development for cardiac arrest patients because it is difficult to conduct clinical trials in these patients due to the urgency of their treatment. Moreover, the procedure used for the identification of candidate drugs in the present study is a very useful method that combines multifaceted evaluation using drug discovery tools and claims databases. To confirm the usefulness of the results and method used in this study and to use the three identified drugs for their therapeutic effect in cardiac arrest patients, it is necessary to validate these results in clinical settings.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

This study was conducted in keeping with the Ministry of Health, Labour, and Welfare's Ethical Guidelines for Epidemiological Research. It was approved by the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences and Okayama University Hospital Ethics Committee (No. 1706-022-001) and conformed to the tenets of the Declaration of Helsinki. Since this study was an observational study with anonymized information, with no treatment intervention and no collection of human samples, obtainment of informed consent was exempted.

AUTHOR CONTRIBUTIONS

YZ contributed to the conception and study design, data acquisition, statistical analysis, interpretation of the data, and the revision of the manuscript. TK and TN contributed to the interpretation of the data and the revision of the manuscript. YS, YI-I, MM, AO, KH, TI, YK, HH, NO, MG, KeT, MC, KoT, SH, MK, and KI contributed to the interpretation of the data and the critical review of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01257/full#supplementary-material

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Incidences of Hypothyroidism Associated With Surgical Procedures for Thyroid Disorders: A Nationwide Population-Based Study

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Background and Aim: Limited information available about different types of thyroid surgeries with risk for postoperative hypothyroidism. This study aimed to investigate the risk of developing early and late-onset postoperative hypothyroidism in patients with thyroid disorders.

Methods: We used a large cohort data from the Taiwan National Health Insurance Research Data Base (NHIRDB) and identified 9,693 (9, 348) patients from January 1998 to December 2010, admitted for thyroid disorder surgeries. We used the surgical procedures time as the index date. Our observational retrospective cohort study excluded the subjects diagnosed with hypoparathyroidism and hypothyroidism before any surgeries. We analyzed the data using the Cox regression model to calculate the hazard ratio.

Result: Postoperative hypothyroidism associated with bilateral-total (HR, 4.27; 95% CI, 3.32–5.50), one-side total and another subtotal (HR, 3.16; 95% CI, 2.59–3.86), bilateral-subtotal (HR, 1.65; 95% CI, 1.37–1.98), and unilateral-total (HR, 1.17; 95% CI, 0.95–1.44) surgical procedures. The time intervals for thyroid disorders were 320 cases developed postoperative hypoparathyroidism in eight weeks, 480 cases the second month, and 1000 cases in the first year after surgery.

Conclusion: Findings suggest that thyroidectomy was associated with transient postoperative hypothyroidism in thyroid disorder patients. The bilateral-total surgical procedure was strongly associated with temporary postoperative hypothyroidism.

Keywords: postoperative hypothyroidism, thyroid disorders, thyroid surgeries, transient hypothyroidism, surgeons experience, BigData analytics, hormones

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INTRODUCTION

Thyroid dysfunction is common around the world. In endocrine practice, thyroid abnormalities take around 30% to 40% of the cases (Garmendia Madariaga et al., 2014). About 5% to 20% of American adult population has thyroid abnormalities (Bennedbæk and Hegedüs, 2000) with 1% to 2% in UK adults (Tunbridge et al., 1977) 17% to 35% in Brazilian women (Tomimori et al., 1995) 10% in Japanese adults (Kasagi et al., 2009) and 1 in 5788 live births occurring in Taiwan (Tsai et al., 1995).

Postoperative hypothyroidism is a major complication after thyroid disorders surgeries, appeared in 32.8% of the cases in the series reported by De Carlucci et al. (2008). Transient hypothyroidism incidence has been estimated to range from 6.9% to 46% (Falk et al., 1988; See and Soo, 1997; Mehrvarz et al., 2014) and permanent hypothyroidism from 0.4% to 33% (Thompson and Harness, 1970; Attie et al., 1979; Wingert et al., 1986; Falk et al., 1988) nevertheless, it depends on patients follow-up interval and their investigators in how they define hypothyroidism (Piper et al., 2005). Hypothyroidism constitutes of several complications such as basal calcification (Posen et al., 1979; Schafer and Ferbert, 1998), formation of cataract (Ireland et al., 1968) electrocardiographic abnormalities (Stathatos and Wartofsky, 2003) and tetany (Scanlon et al., 1981; Dembinski et al., 1994). Several studies reported that the transient and permanent postoperative hypothyroidism are associated with Graves' disease (Van Welsum et al., 1974; FDA Drug Safety Communication, 2015; Sheehan and Doi, 2016), thyrotoxicosis (Querat et al., 2015) recurrent goiter (Wingert et al., 1986; Thomusch et al., 2000) and thyroid cancer (Pattou et al., 1998). Surgical techniques like devascularization or parathyroid glands inadvertent resection are associated with transient and permanent postoperative hypothyroidism (Elmaksoud. et al., 2015; Querat et al., 2015). The incidence of hypothyroidism related to different surgical procedures could be accomplished by estimating the risk of different surgical procedures. Despite of whether or not we know the behavior of patients after surgery their metabolism is still unpredictable.

Limited information exists about the relationship between different surgical procedures and risk to develop postoperative transient or permanent hypothyroidism which, for the most part is still unclear. Therefore, we aim to investigate different surgical procedures for thyroid disorders associated with transient or permanent hypothyroidism in the Taiwanese population.

MATERIALS AND METHODS

Data Source

In this study, we used reimbursement data from the Bureau National Health Insurance (BNHI) system in Taiwan which was implemented on March, 1995 and has registered all the medical claims since 1996. More than 99% of Taiwan's citizens are enrolled in the NHI, which offers mandatory and comprehensive medical care coverage to all Taiwanese residents (Hsing and Ioannidis, 2015). For research and administrative use, the National Research Institute established a randomly selected claim database which represents the whole population, and provides all information of medical services received by each individual yearly, from 1996 to 2012 (Lu and Hsiao, 2003). We randomly selected two million samples from Taiwan's NHI beneficiary claim data during the years 1998 to 2011.

Study Population

For our observational retrospective cohort study, we identified subjects from January 1, 1998 to December 31, 2010 who were hospitalized with surgeries for thyroid diseases [Taiwan National Health Insurance (NHI) codes 82001C, 82002C, 82004B, 82008B, 82015B, 82016B), and used the date of surgical procedures as the index date (see **Table S1** in Appendix). Moreover, subjects diagnosed with hypoparathyroidism and hypothyroidism before any surgical procedures, were excluded in this study. Initially, all eligible subjects were followed-up until a diagnosis of hypothyroidism [International Classification of Disease, Clinical Modification, Ninth Revision [ICD-9-CM) codes 244.0] or until the time subjects were censored for failure to follow-up, or termination of insurance, or a time beyond December 31, 2011 (see **Table S1** in Appendix).

Covariate Assessment

The potential confounders were included in the study. The confounding factors influencing the risk of cancers such as age, gender, location (branch), and socio-economic status (SES) (based on the total amounts of payment to Taiwan's National Health Insurance) were all included in this study. We also identified comorbidities that may be associated with mortality based on diagnostic codes from outpatient datasets prior to the outcome of interest. All diseases were included in the Charlson Comorbidity Index (CCI) and analyzed, except for human immunodeficiency virus (HIV) (Charlson et al., 1987).

Data Analysis

One-way analysis of variance and independent t-test were used to compare each variable among groups undergoing surgery. A p-value of less than 0.05 was considered to be significant. Cumulative incidence curves were estimated by means of the method of Fine and Gray (Fine et al., 1999) were compared with the use of a log-rank test. Cox regression models with the duration (days) as the time scale were used to calculate hazard ratio (HR). The multivariable Cox model was adjusted for these confounders listed in **Table 1**. We used the SPSS 20 software to perform data analysis and the results calculations were expressed as the estimated numbers together with 95% confidence intervals (CIs).

Ethical Approval

This type of study did not require the Institutional Review Board approval according to the policies of the National Health Research Institutes which provides large computerized de-identified data.

TABLE 1 | Characteristic of hypothyroidism patients for each surgical procedures.

	Subtotal		Total		One-side total and another-side subtotal	Radical with unilateral neck	p-value
	Unilateral	Bilateral	Unilateral	Bilateral			
N	1475	3037	2324	537	1216	306	–
Gender, N (%)							<0.001
Female	1192 (80.81)	2572 (84.69)	1857 (79.91)	441 (82.12)	981 (80.67)	240 (78.43)	
Male	283 (19.19)	465 (15.31)	467 (20.09)	96 (17.88)	235 (19.33)	66 (21.57)	
Age							<0.001
Mean (SD)	45.25 (13.87)	41.44 (14.17)	46.35 (14.04)	49.25 (13.49)	45.52 (14.4)	46.54 (14.3)	
Comorbid conditions, N (%)							
Myocardial infarction	11 (0.75)	18 (0.59)	24 (1.03)	4 (0.74)	5 (0.41)	3 (0.98)	0.337
Congestive heart failure	134 (9.08)	196 (6.45)	224 (9.64)	47 (8.75)	103 (8.47)	31 (10.13)	0.001
Peripheral vascular disease	56 (3.8)	98 (3.23)	106 (4.56)	34 (6.33)	65 (5.35)	15 (4.9)	0.002
Cerebrovascular disease	120 (8.14)	188 (6.19)	217 (9.34)	70 (13.04)	130 (10.69)	31 (10.13)	<0.001
Dementia	7 (0.47)	13 (0.43)	15 (0.65)	5 (0.93)	14 (1.15)	4 (1.31)	0.064
COPD	471 (31.93)	956 (31.48)	806 (34.68)	216 (40.22)	481 (39.56)	120 (39.22)	<0.001
Rheumatic disease	57 (3.86)	119 (3.92)	181 (7.79)	56 (10.43)	102 (8.39)	28 (9.15)	<0.001
Peptic ulcer disease	492 (33.36)	1042 (34.31)	897 (38.6)	251 (46.74)	540 (44.41)	132 (43.14)	<0.001
Mild liver disease	383 (25.97)	879 (28.94)	679 (29.22)	214 (39.85)	425 (34.95)	113 (36.93)	<0.001
Diabetes	172 (11.66)	326 (10.73)	354 (15.23)	120 (22.35)	211 (17.35)	54 (17.65)	<0.001
Hemiplegia or paraplegia	22 (1.49)	52 (1.71)	49 (2.11)	21 (3.91)	33 (2.71)	5 (1.63)	0.006
Renal disease	141 (9.56)	218 (7.18)	239 (10.28)	68 (12.66)	115 (9.46)	30 (9.8)	<0.001
Cancer	159 (10.78)	192 (6.32)	608 (26.16)	297 (55.31)	137 (11.27)	279 (91.18)	<0.001
Moderate or severe liver disease	0 (0)	2 (0.07)	5 (0.22)	3 (0.56)	1 (0.08)	0 (0)	0.023
Charlson Comorbidities Index							
Mean (SD)	3.09 (2.93)	2.63 (2.62)	3.79 (3.22)	5.28 (3.41)	3.63 (3.22)	5.53 (3.34)	
Location, N (%)							
Taipei	438 (29.69)	786 (25.88)	758 (32.62)	191 (35.57)	322 (26.48)	115 (37.58)	
Northern	138 (9.36)	334 (11)	330 (14.2)	52 (9.68)	123 (10.12)	24 (7.84)	
Central	238 (16.14)	1009 (33.22)	545 (23.45)	121 (22.53)	281 (23.11)	45 (14.71)	
Southern	257 (17.42)	477 (15.71)	310 (13.34)	60 (11.17)	173 (14.23)	59 (19.28)	
Pingtung	367 (24.88)	377 (12.41)	327 (14.07)	105 (19.55)	287 (23.6)	51 (16.67)	
Eastern	37 (2.51)	54 (1.78)	54 (2.32)	8 (1.49)	30 (2.47)	12 (3.92)	
SES, N (%)							<0.001
INS_AMT< = 20,000	686 (46.51)	1506 (49.59)	1044 (44.92)	220 (40.97)	563 (46.3)	129 (42.16)	
20,000 < INS_AMT< = 40,000	610 (41.36)	1231 (40.53)	890 (38.3)	217 (40.41)	491 (40.38)	130 (42.48)	
INS_AMT > 40,000	179 (12.14)	300 (9.88)	390 (16.78)	100 (18.62)	162 (13.32)	47 (15.36)	

http://nhird.nhri.org.tw/en/. This study contained unidentifiable living individual medical information, that the informed consent is not needed.

RESULTS

In this study, we included 8,895 patients who underwent thyroid diseases surgeries. The mean age of patients who undergoing unilateral-subtotal and bilateral-subtotal surgery were 45.25 and 41.25 years respectively. All the demographic characteristics, confounding comorbidities and other factors which could influence the outcomes of subjects are presented in **Table 1**. In our study analysis, we classified patients into six different thyroid disorder surgery groups and observed a maximum numbers of patients, where the bilateral-subtotal procedure group was compared to other groups. It is noticeable that among all surgery groups, female patients dominate all groups, ranging from 85% to 87%. We also observed the statistically significant difference in comorbid diseases, regional, and socioeconomic status ($P < 0.001$) among different surgical procedures groups.

We analyzed the cumulative incidence for hypothyroidism after adjusting confounding variables among different surgical procedures groups shown in **Figure 1**. It can be seen from the data in **Figure 1** that the bilateral total group revealed 45% cumulative incidence of postoperative hypothyroidism during the 12 years study period. There was no significant difference observed between the bilateral-subtotal (18%) and radical with unilateral neck (17.5%) surgery procedure group. Interestingly the unilateral-subtotal group presented the lowest rate of incidence (10%) among other surgical procedures groups for thyroid disorders.

Table 2 presents incidence of developing hypothyroidism in all six surgical procedures groups. The significantly higher risk for developing hypothyroidism was observed in bilateral-total group (HR, 4.27; 95% CI, 3.32–5.50), for one-side total and another-side-subtotal (HR, 3.16; 95% CI, 2.59–3.86), and for bilateral-subtotal (HR, 1.65; 95% CI, 1.37–1.98) shown in **Table 2**. However, we did not observe any statistically significant

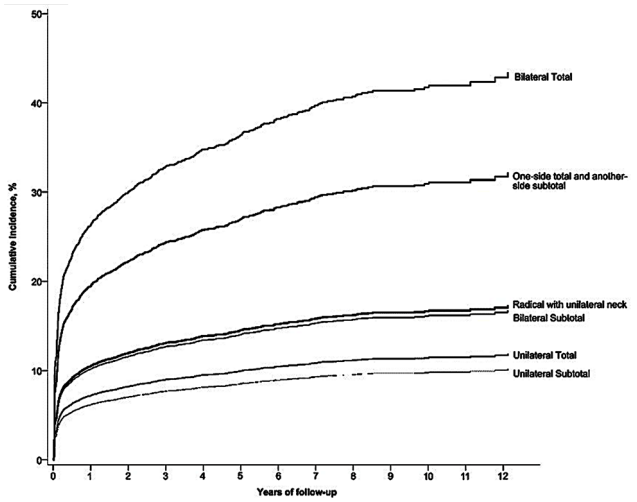

FIGURE 1 | Cumulative incidences of hypothyroidism (ICD-9-CM code 244.0) from different surgical procedures.

association for unilateral-total (HR, 1.17; 95% CI, 0.95–1.44) with postoperative hypothyroidism. In this study, we also investigated the time interval trends associated with weeks, months and years for postoperative hypothyroidism. We found that among a total of 350 subjects after surgery for a period of 1–4 weeks, 340 developed hypoparathyroidism and which subsided within 8 weeks then patients were stable within 12 weeks (**Figure 2A**). Similar trends were observed for periods of 2–12 months while we followed 480 subjects post thyroid disorder surgery, in which symptoms subsided usually within the second month after surgery (**Figure 2B**). For longer periods (1–12 years), 1000 cases of post thyroid surgery became stable within the first year of surgery and no longer had symptoms by the ending of our study's observation period. The rates of postoperative hypothyroidism were observed as significantly associated with increased occurrences just after surgical procedure but subsided shortly after, indicating a transient postoperative hypothyroidism as shown in **Figures 2A–C**. **Table 3** shows the relation of thyroid disorders and the surgical procedures which surgeon selected to perform.

DISCUSSION

We investigated postoperative hypothyroidism's association with thyroid disorder surgeries in the Taiwanese population. The patients with thyroid disorders undergoing six different surgical procedures allowed us to understand the incidence of postsurgical hypothyroidism among thyroidectomies. To our best knowledge, this study provided evidence of association using a large cohort population sample with different surgical procedures for thyroid disorders. Patients' proportion who developed postoperative hypothyroidism is substantial and varying from 10% to 45% depending on the type of surgical procedure.

The findings were startling in that 23.46% were bilateral total 23.76% one-side total and another-side-subtotal surgery associated with greater risk to postoperative hypothyroidism as compared with unilateral-subtotal. However, no significant association was observed in patients with a unilateral total surgical procedure for hypothyroidism. These findings show that the more extensive and substantial surgical procedure would have greater risk to develop postoperative hypothyroidism. Nevertheless, all patients encountered postoperative hypothyroidism temporarily which subsided during the study period which indicates a transient hypothyroidism. Our findings are consistent with Rosato et al. (2004), Michie et al. (1972) and Dunn and Chapman, (1964) that hypothyroidism was a temporary condition and did not reoccur after more than one year post thyroidectomy. However, some studies reported that most of the subtotal thyroidectomy patients showed hypothyroidism after surgery as the long-term outcome (Sung et al., 2015). Hedley et al. (1983) reported that patients undergoing subtotal thyroidectomy are not protected against early or late postoperative hypothyroidism.

In this study, 1048 patients with non-toxic multinodular goiter underwent bilateral-subtotal surgery, 867 patients with unspecified nontoxic nodular goiter had unilateral total surgery and 867 patients underwent thyroidectomy among other thyroid disorders (see **Table 3**). It is revealed that among surgeries, bilateral-subtotal surgical procedures were the most common we observed, with 32.56% patients having non-toxic multinodular goiter. However, most of the patients with thyroid cancer received total thyroidectomy and radioiodine ablation therapy. The reason for these procedures is to prevent a thyroid disorder from becoming hypothyroidism. Usually, multinodular goiter is less likely to develop into thyroid cancer but unilateral nodular goiter has a relatively higher thyroid cancer incidence as compared to multinodular. This might be one of the important reasons that these patients receive unilateral surgery more frequently.

Johner et al. (2011) reported that the incidence of hypothyroidism following thyroid lobectomy is low, and a significant proportion of individuals who become biochemically

TABLE 2 | Hypothyroidism risk after adjusting for surgical procedures.

Operation procedures	No.	Hypothyroidism No.	HR (95% CI)*	P-Value
Unilateral Subtotal	1475	153	148	
Bilateral Subtotal	3037	484	1.65 (1.37 - 1.98)	<0.001
Unilateral Total	2324	219	1.17 (0.95 - 1.44)	0.145
Bilateral Total	537	126	4.27 (3.32 - 5.50)	<0.001
One-side total and another-side subtotal	1216	289	3.16 (2.59 - 3.86)	<0.001
Radical with unilateral neck	306	31	1.70 (1.13 - 2.57)	0.011

Adjusted for variables as in **Table 1.*

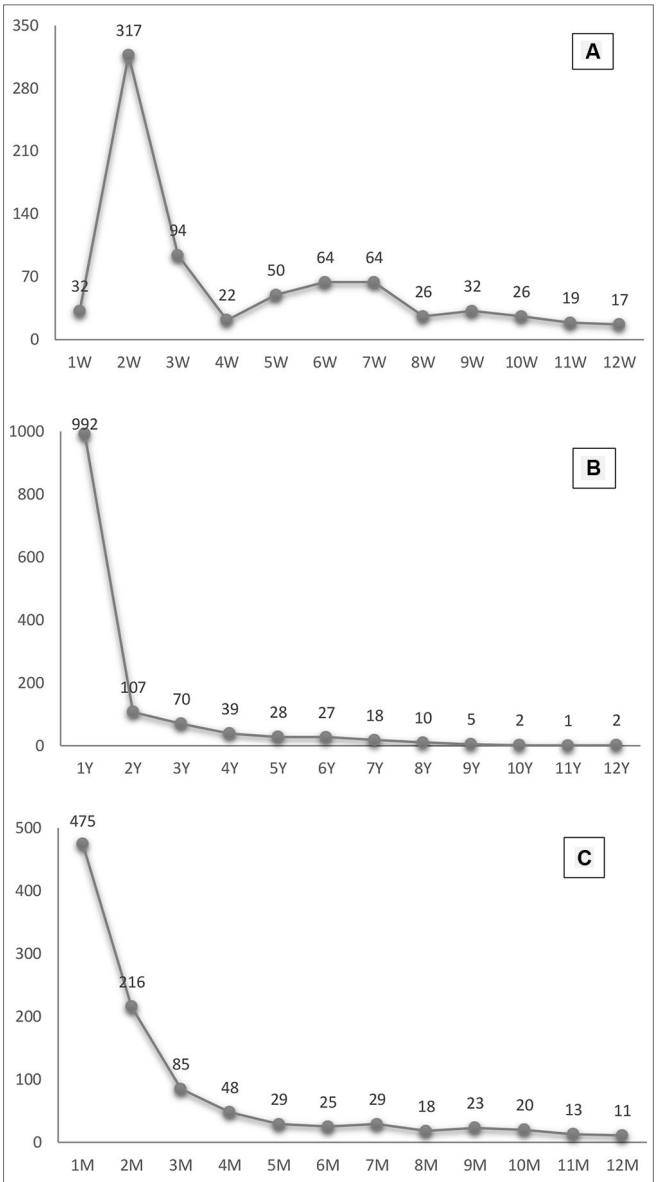

FIGURE 2 | Postoperative hypothyroidism trends in patients for periods of weeks, months and years. **(A)** presents the incidence of hypothyroidism by week in first 12weeks. **(B)** presents the incidence of hypothyroidism by year. **(C)** presents the incidence of hypothyroidism by month.

hypothyroid will reveal only a transient elevation in their TSH levels. We observed that radical thyroidectomy with unilateral neck lymph node dissection had increased incidence of patients with thyroid cancer (88.14%) compared to bilateral total surgical procedure (45.06%). All types of thyroid cancer such as papillary, follicular, medullary, or anaplastic could be removed by using complete thyroid resection surgery. In some cases, if the tissues could not be fully removed, then radioactive iodine therapy is often used to destroy the tissues.

In this current study, we also investigated cumulative incidences of post-operative hypothyroidism in patients undergoing surgery for any thyroid disorder during a time period of weeks, months and years. For a time period of 1–4 weeks, 340 patients developed postoperative hypothyroidism during the second week following thyroid related surgery which subsided to normal within 3 to 8 weeks. For a period of 2–12 months, 480 patients developed postoperative hypothyroidism and which subsided in 2–3 months after thyroid surgery. Similar trends were observed for periods of 1–12 years where almost 1000 patients underwent surgery and developed postoperative hypothyroidism only shortly after surgery, which subsided within the 2–12 years of our study period. Interestingly, we observed almost the same trends for different periods (weeks, months and years) which showed a transient hypothyroidism after surgery for temporarily which subsided afterwards. Our findings are consistent with previous studies in that hypothyroidism is associated with total thyroidectomy and occurred frequently, however, it could be managed as compared to hypoparathyroidism (Mortimore et al., 1998). Verloop et al.'s meta-analysis (Yang et al., 2010) showed that approximately one in five patients will develop hypothyroidism after hemithyroidectomy, with clinical hypothyroidism in one of 25 patients undergoing surgery. Tomoda et al. (2011) observed a 70% incidence of hypothyroidism associated with hemithyroidectomy.

Thomusch et al. (2003) reported that the surgical techniques and extent of resection had a greater influence on permanent postoperative hypoparathyroidism than thyroid pathologic condition. Oda et al. (2016) found that 16.7% transient hypoparathyroidism is associated with tumor enlargement and the appearance of novel lymph node metastases surgeries. Okamoto et al. (1992) and Sugino et al. (1993) reported that 10% of the patients encountered an unexpected permanent postoperative hypothyroidism, despite choosing a surgical procedure to avoid drug usage for longer periods. While medical therapy with an anti-thyroid drug is commonly adopted in European nations and Japan as the first-choice method of therapy, the disease still often occurs. Recent systematic review reported the clinical, behavioral and pharmacogenomic factors could be influence in response to levothyroxine therapy in patients with primary hypothyroidism (Dew et al., 2017).

Moreover, the literature on the opinions of thyroid surgeries experts is somewhat controversial. Sosa et al. (1998) and Shindo et al. (1995) reported that surgeons experience is associated with complication rates. We observed in thyroid cancer patients that half of them took conservative surgeries (unilateral-subtotal, bilateral-subtotal and unilateral-total) and more than half took aggressive surgery (bilateral-total, unilateral-total and unilateral-subtotal, and radical thyroidectomy) with unilateral neck lymph node. The surgical procedure often took place in consideration to the cancer cell type, and stage and size of nodules. After thyroidectomy, the transient hypothyroidism often occurring in patients is associated with blood loss during surgery. This usually subsides once the tissue regeneration and blood perfusion occurs, which leads to the thyroid gland functions returning to normal.

The findings of this study should be interpreted by acknowledging that we did not have access to the type of cancer cells, tumor stage and nodules size information, as we used Taiwan NHI database which only contains claims data. Some limitations may be inevitable in this retrospective

TABLE 3 | The relation of thyroid disorders and the surgical procedures which surgeon selected to perform (Hypothyroidism).

Surgical Procedure/ Thyroid disorders (ICD-9 code)	Unilateral Subtotal N = 1475	Bilateral Subtotal N = 3037	Unilateral Total N = 2324	Bilateral -Total N = 537	One-side total and another-side subtotal N = 1216	Radical with unilateral neck N = 306	Total
193 (Malignant neoplasm)	63 (4.06%)	75 (2.33%)	419 (17.38%)	265 (45.06%)	57 (4.55%)	275 (88.14%)	1154
240 (Simple and unspecified goiter)	1 (0.06%)	0	0	0	0	0	1
240.0 (Goiter, specified as simple)	4 (0.26%)	4 (0.124%)	4 (0.16%)	0	0	0	12
240.9 (Goiter, unspecified)	74 (4.18%)	82 (2.54%)	93 (3.85%)	8 (1.36%)	53 (4.23%)	1 (0.32%)	311
241 (Nontoxic nodular goiter)	4 (0.26%)	3 (0.09%)	0	0	0	0	7
241.0 (Nontoxic uninodular goiter)	137 (8.91%)	44 (1.36%)	225 (9.33%)	3 (0.51%)	31 (2.47%)	11 (3.52%)	451
241.1 (Nontoxic multinodular goiter)	133 (8.65)	1048 (32.56%)	246 (10.20%)	141 (23.97%)	340 (27.15%)	2 (0.64%)	1910
241.9 (Unspecified nontoxic nodular goiter)	654 (42.57%)	504 (15.66%)	867 (35.97%)	53 (9.01%)	278 (22.20%)	4 (1.28%)	2360
242 (Thyrotoxicosis with or without goiter)	0	5 (0.15%)	0	0	0	0	5
242.0 (Toxic diffuse goiter)	0	11 (0.34%)	1 (0.041%)	0	0	0	12
242.1 (Toxic uninodular goiter)	0	0	0	0	0	0	0
242.2 (Toxic multinodular goiter)	0	0	0	0	0	0	0
242.3 (Toxic nodular goiter unspecified type)	1 (0.06%)	0	0	0	0	0	1
242.4	0	0	0	0	0	0	0
242.8 (Thyrotoxicosis of other specified origin)	0	0	0	0	0	0	0
242.9 (Thyrotoxicosis without mention of goiter or other cause)	0	1 (0.03%)	0	0	0	0	1
Other diseases	404 (27.39%)	1260 (41.49%)	469 (20.18%)	67 (12.48%)	457 (37.58%)	13 (4.25%)	2670

NHIRD study. However, our study was still valuable because it is a population-based nationwide long-term study and the NHIRD records included a large sample size (1 million random individuals) with the general representation (covered more than 99% Taiwan citizens). Moreover, the National Health Insurance is a nationwide legislative policy based on National Health Insurance Act and is governed by National Health Insurance Administration, Ministry of Health and Welfare, Republic of China (Taiwan). Insurance claims are scrutinized by official medical specialists and monitored by peer reviewers according to standard diagnostic criteria. Although we used multiple methods during the inclusion process to identify the diagnosis and to minimize misclassification, a few atypical cases may still present difficulties in classification and this issue we further try to deal by ensuring the patients long term follow-up.

CONCLUSION

The most significant findings to emerge from this study is that thyroidectomy was associated with transient postoperative hypothyroidism in thyroid disorder patients. The research has also shown that the risk was associated with different surgical procedures for thyroid disorders as well. Postoperative hypothyroidism usually occurs only temporarily and subsides afterwards.

DATA AVAILABILITY STATEMENT

The raw data supporting this manuscript's findings will be available upon request to any qualified scientist by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

W-SJ designed the study, enrolled patients, interpreted data, wrote the report, and approved the final draft. UI designed the study, searched the published work, analyzed and interpreted data, reviewed the manuscript, and approved the final draft. H-PM, Y-CW and P-HC recruited patients, collected and interpreted data, reviewed the manuscript, and approved the final draft. P-AN, C-LH, AH, W-SJ and UI interpreted data, reviewed the manuscript, and approved the final draft. C-LH,

S-CC and RNA recruited participants, reviewed the report and approved the final draft. S-HT designed the study, analyzed and interpreted data, reviewed the report, and approved the final draft.

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ORIGINAL RESEARCH
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Effectiveness of Subcutaneous Tumor Necrosis Factor Inhibitors in Patients With Ankylosing Spondylitis: A Real-World Prospective Observational Cohort Study in China

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Objective: This prospective observational study investigated the efficacy of tumor necrosis factor inhibitors (TNFis) on disease activity, physical functionality, and mobility in patients with ankylosing spondylitis (AS) in a real-world setting.

Methods: The Chinese Ankylosing Spondylitis Prospective Imaging Cohort (CASPIC) is an ongoing cohort study. Patients with AS were included to one of two groups: the TNFi user group included those who received TNFi at any time point; the non-TNFi user group included those who did not receive TNFi. Disease activity, physical functionality, and mobility were assessed by AS Disease Activity Scores (ASDAS), Bath AS Functional Index (BASFI), and Bath AS Metrology Index (BASMI), respectively.

Results: A total of 804 patients with AS (241 TNFi users and 563 non-TNFi users) were recruited. For TNFi users, 83% received an etanercept biosimilar and 17.0% received adalimumab. Seventy-three TNFi users (30.3%) discontinued TNFis during the follow-up period; the mean duration of TNFi treatment was 6.9 ± 3.2 months. Reductions in ASDAS were significantly greater in TNFi users than in nonusers at 3, 6, and 12 months (differences in ASDAS reduction were 0.61, 0.56, and 0.46 units, respectively, all $P < 0.05$). Similarly, the improvement in BASFI was significantly greater in users than in nonusers at 3, 6, and 12 months (differences in BASFI improvement: 0.31, 0.75, and 0.74 units, respectively, all $P < 0.05$). BASMI increased in nonusers at 6 and 12 months (0.27, $P = 0.47$; 0.66, $P < 0.001$, respectively), but did not change in users (–0.16 and –0.13, respectively, both $P > 0.05$). At 12 months, changes in BASMI were significantly greater in nonusers than in users (–0.60, $P = 0.47$).

Conclusion: TNFis are effective against disease activity and improve the physical functionality of patients with AS, even in those who taper or discontinue TNFis. Thus, TNFis may retard the progression of spinal mobility dysfunction in AS patients. TNF may maintain spinal mobility as indicated by the BASMI.

Keywords: tumor necrosis factor, ankylosing spondylitis disease activity, real-world study, adalimumab, biosimilar etanercept

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INTRODUCTION

The most common form of spondyloarthritis is ankylosing spondylitis (AS), a chronic and progressive condition characterized by radiographic changes in sacroiliac joints (Braun and Sieper, 2007; Machado et al., 2010). Inflammation in AS mainly affects the axial skeleton, resulting in inflammatory back pain, bony fusion, and new bone formation in the spine. The peripheral joints, eyes, bowels, and lungs can also be affected by AS. In addition, AS, which has a global prevalence of 0.1–1.4%, (Dean et al., 2014) more commonly develops in young men.

Several randomized controlled trials have shown that tumor necrosis factor inhibitors (TNFis) improve the treatment of AS and spondyloarthritis (Calin et al., 2004; van der Heijde et al., 2005; van der Heijde et al., 2006; van den Berg et al., 2011; Ward et al., 2016; van der Heijde et al., 2017). In many randomized controlled trials, TNFis rapidly and significantly reduced disease activity, and their long-term use even delayed radiological progression of the spine (van der Heijde et al., 2015; Poddubnyy et al., 2016; Wei et al., 2018). However, in clinical settings, TNFi treatment may be tapered or even discontinued for various reasons, especially when TNFis are not fully covered by local healthcare services, such as in some developing countries like China (Glintborg et al., 2010; Kristensen et al., 2010).

The cost-effectiveness of TNFi therapy is an important factor in treatment decisions for both patients and rheumatologists (Braun et al., 2006). Although TNFi costs vary tremendously across countries, it is a financial burden worldwide for societies, families, and patients with AS (Westhovens and Annemans, 2016). In Western countries, the pressure to reduce medical cost has increased greatly (van der Heijde et al., 2017). In areas where TNFis are not fully covered by local healthcare services, including China and many other developing countries, this burden is higher and mainly borne by the patients.

In addition to cost considerations, long-term TNFi therapy is associated with increasing vulnerability to severe infectious diseases, including tuberculosis and hepatitis B infection, as well strains of carcinomas. Thus, in 2016, the Assessment of SpondyloArthritis International Society (ASAS)–European League Against Rheumatism (EULAR) recommended that tapering of TNFis should be considered in patients with sustained remission (at least 6 months) (van der Heijde et al., 2017). Because of the diversity of real-world settings and the inevitable existence of TNFi discontinuation or tapering, it is important to investigate the efficacy of TNFis in AS patients who had discontinued or tapered TNFi therapy. The primary purpose of this study was to model TNFi-related improvements in AS disease activity, physical functionality, and disability over a 12-month period. A secondary objective was to assess the effect of tapered TNFi therapy on outcomes in AS patients.

PATIENTS AND METHODS

Patient Population and Inclusion Criteria

The Chinese Ankylosing Spondylitis Prospective Imaging Cohort (CASPIC) is a nationwide, ongoing, prospective, and state-funded cohort study which was launched in conjunction with Smart-phone SpondyloArthritis Management System, a mobile health (mHealth) (**Supplementary Figure 1**) (Ji et al., 2019). To observe the whole disease process, patients of any age and disease duration were enrolled, and their prognoses for AS were comprehensively evaluated. All data were obtained from the Chinese People's Liberation Army (PLA) General Hospital, a prominent tertiary referral center in Beijing to which patients were referred from throughout the country. Patients were recruited consecutively from outpatient rheumatology clinics, irrespective of the presence of concomitant acute anterior uveitis, psoriasis, or inflammatory bowel disease. Eligible individuals for this study were patients who 1) fulfilled the 1984 modified New York criteria (van der Linden et al., 1984), 2) had complete clinical data including medical history, and 3) had at least one follow-up visit after the initial visit between April 2016 and April 2018. Exclusion criteria were patients who 1) refused to complete the survey and 2) had invalid/missing data on registration.

Drug Exposure

TNFi users were defined as patients who were treated with TNFi at any point during the first and follow-up visits; the baseline was defined as the time of enrollment for patients who were using TNFi at the time of enrollment or as the time at which patients started using TNFi after enrollment. TNFi users received subcutaneous administration of TNFis, including biosimilar etanercept (ETN; Yisaipu®; Sunshine Guojian Pharmaceutical Co., Ltd.; Shanghai, China) or adalimumab (ADA; AbbVie, Ludwigshafen, Germany), a recombinant human tumor necrosis factor receptor–antibody fusion protein that is widely used in China (Huang et al., 2010; Huang et al., 2011; Li et al., 2018). Non-TNFi users were defined as patients who did not receive treatment with any type of TNFi during the observation period, and their baseline was defined as the time of enrollment in the registry. Patients who had at least one visit during the follow-up after the baseline were included for further analysis.

The use of other medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) such as methotrexate (Sine, Shanghai, China), sulfasalazine (Sine, Shanghai, China), and thalidomide (Changzhou Pharmaceutical Factory Co., Ltd., Changzhou, China), was also analyzed.

Outcomes

The Ankylosing Spondylitis Disease Activity Score (ASDAS), a good indicator of disease activity, was used to evaluate the outcomes of AS patients (Aydin et al., 2010). It was calculated using a formula defined for assessing disease activity in AS patients (Garrett et al., 1994; Lukas et al., 2009; van der Heijde et al., 2009). The Bath Ankylosing Spondylitis Functional Index (BASFI), which includes 10 questions on 0–10 numeric rating scales) (Garrett et al., 1994), was used to assess patients' daily life functions. The questionnaire was completed by patients at each clinic visit. The Bath Ankylosing Spondylitis Metrology Index (BASMI) (Jenkinson et al., 1994) was used to assess the mobility of the spine and hips of AS patients and was determined by

trained rheumatologists at each clinic visit. The primary outcome was the improvement in ASDAS during the follow-up period. The secondary outcomes were changes in BASFI and BASMI values.

The demographic characteristics of patients, including age, sex, height, weight, smoking status, comorbidities, past medical history, onset date for back pain, human leukocyte antigen (HLA)-B27 status, presence of AS features (acute anterior uveitis, psoriasis, and colonoscopy- and pathology-confirmed diagnoses of inflammatory bowel disease), family history, enthesitis, and peripheral arthritis, were recorded. The follow-up assessment for AS included examination of inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein. When C-reactive protein levels were below the limit of detection, a constant value of 2 mg/l was used to calculate ASDAS (Machado et al., 2015).

Subsequent visits were scheduled according to the patients' needs (1–12 months) and were determined at each visit. Discontinuation of TNFi treatment was defined as a ≥45-day gap without TNFi treatment. Safety was evaluated at every visit, including the monitoring of adverse events and assessment of clinical laboratory results. The reasons for TNFi discontinuation were also recorded, including shared patient–physician decision after remission, patient decision without the physician's guidance, adverse effects, lack of efficacy following sufficient dosage for more than 1 month, and other reasons, such as pregnancy plans and surgery.

Dose Tapering Strategy

Treatment adherence was defined as the number of days of continuous use of TNFis. Tapering of TNFis was considered for cases in which disease activity was stable (ASDAS < 1.3) after 3 months on the standard dose regimen. Each patient's preference was fully considered in the tapering strategy. The dosage was reduced in a step-by-step and patient-tailored manner, starting at 10-day intervals for ETN and 20-day intervals for ADA; intervals generally did not exceed 1 month.

Statistical Analysis

All analyses were performed using Empower (R) (www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) and R (http://www.R-project.org). Quantitative data are presented as means and standard deviation (SD). Student's *t* test or one-way analysis of variance was used to identify significant differences in quantitative variables. Categorical data are presented as percentage (%). Chi-square tests were used to identify significant differences in categorical data.

General additive mixed models with smooth curve fitting are optimal for analyzing repeated measurements (Lin and Zhang, 1999). General additive mixed models were used to assess the relationship between follow-up duration (independent variable) and ASDAS, BASFI, and BASMI (dependent variables), stratified by TNFi treatment. Intercept and time were included as random terms. In these models, ASDAS, BASFI, and BASMI were assessed at the baseline visit and during all follow-up visits. All models used the same set of fixed effects that have been widely used in studies of TNFi and AS disease outcomes (Molnar et al., 2018). The following variables were measured or calculated at the baseline visit and entered into adjusted models as fixed effects: gender, disease duration, body mass index, HLA-B27 status, smoking status (self-reported as never or former/current), peripheral arthritis, and treatment with NSAIDs (user or nonuser) and csDMARDs (user or nonuser). The interaction terms between follow-up time and TNFi treatment were also evaluated.

General additive mixed models were also used to assess the relationship between follow-up duration and ASDAS among TNFi users stratified by enthesitis. A two-tailed P value <0.05 was considered statistically significant.

RESULTS

Study Population

A total of 1,201 patients with AS were recruited between April 2016 and April 2018. Sixty-eight patients (5.7%) withdrew or had no medication records; 329 patients had no follow-up visits, including 91 (7.6%) TNFi users and 238 (19.8%) non-TNFi users. Those patients were excluded from the study (**Figure 1**). Finally, 804 patients with at least two follow-up visits were included in the study, including 241 TNFi users and 563 non-TNFi users. The mean patient age was 30.5 ± 8.8 years, and the majority of the patients were male (83.1%). The HLA-B27-positive rate was 88.7%. The median follow-up duration was 7.9 months (interquartile range, 0.9–12.0 months) in the TNFi user group and 7.5 months (interquartile range, 0.7–12.0 months) in the non-TNFi user group (P = 0.228). In the TNFi user group, 200 (83%) patients were given an ETN biosimilar and 41 (17.0%) patients were treated with ADA. The mean duration of TNFi treatment was 6.9 ± 3.2 months.

Baseline Characteristics

The demographic characteristics of the 241 (30.0%) TNFi users and 563 (70.0%) nonusers are summarized in **Table 1**. The mean age was 29.0 ± 7.8 years for TNFi users and 31.1 ± 9.1 years for nonusers (P = 0.002). Compared with TNFi nonusers, TNFi users were younger and more likely to present with enthesitis and peripheral arthritis. In addition, TNFi nonusers had higher baseline disease activity scores (ASDAS), higher acute-phase reactant levels (higher erythrocyte sedimentation rate), more inflammation (higher C-reactive protein), and were less likely to receive NSAIDs and csDMARDs (all P < 0.05) than TNFi users. At baseline, ASDAS, BASFI, and BASMI scores significantly differed between non-TNFi users (2.0 ± 1.0, 1.5 ± 1.7, and 1.4 ± 2.0, respectively) and TNFi users (2.4 ± 1.1, 1.9 ± 1.8, and 2.0 ± 2.2, respectively; P < 0.05).

Combination Therapy

Table 2 summarizes the medications used by AS patients at baseline. NSAIDs were administered to 97.2% of patients and csDMARDs were administered to 64.8% patients; the csDMARDs included sulfasalazine (22.6% of patients), leflunomide (15.4% of patients), methotrexate (4.1% of patients), and thalidomide

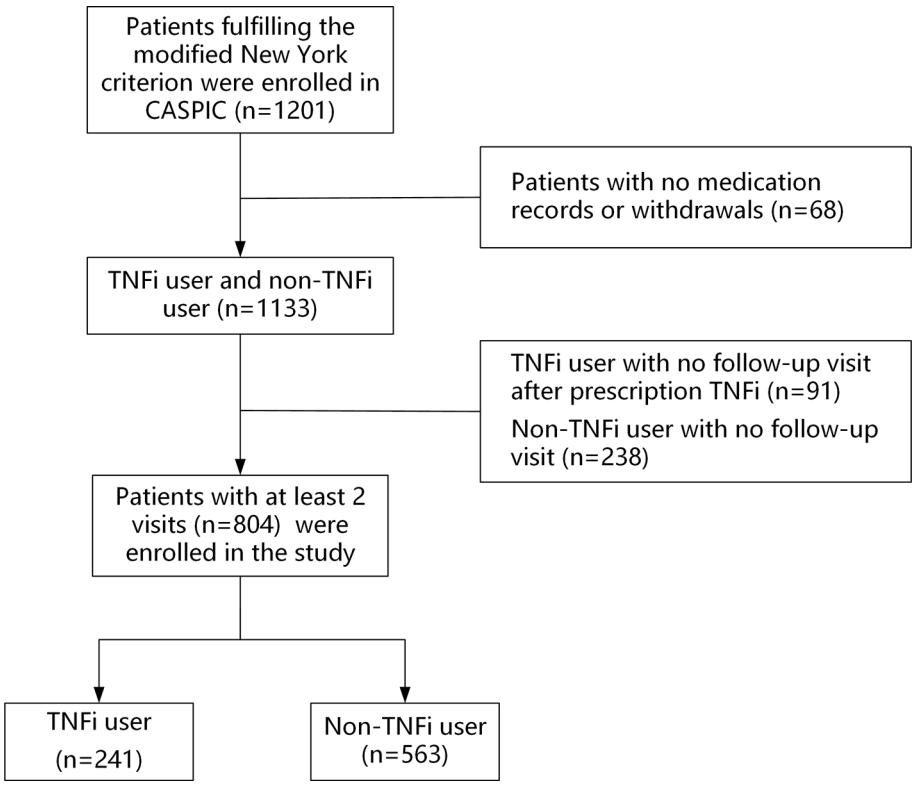

FIGURE 1 | Participant selection flowchart. *CASPIC,* Chinese Ankylosing Spondylitis Prospective Imaging Cohort; *TNFi,* tumor necrosis factor inhibitor.

TABLE 1 | Baseline characteristics of AS patients who were users and nonusers of tumor necrosis factor inhibitors.

Characteristics mean (SD) or %	Non-TNFi users (*n* = 563)	TNFi users (*n* = 241)	*P*
Male sex	83.8%	81.3%	0.385
Age, years	31.1 (9.1)	29.0 (7.8)	0.002
Disease duration, years	8.2 (6.5)	8.4 (5.6)	0.692
BMI, kg/m^2	23.7 (3.9)	23.5 (3.9)	0.452
HLA-B27 positive	87.5%	91.5%	0.113
Smoker	32.4%	30.1%	0.521
BASFI	1.5 (1.7)	1.9 (1.8)	0.003
BASMI	1.4 (2.0)	2.0 (2.2)	0.006
ESR, mm/h	13.9 (15.8)	24.2 (24.7)	<0.001
CRP, mg/l	11.8 (25.1)	21.5 (28.1)	<0.001
ASDAS	2.0 (1.0)	2.4 (1.1)	<0.001
AAU	19.5%	23.8%	0.169
IBD	8.2%	12.6%	0.058
Psoriasis	3.9%	4.6%	0.668
Enthesitis	19.2%	32.4%	<0.001
Peripheral arthritis	9.2%	24.0%	<0.001
NSAID	98.9%	94.2%	<0.001
csDMARDs	71.8%	51.9%	<0.001

TNFi, tumor necrosis factor inhibitor; SD, standard deviation; BMI, body mass index; HLA-B27, human leukocyte antigen B27; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ASDAS, Ankylosing Spondylitis Disease Activity Score; AAU, acute anterior uveitis; IBD, inflammatory bowel disease; NSAID, nonsteroidal anti-inflammatory drug; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs.

TABLE 2 | Baseline medications of patients on combination therapies and monotherapies.

Characteristics (%)	Overall (*n* = 804)
NSAIDs	97.2
TNFi	35.4
csDMARDs	64.8
Sulfasalazine	22.6
Leflunomide	15.4
Methotrexate	4.1
Thalidomide	23.5
Drug combination	
NSAID monotherapy	18.0
TNFi monotherapy	1.3
NSAID + csDMARD	46.2
NSAID + TNFi	15.8
NSAID + TNFi + csDMARD	17.7

NSAID, nonsteroidal anti-inflammatory drug; TNFi, tumor necrosis factor inhibitor; csDMARD, conventional synthetic disease-modifying antirheumatic drug.

(23.5% of patients). TNFis were administered to 35.4% of patients. NSAID monotherapy and TNFi monotherapy were used in 18.0% and 1.3% of patients, respectively. NSAID plus csDMARD (46.2%) was the most common therapeutic regimen, followed by NSAID plus TNFi (15.8%). A combination regimen with three drugs was administered to 17.7% of patients. NSAIDs and thalidomide were less likely to be administered to female patients, who were more likely to receive TNFis as a monotherapy (4.2%).

Efficacy

Figure 2 shows the smoothing curve analyses of ASDAS, BASFI, and BASMI values for TNFi users and non-TNFi users during the 12-month follow-up period. The adjusted decline in ASDAS among TNFi users and non-TNFi users over 3 months was 0.84 units [95% confidence interval (CI), 0.48–1.21; $P < 0.001$] and 0.21 units (95% CI, 0.015–0.58; $P = 0.259$), respectively (**Table 3**).

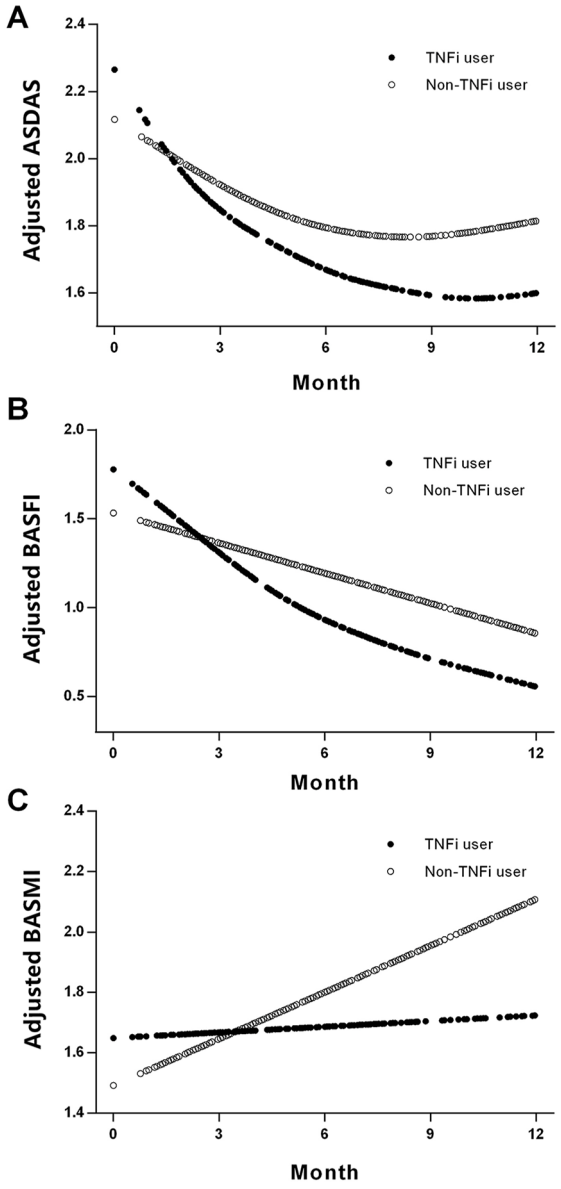

FIGURE 2 | Smoothing analysis curves for ASDAS **(A)**, BASFI **(B)**, and BASMI **(C)** values during the follow-up of 12 months in TNFi and non-TNFi users. All of the models were adjusted for sex, symptom duration, human leukocyte antigen B27, body mass index, smoking status, peripheral arthritis, and treatment with nonsteroidal anti-inflammatory drugs and disease-modifying antirheumatic drugs. ASDAS, Ankylosing Spondylitis Disease Activity Score; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; TNFi, tumor necrosis factor inhibitor.

There were significant differences in ASDAS decline between TNFi users and non-TNFi users during the 0- to 3-month, 0- to 6-month, and 0- to 12-month follow-up periods (0.61 units, $P = 0.017$; 0.56 units, $P < 0.001$; 0.46 units, $P < 0.001$, respectively).

For non-TNFi users, BASFI did not significantly decline during the first 3 months (0.02 units, $P = 0.482$), but they did significantly decline during the 0- to 6-month and 0- to 12-month follow-up periods (0.48 and 0.67 units, respectively, both $P < 0.001$). For TNFi users, BASFI significantly declined during the 0- to 3-month, 0- to 6-month, and 0- to 12-month follow-up periods (0.28 units, $P = 0.021$; 1.20 units, $P < 0.001$; 1.45 units, $P < 0.001$, respectively). The differences in BASFI reduction rates between TNFi users and non-TNFi users during each follow-up period were significant (**Table 3**).

For non-TNFi users, BASMI significantly increased during the 0- to 6-month and 0- to 12-month follow-up periods (0.27 units, $P = 0.047$, and 0.66 units, $P < 0.001$, respectively), whereas for TNFi users, the BASMI was stable—even slightly improved—during the same follow-up periods (0.06, −0.16, and −0.13 units, all $P > 0.05$). Differences in the reduction of BASMI between TNFi users and non-TNFi users were significant for the 12-month follow-up period (0.60 units, $P = 0.047$; **Table 3**).

The adjusted decline in ASDAS among TNFi users with and without enthesitis was 1.66 units (95% CI, 1.02–2.31; $P < 0.001$) and 0.60 units (95% CI, 0.22–0.99; $P = 0.003$), respectively, during the 12-month follow-up periods. Differences in the reduction of ASDAS between the two groups were significant for the 12-month follow-up period (1.13 units, $P = 0.002$; **Supplementary Table 1**). **Supplementary Figure 2** shows smoothing analysis curves for ASDAS during the follow-up of 12 months among patients with and without enthesitis among TNFi users.

Discontinuation of TNFi

No other serious adverse events were observed during the follow-up period. TNFi-related adverse events occurred among six patients in the TNFi user group; therefore, TNFi was discontinued for those patients, including two patients with infections in the upper respiratory tract and one patient each with pulmonary tuberculosis, new-onset uveitis, palmoplantar pustulosis, and subacute thyroiditis. All of them were receiving adequate TNFi dosages (ADA: 40 mg/14 days; ETN biosimilars: 50 mg/7 days).

TNFi-Related Adverse Events

No serious adverse events were observed during the follow-up period. TNFi-related adverse events occurred among six patients in the TNFi user group; therefore, TNFi was discontinued for these patients, including two patients with infections in the upper respiratory tract, one patient with pulmonary tuberculosis, one patient with new-onset uveitis, one patient with palmoplantar pustulosis, and one patient with subacute thyroiditis. All of them received adequate TNFi dosages (ADA: 40 mg/14 days; ETN biosimilars: 50 mg/7 days).

DISCUSSION

In this real-world cohort study of AS patients, those who received TNFi treatment showed better improvement in disease activity

TABLE 3 | Unadjusted and adjusted changes in ASDAS, BASFI, and BASMI among TNFi users and nonusers.

	0–3 months		0–6 months		0–12 months	
	β (95% CI)	*P*	*β* (95% CI)	*P*	*β* (95% CI)	*P*
ASDAS						
Unadjusted						
Non-TNFi users	−0.38 (−0.66, −0.09)	0.012*	−0.49 (−0.66, −0.32)	0.018*	−0.41 (−0.54, −0.28)	<0.001*
TNFi users	−1.11 (−1.46, −0.77)	<0.001*	−0.90 (−1.16, −0.64)	<0.001*	−0.74 (−0.95, −0.52)	<0.001*
Difference	−0.80 (−1.24, −0.36)	<0.001*	−0.42 (−0.73, −0.12)	0.005*	−0.33 (−0.57, −0.09)	<0.001*
Adjusted						
Non-TNFi users	−0.21 (−0.58, 0.15)	0.259	−0.40 (−0.60, −0.21)	<0.001*	−0.44 (−0.63, −0.25)	<0.001*
TNFi users	−0.84 (−1.21, −0.48)	<0.001*	−0.94 (−1.22, −0.66)	<0.001*	−0.93 (−1.26, −0.41)	<0.001*
Difference	−0.61 (−1.13, −0.10)	0.023*	−0.56 (−0.89, −0.24)	<0.001*	−0.46 (−0.81, −0.10)	0.012*
BASFI						
Unadjusted						
Non-TNFi users	0.00 (−0.07, 0.07)	0.933	−0.56 (−0.74, −0.38)	<0.001*	−0.67 (−0.82, −0.52)	<0.001*
TNFi users	−0.22(−0.41, −0.03)	0.028*	−1.24 (−1.54, −0.94)	<0.001*	−1.25 (−1.52, −0.99)	<0.001*
Difference	−0.24(−0.45, −0.03)	0.024*	−0.69 (−1.02, −0.36)	<0.001*	−0.58 (−0.87, −0.29)	<0.001*
Adjusted						
Non-TNFi users	−0.02 (−2.62, 5.54)	0.482	−0.48 (−0.67, −0.29)	<0.001*	−0.67 (−0.92, −0.44)	<0.001*
TNFi users	−0.28 (−0.51, −0.05)	0.021*	−1.20 (−1.54, −0.86)	<0.001*	−1.45 (−1.87, −1.03)	<0.001*
Difference	−0.31 (−0.57, −0.05)	0.020*	−0.75 (−1.13, −0.38)	<0.001*	−0.74 (−1.18, −0.29)	0.001*
BASMI						
Unadjusted						
Non-TNFi users	0.12 (−0.38, 0.63)	0.630	0.23 (−0.09, 0.56)	0.158	0.68 (0.44, 0.93)	<0.001*
TNFi users	0.24 (−0.45, 0.94)	0.499	−0.06 (−0.64, 0.51)	0.833	0.06 (−0.50, 0.62)	0.840
Difference	0.15 (−0.68, 0.99)	0.718	−0.24 (−0.86, 0.38)	0.453	−0.53 (−1.08, 0.02)	0.060
Adjusted						
Non-TNFi users	0.10 (−0.34, 0.53)	0.670	0.27 (0.01, 0.53)	0.047*	0.66 (0.41, 0.91)	<0.001*
TNFi users	0.06 (−0.39, 0.50)	0.811	−0.16 (−0.82, 0.51)	0.639	−0.13 (−0.78, 0.51)	0.688
Difference	−0.11 (−0.74, 0.52)	0.729	−0.26 (−0.93, 0.40)	0.443	−0.60 (−1.19, −0.01)	0.047*

The model was adjusted for sex, symptom duration, human leukocyte antigen B27, body mass index, smoking status, peripheral arthritis, and treatment with non-steroidal anti-inflammatory drugs and disease-modifying antirheumatic drugs.

CI, confidence interval; ASDAS, Ankylosing Spondylitis Disease Activity Score; TNFi, tumor necrosis factor inhibitor; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index.

**P < 0.05.*

and physical functionality, and were more likely to maintain mobility, than those not treated with TNFis.

Clinical practice guidelines in 2016 recommended that tapering of TNFi therapy should be considered in patients with sustained remission (minimum of 6 months), owing to the high cost and risks of severe infectious disease associated with TNFi therapy (van der Heijde et al., 2017). Previous studies in Europe have reported that the first-year survival rate for AS patients with TNFi treatment is 75–88% (Pavelka et al., 2009; Lie et al., 2011; Heinonen et al., 2015). In countries where medical insurance does not cover the cost of TNFis, patients may not be able to afford the high cost of TNFi therapy for prolonged periods, and dose adjustments are important and pressing. In the present study, 30.3% of TNFi users discontinued TNFis by their final visit. At least 17.8% of those patients discontinued TNFis on their own for economic reasons. Economic factors also influenced shared patient–physician decisions to taper TNFi therapy after 3 months of full-dose treatment and to discontinue after 6 months if clinical improvement was achieved. Adverse events were also a reason for the discontinuation of TNFi therapy. Overall, the treatment with TNFi was well tolerated and only a few patients discontinued because of adverse events. In our study, six patients discontinued treatment due to adverse events, accounting for 8.2% of all discontinued patients treated with TNFi therapy. The proportion of discontinuation due to adverse events was similar to that reported of 8% (69/310) in the Danish nationwide DANBIO registry (Glintborg et al., 2010) and less than that reported of 27% in another observational study (Arends et al., 2011). Treatment and discontinuation strategies vary across countries. However, our results confirmed the real-world efficacy of TNFi for the treatment of AS, with respect to disease activity, physical functionality, and mobility. However, our results confirmed the real-world efficacy of TNFis for the treatment of AS, with regard to disease activity and physical functionality. Poddubnyy (Poddubnyy et al., 2016; Poddubnyy et al., 2018) reported that the functional status and spinal mobility of patients with established AS remained stable during long-term TNFi therapy during the observation period of 10 years. In our cohort, we also found that the BASMI of TNFi users was well maintained.

Although csDMARDs are not included in the ASAS-EULAR management recommendations (van der Heijde et al., 2017), rheumatologists have continued to use them in combination therapy according to national guidelines and their treatment experience in clinical practice. A Finnish observational study showed that 78% of patients with AS receive csDMARDs (Heinonen et al., 2015), whereas a Swedish study reported that 61% of patients with AS receive csDMARDs (Kristensen et al., 2010). In our study, csDMARDs were administered to 64.8% of

patients, which is similar to those previous studies. TNFi users were less likely to use csDMARDs than non-TNFi users (51.9% and 71.8%, respectively, $P < 0.001$). Despite their lower rate of csDMARD use, TNFi users exhibited greater improvement in AS disease activity, physical functionality, and disability than non-TNFi users did during the 12-month follow-up period. More importantly, the decline in disease activity at the 3-month follow-up was four times greater for TNFi users than that for non-TNFi users. Therefore, the use of concomitant csDMARDs did not alter the efficacy of TNFis in this study.

Compared to male patients, female patients were much more likely to be prescribed monotherapy and had significantly lower NSAID and thalidomide intake rates. This may be because female patients have better spinal mobility and less severe structural damage than male patients do (van der Horst-Bruinsma et al., 2013; van der Slik et al., 2019). In addition, thalidomide has significant teratogenic toxicity and is not recommended for female patients of reproductive age.

Strengths of our study lie in the availability of data from an established observational cohort that included a continuous included sample of patients with AS, which may have aided in the reduction of biases in selection and observation. In addition, we used generalized additive mixed models to effectively analyze repeated measurements and individually aggregated data (Lin and Zhang, 1999). Despite the strengths of this study, there are still limitations to note. First, because it is a real-world, non-randomized comparative effectiveness study, the comparability of the TNFi and non-TNFi groups is potentially limited owing to confounding factors. Thus, to improve the comparability, we have adjusted several confounding factors including gender, disease duration, body mass index, HLA-B27 status, smoking status, peripheral arthritis, and treatment with NSAIDs and csDMARDs in the longitudinal model. Second, ADA and ETN were the only types of TNFis included in our study, and infliximab was not included because the number of patients using infliximab on an outpatient basis was very low. Other types of TNFis are unavailable in China. Third, the sample was included in a single tertiary academic center, which may limit the ability to extrapolate our findings to other clinical settings.

CONCLUSIONS

In summary, we found that after adjustment for mixed factors, short-term treatment with TNFis was associated with significant improvements in disease activity and physical functionality in patients with AS, whereas therapies using only NSAIDs and csDMARDs (non-TNFi users) were significantly less effective than TNFis for improving disease activity, increasing physical functionality, and maintaining spinal mobility as indicated by the BASMI.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the Chinese PLA General Hospital (S2016-049-02). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conception and design: FH. Administrative support: JZhu and JZha. Data analysis and interpretation: XJ. Data collection, manuscript writing and final approval of the manuscript: XJ, YiW, ZH, YM, SM, KL, YaW, JZhu, JZha and FH.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01476/full#supplementary-material

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Flunarizine Induced Parkinsonism in Migraine Group: A Nationwide Population-Based Study

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Background: Flunarizine (Fz) is a first-line prophylactic medication that is widely used in migraine. However, Fz has been recognized as a potential cause of drug-induced parkinsonism for a long time. However, to our knowledge, there has been no population-based subgroup analyses for Fz-induced parkinsonism (FIP) in migraine patients.

Methods: Data were obtained from the Taiwan's National Health Insurance Research Database. The study comprised 6,470 migraine patients who were divided into two groups, based on their exposure or non-exposure to Fz.

Results: During the study period (2000–2012), the incidence rate of parkinsonism was 1.92 and 8.72 per 1,000 person-years in the control and Fz -treated groups, respectively. In the study population, the adjusted hazard ratio was 4.07 (95% confidence interval CI: 2.84–5.85). In 45–64-year old subjects and ≥ 65-year old subjects, the risk of FIP was 3.18 times (95% CI = 1.63–6.20) and 4.89 times (95% CI = 3.09–7.72) more than that in the controls. The Fz-treated subjects with comorbidities also had a higher risk (4.54, 95% CI: 3.14-6.57). An average annual cumulative Fz dose > 445 mg was accompanied by the greatest risk of FIP; Fz use for >60 days is a cut-off point for predicting future FIP.

Conclusion: At the population level, this study showed a complete picture of FIP in migraine patients. FIP is associated with older age, history of comorbidities, exposure to high-dose of Fz, and longer duration of exposure to Fz.

Keywords: flunarizine, parkinsonism, drug-induced parkinsonism, migraine, population-based

Abbreviations: Fz, Flunarizine; FIP, Fz-induced parkinsonism; DIP, drug-induced parkinsonism; LHID, Longitudinal Health Insurance Database; NHIRD, National Health Insurance Research Database; NHRI, National Institutes of Health; PD, Parkinson's disease; HR, hazard ratios.

INTRODUCTION

Flunarizine (Fz) is a derivative of piperazine and exerts calcium-channel blocking, anti-histaminic, anti-serotoninergic, and anti-dopaminergic properties. Fz is widely used for migraine, vestibular dysfunction, insomnia, and neuroprotection (Holmes et al., 1984; Brucke et al., 1995). For adult migraine, Fz is considered the first-line prophylactic choice as per several guidelines (Pringsheim et al., 2012; Silberstein et al., 2012; Charles, 2017). A meta-analysis estimated that Fz reduces the headache frequency by 0.4 attacks per 4 weeks (Stubberud et al., 2019). Fz also improves the clocking tinnitus in migraine patients (Chen et al., 2019).

Fz has common adverse effects, such as sedation, weight gain, and depression (Charles, 2017). Drug-induced parkinsonism (DIP) is a serious adverse effect that has been reported from 1984 until today (De Melo-Souza, 1984; Chouza et al., 1986; Micheli et al., 1987; Benvenuti et al., 1988; Capella et al., 1988; Moretti and Lucantoni, 1988; Micheli et al., 1989; Brucke et al., 1995; Negrotti and Calzetti, 1997; Fabiani et al., 2004). However, such studies about Fz-induced parkinsonism (FIP) are limited because of the small sample sizes. Recently, four big data-based studies have focused on the issue (Lin et al., 2017; Liang et al., 2018; Jhang et al., 2019; Kim et al., 2019). The first study concluded that Fz obviously increased the FIP risk, especially in aging, diabetic, or stroke patients (Lin et al., 2017). The second research found that Fz was a potential risk factor for FIP in patients with newly diagnosed type 2 diabetes (Liang et al., 2018). The third indicated that FIP disorders were associated with high-dose or longer exposure, older age, essential tremor, and cardiovascular disease (Jhang et al., 2019). The most recent trial has shown that propulsives, antipsychotics, and Fz are significantly associated with an increased risk of FIP, depending on drug exposure duration and the cumulative dose amount (Kim et al., 2019).

Migraine is a primary headache disorder and the seventh leading cause of time spent disabled in the world (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). However, to the best of our knowledge, no population-based analyses have been performed for FIP in migraine patients. This study aimed to investigate the risk factors and cumulative daily dose associated with FIP.

METHODS

Data Source and Study Design

This cohort study utilized the data from the Longitudinal Health Insurance Database (LHID), one of the data subsets of the National Health Insurance Research Database (NHIRD). The NHIRD was established by the National Institutes of Health (NHRI) and has recorded the health information of 99% of the residents in Taiwan. The LHID contained the medical records of one million beneficiaries randomly selected from the National Health Insurance program. The definition of the disease in the LHID was according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). The index date was defined as the date of receiving Fz therapy. Each migraine patient (ICD-9-CM 346) who received Fz treatment for more than one month was matched to a subject without Fz treatment by age, sex, index year, and the interval between the onset of migraine and the first Fz consultation. People aged < 20 years or > 90 years were excluded. Moreover, subjects with Parkinson's disease (PD, ICD-9-CM 332), parkinsonism (ICD-9-CM 333), stroke (ICD-9-CM 430-438), dementia (ICD-9-CM 290, 294.1, and 331.0), head injury (ICD-9-CM 850-854, 959.01), or hydrocephalus (ICD-9 331.3-331.5) and those who used anti-psychotics drugs during the study period were also excluded (**Figure 1**).

Main Outcome and Covariates

PD or parkinsonism (ICD-9-CM codes 332 and 333, excluding 333.1–333.8) was the main outcome in the present study. The study period was defined as the interval between the index date to the diagnosis of parkinsonism, missing, or death. The PD-related comorbidities, including diabetes (ICD-9-CM 250), hypertension (ICD-9-CM 401-405), hyperlipidemia (ICD-9-CM 272), depression (ICD-9-CM 296.2, 296.3, 300.4, and 311), anxiety (ICD-9-CM 300.00), and sleep disorder (ICD-9-CM 307.4 and 780.5) before the index date were considered covariates.

Statistical Analyses

Chi-square tests were used to compare the differences in the demographic variables and the comorbidity status between the two cohorts. The mean age in the two groups was examined with a two-sample test. The incidence rates of parkinsonism in difference variables were calculated and the hazard ratios (HR) were also estimated by Cox proportional regression model. We adjusted the HR with the multivariable Cox model that included variables of age; sex; as well as comorbidity of hypertension, diabetes, hyperlipidemia, sleep disorder, anxiety, and depression. A stratified analysis of the duration, average dose, average DDD (defined daily dose), and cumulative DDD (cDDD) of Fz therapy per year was also performed. The Kaplan-Meier method was applied to obtain the cumulative incidence of parkinsonism and compare the difference between the two cohorts with the log-rank test. P-value < 0.05 was considered statistically significant in all the analyses.

RESULT

Total 6,470 subjects were included in this study. The distribution of the demographic and clinical comorbidity status between the two cohorts is shown in **Table 1**. The age and sex in the two cohorts was not significantly different. Most subjects were in the age group of 45–64 years (48.7%), and 74.3% were women. Subjects with Fz treatment had a higher incidence of comorbidities than those without Fz treatment. **Table 2** represents the incidence rate and HR of parkinsonism among the two study cohorts. In those aged 45–64 years and those

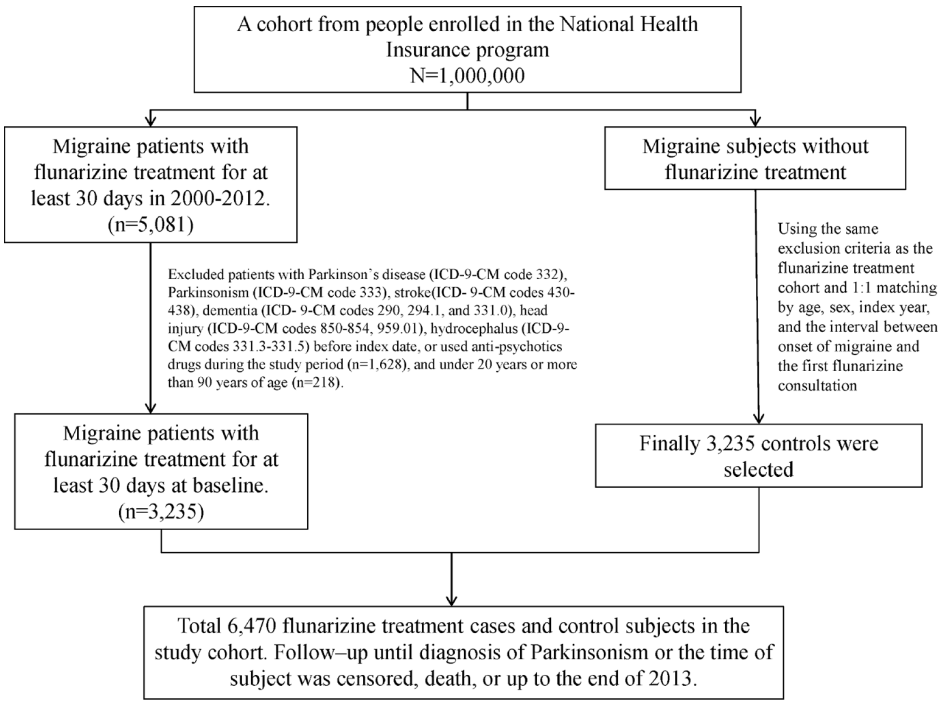

FIGURE 1 | Shows the selection process of the participants in the two study cohorts.

TABLE 1 | Distributions of demographic and clinical comorbid status among migraine patients.

	Flunarizine				p-value
	No N = 3235		Yes N = 3235		
	n	%	n	%	
Age, years					0.99
<45	827	25.6	827	25.6	
45-64	1575	48.7	1575	48.7	
≥65	833	25.8	833	25.8	
Mean ± SD[a]	54.1 ± 14.4		54.6 ± 14.3		0.75
Gender					0.99
Women	2405	74.3	2405	74.3	
Men	830	25.7	830	25.7	
Comorbidity					
Hypertension	1360	42.0	1807	55.9	<0.001
Diabetes	220	6.80	313	9.68	<0.001
Hyperlipidemia	1128	34.9	1456	45.0	<0.001
Sleep disorder	1552	48.0	2089	64.6	<0.001
Anxiety	1005	31.1	1769	54.7	<0.001
Depression	461	14.3	901	27.9	<0.001

Chi-square test, [a] t-test.

aged > 65 years, the risk of parkinsonism with Fz treatment was 3.18 times (95% CI = 1.63–6.20) and 4.89 times (95% CI = 3.09–7.72) higher than that in the controls. The adjusted HR of parkinsonism for women in the case group was 4.24-fold (95% CI = 2.69–6.68) higher than that for those in the control group and that for men was 3.89-fold (95% CI = 2.14–7.09) higher. Fz-treated patients with comorbidities had a greater risk of developing parkinsonism (adjusted HR = 4.54, 95% CI = 3.14–6.57). Cox regression analysis stratified by duration and average dose of Fz therapy is displayed in **Table 3**. Patients who received Fz treatment for at least 60 days were more likely to develop parkinsonism than those who had not undergone Fz treatment (adjusted HR = 8.49, 95% CI = 5.86–12.3). The adjusted HR of parkinsonism for patients with Fz dose ≥ 445 mg was 7.69 (95% CI = 5.31–11.1), with Fz average DDD ≥ 45 DDD was 7.77 (95% CI = 5.36-11.3), and with Fz cDDD ≥ 200 cDDD was 4.32 (95%

TABLE 2 | Incidence and hazard ratio of Parkinsonism for individuals with and without flunarizine among migraine patients.

	Flunarizine						Crude HR (95% CI)	Adjusted HR[a] (95% CI)
	No			Yes				
	Event	PY	Rate[#]	Event	PY	Rate[#]		
All	38	19780	1.92	166	19030	8.72	4.52(3.18, 6.43)***	4.07(2.84, 5.85)***
Age								
<45	3	5267	0.57	9	5211	1.73	3.05(0.83, 11.3)	1.70(0.44, 6.50)
45-64	12	9703	1.24	44	9502	4.63	3.74(1.98, 7.08)***	3.18(1.63, 6.20)***
≥65	23	4810	4.78	113	4317	26.2	5.39(3.44, 8.44)***	4.89(3.09, 7.72)***
Gender								
Women	24	14596	1.64	108	14172	7.62	4.62(2.97, 7.19)***	4.24(2.69, 6.68)***
Men	14	5184	2.70	58	4858	11.9	4.37(2.44, 7.84)***	3.89(2.14, 7.09)***
Comorbidity[§]								
No	4	5111	0.78	2	1851	1.08	1.42(0.26, 7.75)	1.95(0.35, 10.8)
Yes	34	14669	2.32	164	17179	9.55	4.12(2.85, 5.96)***	4.54(3.14, 6.57)***

CI, confidence interval; HR, hazard ratio; PY, person-years; cHR, crude hazard ratio; aHR, adjusted hazard ratio.
[a]Adjusting for age, gender, comorbidity of hypertension, diabetes, hyperlipidemia, sleep disorder, anxiety, and depression.
[#]Rate, incidence rate per 1000 person-years.
[§]Patients with any one of the comorbidities hypertension, diabetes, hyperlipidemia, sleep disorder, anxiety, and depression as the comorbidity group.

TABLE 3 | Incidence and adjusted hazard ratio of parkinsonism stratified by duration, average dose, average defined daily dose, and cumulative defined daily dose of flunarizine therapy per year in migraine patients.

Medication exposed	N	Event	Person-year	Rate	aHR (95% CI)[a]
Fz[#]	3235	38	19780	1.92	1.00
Non- Fz					
<60 days	1660	27	12046	2.24	1.12(0.68, 1.85)
≥60 days	1575	139	6984	19.9	8.49(5.86, 12.3)***
<445 mg	1616	27	11617	2.32	1.21(0.73, 1.99)
≥445 mg	1619	139	7413	18.8	7.69(5.31, 11.1)***
<45 DDD	1630	28	11715	2.39	1.24(0.76, 2.04)
≥45 DDD	1605	138	7315	18.9	7.77(5.36, 11.3)***
<200 cDDD	1661	66	8683	7.60	3.75(2.49, 5.64)***
≥200 cDDD	1574	100	10348	9.66	4.32(2.94, 6.33)***

CI, confidence interval; Fz, Flunarizine; DDD, defined daily dose; cDDD, cumulative defined daily dose.
[#]The average use day and average dose are partitioned in to 2 segments by median.
[a]Adjusting for age, gender, comorbidity of hypertension, diabetes, hyperlipidemia, sleep disorder, anxiety, and depression.
**p < 0.05, **p < 0.01, ***p < 0.001.*

CI = 2.94-6.33). Cumulative incidences of parkinsonism in the two cohorts are shown in **Figure 2**. The curve of the Fz cohort was significantly higher than that of the control cohort, and the p-value of log-rank test was <0.001.

DISCUSSION

Fz, with molecular formula C26H26F2N2, is a difluorinated piperazine derivative. It is highly lipophilic, crosses the blood-brain barrier, and is found in higher concentrations in the tissue than in the blood (Leone et al., 1991). Pharmacodynamic studies have shown selective blocking of the entry of calcium into the cells in situations where calcium is stimulated to enter the cell in excess (Holmes et al., 1984). With respect to the possible mechanism of FIP, in addition to the more D2 receptor blocker, loss of tyrosine hydroxylase in the monoaminergic presynaptic neuron may lead to dopamine deletion and cause movement disorders (Negrotti and Calzetti, 1997; Fabiani et al., 2004; Lin et al., 2017).

To our knowledge, this is the first population-based study to investigate the risk of FIP within a 13-year follow-up time in patients with migraine. Our study showed an incidence rate of 8.72% and 1.92% for parkinsonism in the Fz-treated and control groups, respectively. In another study, the incidence rate was 2.9% due to only 3-year follow-up time. For the Fz-treated group, the adjusted HRs were 4.07 and 5.12 (95% CI: 2.84–5.85 and 3.76–6.97) in our migraine subgroup study and another general population-base group, respectively (Lin et al., 2017). There are facts that midlife migraine patients, particularly migraine with aura, have higher risk of PD or parkinsonism in old age more than twice in comparison to people without migraine (Scher et al., 2014). The incidence rate of FIP in the migraine group seems higher then general people. So physicians must look carefully for early signs of PD or parkinsonism in migraine patients treated by Fz.

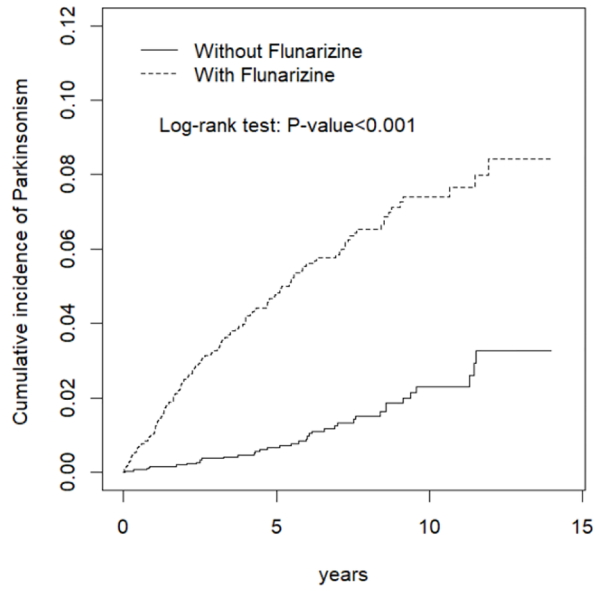

FIGURE 2 | Kaplan-Meir method determined cumulative incidence of parkinsonism compared between Flunarizine cohort and comparisons without Flunarizine.

The higher risk of parkinsonism among the Fz-treated patients was independent of age and comorbidities. The increased incidence rates were 1.73, 4.63, and 26.2 per 1,000 person-years and the adjusted HRs were 1.70, 3.18, and 4.89 (95% CI: 0.44–6.50, 1.63–6.20 and 3.09–7.72) of FIP in young (20–44-year old), middle-aged (45–64-year old), and old (≥65-year old) subjects, respectively. The Fz-treated group with comorbidities had higher incidence (9.55%) and significantly higher adjusted HRs (4.54, 95% CI: 3.14-6.57). Lin et al. reported that elderly, diabetic, or stroke patients had a higher risk. Liang et al. concluded that newly diagnosed type 2 diabetes patients had higher risk of FIP (Lin et al., 2017; Liang et al., 2018). Our results are similar to these previous population-based reports.

Previous studies ever proposed women had higher risk of FIP (Micheli et al., 1987; Teive et al., 2004). Female sex is considered a risk factor for DIP because estrogen can suppress the expression of dopamine receptors (Bedard et al., 1977; Shin and Chung, 2012). But in our study, both male and female patients with Fz treatment showed had a higher tendency to develop parkinsonism compared to the control subjects.

Our study indicates that an average annual Fz cumulative dosage of >445 mg indicates the greatest risk of future parkinsonism. Fz used for >60 days or ≥45 DDD is a cut-off point for predicting future parkinsonism. At the same time, the adjusted HRs changes from 3.75 (95%CI = 2.49–5.64) up to 4.32 (95%CI = 2.94–6.33) in cDDD from <200 to ≥200. Three studies have reported similar results. Liang et al. found that the odds ratio (OR) was 1.77 for patients who used Fz for <1 month, and the OR was up to 7.03 when the exposure period was >3 months. The cumulative dose of Fz also had a linear dose-response effect (Liang et al., 2018). Jhang et al. found that the OR was 3.80 (95% CI: 2.61–5.52) if the cumulative defined daily dose (cDDD) was ≥ 87.75/day. The optimal value of cDDD to predict movement disorders was 58.5 (sensitivity: 0.67, specificity: 0.60), indicating an overall exposure of 585 mg (Jhang et al., 2019). Kim et al. also concluded that Fz had a significant association with increased risk of FIP, depending on the cumulative dose (Kim et al., 2019). Our research provides safety advice of Fz for migraine patients.

Migraine patients are the main users of Fz. Fz is not available in the United States of America but is widely used in Europe as the first-line preventive measure for migraine (Pringsheim et al., 2012; Silberstein et al., 2012; Charles, 2017). One retrospective cohort study by Karsan, N. et al. on 200 migraine patients treated with Fz stated that Fz is generally effective, with only 24% (n = 47) of the patients reporting no clinical effect. The most common dose used was 10 mg per day. Information on treatment duration was available for 39% (n = 78) of the patients. Of these patients, 64% (n = 50) continued treatment for > 1 year. Doses up to 15 mg were generally well tolerated, with only 10.5% (n = 21) of the patients discontinuing treatment because of adverse effects. The most common adverse effects were fatigue (18%), mood change (17%), and weight gain (16%); other less common side effects included tremor (4.5%), dizziness (4%), constipation (2.5%), and nausea (2%) (Karsan et al., 2018). Physicians should carefully weigh the efficacy and adverse effects of Fz when the drug is recommended for long-term use in migraine patients.

This study has certain limitations that should be considered while interpreting the results. First, the NHIRD does not contain detailed information regarding smoking habits, alcohol consumption, socioeconomic status, diet, inactivity, or family history, despite these factors being potential risk factors for parkinsonism. Changes in the lifestyle and diet may affect the results. Second, although the secondary database research lacked clinical information, such as the history, neurological evaluation, clinical course and imaging, some patients may have been wrongly classified. As far as possible to eliminate this limitation, we excluded migraine subjects before Fz treatment with history including PD (ICD-9-CM 332), parkinsonism (ICD-9-CM 333), stroke (ICD-9-CM 430-438), dementia (ICD-9-CM 290, 294.1, and 331.0), head injury (ICD-9-CM 850-854, 959.01), and hydrocephalus (ICD-9 331.3-331.5). After Fz treatment for more than 1 month, we included subjects with PD and parkinsonism. Third, all the data in the NHIRD are anonymous; therefore, relevant clinical variables, such as body mass index, imaging results, and serum laboratory data were unavailable for the study subjects. However, data related to Fz and parkinsonism diagnosis were highly reliable.

In conclusion, Fz is frequently prescribed and is effective for migraine patients. However, FIP is associated with older age, history of comorbidities, a high-dose exposure, and longer exposure duration. Physicians should be aware of the neurogenic adverse effects, especially when the drug is used continuously for >60 days.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Research Ethics Committee (REC) II of China Medical University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WL, C-LL, and C-YW participated in the design of the study. C-LL was involved in collecting data and producing tables. WL, C-LL, and C-YW produced the initial draft of the manuscript that was further revised by CH and C-YW. All co-authors reviewed and approved the final version of the manuscript.

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A Proteotranscriptomic-Based Computational Drug-Repositioning Method for Alzheimer's Disease

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Numerous clinical trials of drug candidates for Alzheimer's disease (AD) have failed, and computational drug repositioning approaches using omics data have been proposed as effective alternative approaches to the discovery of drug candidates. However, little multi-omics data is available for AD, due to limited availability of brain tissues. Even if omics data exist, systematic drug repurposing study for AD has suffered from lack of big data, insufficient clinical information, and difficulty in data integration on account of sample heterogeneity derived from poor diagnosis or shortage of qualified post-mortem tissue. In this study, we developed a proteotranscriptomic-based computational drug repositioning method named Drug Repositioning Perturbation Score/Class (DRPS/C) based on inverse associations between disease- and drug-induced gene and protein perturbation patterns, incorporating pharmacogenomic knowledge. We constructed a Drug-induced Gene Perturbation Signature Database (DGPSD) comprised of 61,019 gene signatures perturbed by 1,520 drugs from the Connectivity Map (CMap) and the L1000 CMap. Drugs were classified into three DRPCs (High, Intermediate, and Low) according to DRPSs that were calculated using drug- and disease-induced gene perturbation signatures from DGPSD and The Cancer Genome Atlas (TCGA), respectively. The DRPS/C method was evaluated using the area under the ROC curve, with a prescribed drug list from TCGA as the gold standard. Glioblastoma had the highest AUC. To predict anti-AD drugs, DRPS were calculated using DGPSD and AD-induced gene/protein perturbation signatures generated from RNA-seq, microarray and proteomic datasets in the Synapse database, and the drugs were classified into DRPCs. We predicted 31 potential anti-AD drug candidates commonly belonged to high DRPCs of transcriptomic and proteomic signatures. Of these, four drugs classified into the nervous system group of Anatomical Therapeutic Chemical (ATC) system are voltage-gated sodium channel blockers (bupivacaine, topiramate) and monamine oxidase inhibitors (selegiline, iproniazid), and their mechanism of action was inferred from a potential anti-AD drug perspective. Our approach suggests a shortcut to discover new efficacy of drugs for AD.

Keywords: drug repositioning, Alzheimer disease, proteotranscriptomics, transcriptomics, proteomics, computational drug repositioning, drug discovery, system based approach

INTRODUCTION

AD is the most common type of dementia, and is characterized by progressive declines in memory and cognition. The prevalence of AD is increasing rapidly as population ages. There are currently approximately 50 million people worldwide with dementia, and the cost of treating and caring for people with dementia is estimated to be about US$1 trillion per year (Patterson, 2018). Although the precise cause of AD is still unclear, the disease is characterized by the presence of amyloid plaques comprised of beta-amyloid (Aß) and neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau in the brain. Most drugs under development target these two pathological hallmarks. However, the success rate of newly-developed AD drugs has been very low, about 0.4%, and there have been hundreds of failures of clinical trials (Cummings and Science, 2018). Of all the potential drugs developed for the treatment of AD, only drugs such as cholinesterase inhibitors and memantine have been approved by the U.S. Food and Drug Administration (FDA) to relieve some of the symptoms of the disease. Given the impact of AD, it is therefore important to explore new drug development strategies for this condition.

Numerous drug repositioning methods have been suggested to repurpose already-approved drugs, and several compounds have been identified as innovative approaches to different diseases. Drugs that have been repositioned have undergone clinical trials, and so have confirmed pharmacokinetics, pharmacodynamics, and well-understood toxicity mechanisms, and have been approved by the U.S. FDA. Drug repositioning takes advantage of the reduced toxicity, side effects, and costs of clinical trials. Many computational drug repositioning methods based on transcriptomic data have been developed to identify potential new indications for drugs. Each method has applied techniques such as comparison of gene expression profiles between a disease model and the drug-treated condition (Chen et al., 2017), network integration (Luo et al., 2017), prediction of drug-protein interactions (Yang and Agarwal, 2011), and utilization of genotype-phenotype associations (Zhang et al., 2016). Systematic computational drug repositioning methods using large transcriptomic datasets perturbed by drugs have been developed (Dudley et al., 2011), and many promising drug candidates have been identified for diverse diseases (Végner et al., 2013; Zerbini et al., 2014). To assist in this endeavour, CMap (Lamb et al., 2006) and L1000 of the Integrated Network-based Cellular Signatures (LINCS) project (Subramanian et al., 2017) have been widely used. The CMap database was first released in 2006 and consisted of data relating to 564 gene expression signatures as perturbed by 164 bioactive small molecules. In 2010, the NIH LINCS consortium launched L1000, a database comprising approximately one million gene expression profiles of human cell lines as perturbed by about 15,000 drugs or small molecules. TCGA is the largest public data set related to human cancer genomes, and consists of multi-omics data generated by RNA-seq, copy-number variation analysis, genomic mutation, and DNA methylation, generated from 11,000 patients across 33 tumor types, together with relevant clinical information, including list of prescribed drugs (Nagaraj et al., 2018). Several studies developed and validated their methods based on anti-correlation between disease- and drug-induced gene expression profiles from these datasets. (Chen et al., 2017; Srivastava et al., 2018).

Most computational drug repositioning methods have been developed for a few diseases, such as cancers, since there are considerable amounts of gene and protein expression data available for these diseases, with clinical and pharmacological information, in databases such as TCGA. In the case of AD, little multi-omics data with clinical information have been produced, due to limitations in tissue availability from patients with clear clinical diagnoses. There have been several studies on the relationship between cancer and neurodegenerative diseases including AD, Parkinson's disease (PD), and Huntington's disease. Epidemiological studies have reported an inverse association between neurodegeneration and cancer, in that individuals with neurodegenerative diseases appear to have a lower risk of developing cancer and vice versa (Catalá-López et al., 2014; Seddighi et al., 2019). In addition, ageing-associated transcriptomic alterations are similar to those observed in neurodegeneration, but are opposite to those observed in cancer (Irizar et al., 2018). The expression of several genes that contribute to cell growth and proliferation is increased in cancer and decreased in AD (Shafi, 2016). There has, however, been some evidence of a positive association between AD and cancer. There appears to be a positive correlation between the mortality rates in AD and Glioma (Lehrer, 2018). This observation suggests a role of gene expression regulators in the shared genetic etiology between AD and cancer, and implies that some shared variants modulate disease risk. Increasing evidence suggest that there are common pathophysiological features in both diseases, such as DNA damage, oxidative stress, mitochondrial dysfunction, metabolic dysregulation, and inflammation (Driver, 2014; Houck et al., 2018). Moreover, single nucleotide polymorphism (SNP)-trait genome-wide association studies (GWAS) have shown positive genetic correlations between AD and cancer (Feng et al., 2017). Although the relationship between AD and cancer remains controversial, the analysis of large cancer multi-omics datasets and associated clinical information should provide insights into developing new drugs for AD.

In this study, we developed a new DRPS and new DRPC based on pharmacogenomic knowledge, along with the information that disease- and drug-induced gene and protein expression signatures have an inverse association. We first standardized drug names by PubChem compound identifier (CID) (Cheng et al., 2014b). Then we constructed a DGPSD comprised of 61,019 Drug-induced Gene Perturbation

Abbreviation: DRPS, Drug Repositioning Perturbation Score; DRPC, Drug Repositioning Perturbation Class; DGPSD, Drug-induced Gene Perturbation Signature Database; CGPS, Cancer-induced Gene Perturbation Signatures; DGPS, Drug-induced Gene Perturbation Signature; AGPS, AD-induced Gene Perturbation Signature; APPS, AD-induced Protein Perturbation Signature.

Signatures (DGPSs) generated by 1,520 compounds in 26 cell lines collected from CMap and L1000. DRPS was calculated using nine Cancer-induced Gene Perturbation Signatures (CGPSs) from 4,948 cancer and normal profiles (BRCA, UCEC, KIRC, LUAD, LUSC, COAD, STAD, CESC, and GBM) perturbed by 152 drugs, using data from TCGA, and each drug was classified into one of three DRPC (high, intermediate, low) by DRPS. The DRPS/C method was validated by calculating the AUC of each DRPC using DRPS as an input, and the prescribed drug list with CID as the gold standard. Glioblastoma (GBM) was found to have the highest AUC (0.708). Since GBM shared gene expression patterns and related pathways with AD, we applied the DRPS/C method to the prediction of anti-AD drugs using multi-omics datasets from AD patients. Two AD-induced Gene Perturbation Signature (AGPS) and one AD-induced Protein Perturbation Signature (APPS) were calculated from 159 RNA-seq, 108 microarray, and 17 proteomic datasets, respectively. We predicted 31 potential anti-AD drug candidates belonging to the intersection of high DRPCs that were calculated from AGPS and APPS. Of these, the mechanism of action of the drugs belonging to the nervous system class of ATC system was inferred from a potential anti-AD drug perspective. Our DRPS/C method may provide a shortcut to discover new efficacy of drugs for AD.

MATERIALS AND METHODS

Standardization of Compound Names Based on PubChem Identifiers

When we investigated the collected compound lists that contained various nomenclature problems including uncertain naming, spelling errors, and the use of diverse synonymous. To solve these problems, we conducted cleaning and standardization of 1,858 compound names using CID of PubChem as follows. First, we selected 312 compounds that had the compound ("trt-cp") or controls-vehicle ("ctl_vehicle") perturbation type from LINCS level 3 data (GSE92742) (**Supplementary Table 3**). 159 and 1,387 compounds were extracted from the prescribed drug list of TCGA and CMap compounds list, respectively. Next, we converted compound names to CID using the PUG REST service provided by PubChem. For the un-mapped terms, we performed standardization of compound names into CIDs with human curation. Finally, we collected 1,608 compound names with CIDs (**Supplementary Figure 1**).

Drug-Induced Gene Perturbation Signature Database (DGPSD)

Build02 (2009) of the CMap data was downloaded (https://portals.broadinstitute.org/cmap/index.jsp) and processed. We normalized the data with the MAS and quantile method using the affy R package (version 1.58.0). LINCS level 3 data were downloaded from the Gene Expression Omnibus (GSE92742). We selected 61,019 gene expression profiles from CMap (5,819) and L1000 (55,200), that have treated compounds with associated CID identifiers from our drug-CID mapping table (**Supplementary Figures 2A** and **3A**). In the case of the same experimental conditions (compound, treatment time, dosing, and cell line), we adopted the average of each gene expression value as a representative value of an experiment. To obtain DGPSs, the perturbed gene expression profiles induced by drugs, we calculated the log2 fold change in gene expression for each control versus compound-dosing-time experimental condition within the same cell line. To standardize the gene identifiers, all gene identifiers were converted to Ensembl gene IDs, using the BioMart R package (version 3.7).

Analysis of Omics Expression Signatures

We downloaded prescribed drugs (40 types), patient information, prescribed drug list and normalized RNA-seq gene expression profiles from the TCGA (https://portal.gdc.cancer.gov, May, 2018). From these, we selected 4,948 gene expression data of nine cancer types (BRCA, GBM, CESC, COAD, KIRC, LUAD, UCEC, LUSC, STAD), which satisfied three conditions: (1) A cancer type dataset contained more than three normal samples with sample code 10 (solid tissue normal) or 13 (EBV immortalized normal); (2) the dataset included more than ten prescribed drugs; (3) It had one or more shared drugs from the drug lists from TCGA and CMap or L1000. In case of AD, 159 RNA-seq, 108 microarray, and 17 LC-MS/MS datasets were collected from Synapse (syn8690904), ArrayExpress (E-TABM-185), and PRIDE Archive (PXD006122, 4/6/2018), respectively (**Supplementary Data 4**).

The RNA-seq data were analyzed using Generalized linear models (GLM) in cancer versus unpaired normal samples (adjusted p-value < 0.05) using the R package EdgeR (release 3.7). All gene identifiers were transformed into Ensembl gene IDs using the BioMart R package (version 3.7) (**Supplementary Figures 2B**, **4A, B**, and **Supplementary Data 1**). Microarray data were normalized using the Robust Multi-array Average (RMA) algorithm and 817 differentially expressed genes were identified using t-test with a false discovery rate (FDR) correction (q-value < 0.05). The probe sets were summarized to Ensemble gene symbol using the HT HG-U133 database (version 3.7) and BioMart R package (version 3.7). The raw mass spectrometry data of AD human brain proteomics datasets were processed using Proteome Discoverer (Thermo Scientific, version 2.2) with a Uniprot human database (2017_08). Searches were performed using a 10 ppm precursor tolerance, and 0.05 Da fragment tolerance. Two missed cleavages were accepted. TMT tags on lysine residues and peptide N-termini (+ 229.162932 Da) and carbamidomethylation of cysteine (+ 57.02146 Da) were set as static modifications, while oxidation of methionine (+ 15.99492 Da) was set as a variable modification. Results were filtered to a 1% FDR at the peptide and protein levels. We normalized the quantitative proteome data using the VSN package (version 3.50.0) and performed t-test (p-value ≤ 0.05). Finally, we generated APPS based on 175 DEPs (**Supplementary Figures 4C, D**, and **Supplementary Data 2**). To comparison of biological characterization between AD and cancer, we performed pathway enrichment and PPI network analysis using GSEA v3.0 (MSigDB version 6.2, permutation method: 1,000 gene set) and STRING database (v10.5; confidence score of ≥ 0.8).

Drug Repositioning Perturbation Score/Class

To calculate DRPS, we undertook the following analysis, First, for every gene k in each drug or disease gene expression profile perturbed by drug j, or disease d, we calculated log2 fold change (F_{drg_k}; F_{dg_k}) between drug-treated (E_{kj}) and control (E_{kc}); disease (E_{kd}) and normal(E_{kn}) gene expression profile, shown as Eq. 1 (Detail descriptions of the symbols in **Supplementary Table 1**).

$$F_{drg_k} = \log_2\left(\frac{E_{kj}}{E_{kc}}\right), F_{dg_k} = \log_2\left(\frac{E_{kd}}{E_{kn}}\right) \quad (1)$$

Second, we identified differentially expressed genes in the intersection of DGPS and C/AGPSs Third, if gene k had an inverse signature expression pattern ($F_{drg_k} > 0$ & $F_{dg_k} < 0$, $F_{drg_k} < 0$ & $F_{dg_k} > 0$) between DGPS j and CGPS or AGPS d, we compute a Perturbation Score (PS) of gene k as follow Eq2.

$$PS_{g_k} = |F_{dg_k}| - |F_{drg_k}| \quad (2)$$

To assign a weighted value to an influential pharmacogene using pharmacogenomic knowledge, we downloaded data from 11,922 pharmacogenes, including target, enzyme, transporter, and carrier from Drugbank (version 5.1.1; (Wishart et al., 2018) and defined these genes as our "PharmacoGene List" (PGL). We extracted pharmacogene (pg)s from DGPS j, or CGPS or AGPS d based on PGL (Eq.3) and computed log2 fold change (F_{drg_k}/F_{dg_k}) for every pg i as follow Eq.4. E_{ij} and E_{ic} is the gene expression value of pg i in drug-treated (j) and control(c) gene expression profiles. E_{id} and E_{in} are the gene expression value of pg i in disease(d) and normal(n) gene expression profiles.

$$PG = \{g_k \in PGL | pg_1 \ldots pg_i\} \quad (3)$$

$$F_{drpg_i} = \log_2\left(\frac{E_{ij}}{E_{ic}}\right), F_{dpg_i} = \log_2\left(\frac{E_{id}}{E_{in}}\right) \quad (4)$$

The PS of pg i was calculated in the same manner ($F_{drpg_i} > 0$ & $F_{dpg_i} < 0$, $F_{drpg_i} < 0$ & $F_{dpg_i} > 0$) as that of gene k (Eq.5).

$$PS_{pg_i} = |F_{dpg_i}| - |F_{drpg_i}| \quad (5)$$

We calculated the DRPS of drug j ($DRPS_{drug_j}$) as follows (Eq. 6). If drug j had multiple experimental conditions (dosing, time), we selected the maximum score among the DRPSs calculated from several experiment conditions (e). n and m are the total number of genes and pharmacogenes in the gene expression profile of DGPS j and CGPS or AGPS d.

$$DRPS_{drug_j} = \max_{e=1}^{h}\left[\frac{1}{n}\sum_{k=1}^{n} PS_{g_k} \times \frac{1}{m}\sum_{i=1}^{m} PS_{pg_i}\right] \quad (6)$$

After sorted drugs by DRPS in ascending order, we classified into three DRPC ("high", "intermediate", "low") based on DRPS.

RESULTS

Compound Label Standardization and DGPSD Construction

We collected 74,171 gene expression profiles perturbed by 2,021 compounds from CMap and L1000 to construct DGPSD. The DGPSD contained 15,137 and 14,123 genes from CMap and L1000, respectively. We standardized 1,858 compound labels from CMap (1,387), L1000 (312) and TCGA (159) based on the CID of PubChem, using the PUG REST service and human curation (**Supplementary Figure 1**). We selected gene expression profiles according to predefined criteria including drug name and CID. All gene expression profiles were converted into DGPS by calculating the log2-ratios of expression values between control and compound treated samples. The final DGPSD was made up 61,019 DGPS perturbed by 1,520 compounds in 26 cell lines (**Figure 1**; **Supplementary Figures 2A**, **3**).

DRPS/C Method Development and Validation

In order to generate CGPS, we selected gene expression profiles, meeting the criteria: the associated clinical data must include at least 20 kinds of prescribed drugs, and gene expression profiles should have one or more normal data sets. We computed nine CGPS through statistical analysis (Generalized linear models; adjusted P-value < 0.05; release 3.7) using 4,948 cancer and normal gene expression profiles perturbed by 152 drugs in nine cancer types (BRCA, UCEC, KIRC, LUAD, LUSC, COAD, STAD, CESC, and GBM) (**Supplementary Figure 2B**). Each CGPS included between 1221 and 4502 differential expressed genes (**Supplementary Data 1**, **Supplementary Table 2**, and **Supplementary Figure 4B**). We computed DRPS using DGPSD and nine CGPS. (**Supplementary Data 3**). The DRPS is a score that weights the pharmacogenomic knowledge supporting the value that measures an inverse association between each DGPS and CGPS.A higher DRPS means that the drug has not merely a higher inverse signature expression pattern between DGPS and CGPS, but also many influential pharmacogenes were perturbed. To select optimal drugs based on gene/protein expression data, we classified drugs based on DRPS and DRPC. To evaluate the performance of our method, we calculated the area under the ROC curve (AUC) of each DRPC for each cancer type using predicted repositioning candidate drugs ordered descending by DRPS score and prescribed drugs with CID from TCGA as a gold standard (**Figure 2**). The results show that the all AUCs in the nine cancer types were ordered as high, intermediate, and low class consistently. Based on these results, we assessed that the DRPS methods robustly predicts drugs based on inverse signature expression pattern. The highest AUC (0.708) was observed for GBM in the high class. We concluded that our DRPS is more valuable in drug repositioning analysis using brain gene expression data than when using data from other organs.

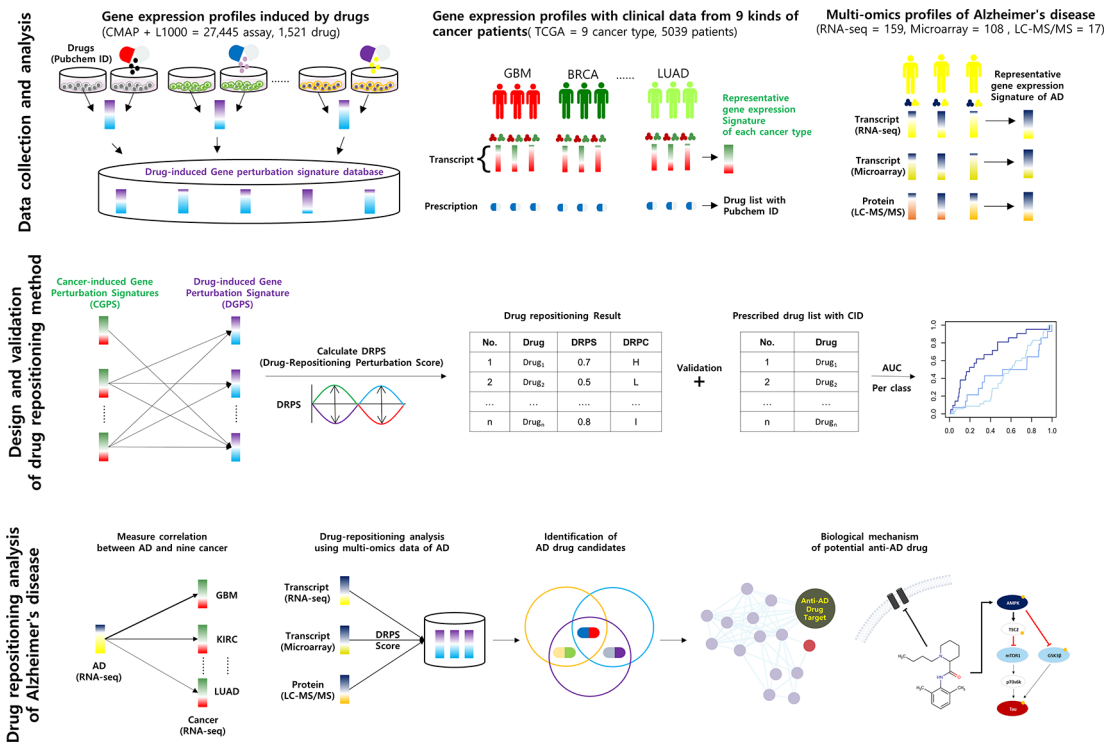

FIGURE 1 | Schematic of the calculation of DRPS based on the inverse association between disease- and drug-induced transcript/protein perturbation signatures Higher DRPS means that the drug has not only a higher contrary correlation between drug-induced and cancer multi-omics signatures but also many influential pharmacogenes with high perturbation.

Interrelation Between Cancer and AD

To estimate the possibility of applying our method to AD drug repositioning, the relationship between cancer and AD was investigated at the transcription level. We downloaded 159 gene expression profiles generated by RNA-seq of AD brain tissues (AD: 88, Normal: 77) from the synapse portal, and computed AGPS comprised of 9,603 differentially expressed genes (DEGs). We measured the rate of shared DEGs between AGPS and CGPS per fold change (**Figure 3A**). GBM had the lowest reduction degree of shared DEG rates according to increasing fold change. These results indicate that AD and GBM share highly perturbed DEGs. We also calculated the rate of genes with same expression direction (overexpressed or underexpressed) in AGPS and CGPS. GBM (0.49) had highest similarity with AD. CESC (0.47) and STAD (0.46) followed (**Figure 3B**, **Supplementary Figure 5**). To assess whether AD and the nine cancer types share similar biological processes, we compared the significantly enriched pathways between AGPS and CGPS using the KEGG pathway gene sets in MSigDB (ver. 6.2) and GSEA v3.0. KIRC and GBM had the largest number of shared pathways with AD. *JAK-STAT* signaling pathway (map04630) and cytokine-cytokine receptor interaction (map04060) that were involved in long-term memory (Copf et al., 2011) were shared only in GBM and KIRC with AD (**Figure 3C**). In comparison of shared genes between CGPS with AD-related genes from Ingenuity Pathway Analysis (IPA) (Krämer et al., 2013), the GBM had the highest number of shared genes with AD (**Figure 3D**). PPI network analysis was also performed using the shared genes as an input for STRING, and the shared genes were linked with neurotransmitter receptors such as the glutamate, cholinergic receptor, and gamma-aminobutyric acid receptors (**Supplementary Figure 6**). Taken together, GBM showed a consistent strong correlation with AD.

Drug Repositioning Analysis for AD Drug Discovery

To identify novel anti-AD drug candidates using DRPS/C, we further downloaded 108 and 17 gene expression profiles of brain tissues from AD patients generated by microarray, and LC-MS/MS from ArrayExpress database and PRIDE archive. The microarray data consisted of 22 AD and 86 normal samples, respectively. We computed microarray and RNA-seq AGPSs composed of 817 and 9,603 DEGs. Proteomic data included samples of 9 AD and 8 normal, and we generated AD-induced Protein APPS using 175 differentially expressed proteins (DEPs) (**Supplementary Data 2**). We then calculated the DRPS for each drug using 3 kind version of gene/protein expression signature AGPS (**Supplementary Data 4**). We found that 1,047 drugs were at least once ranked as high class (**Supplementary Data 3**). Among these drugs, 492 drugs had ATC code (ver. 2018). The

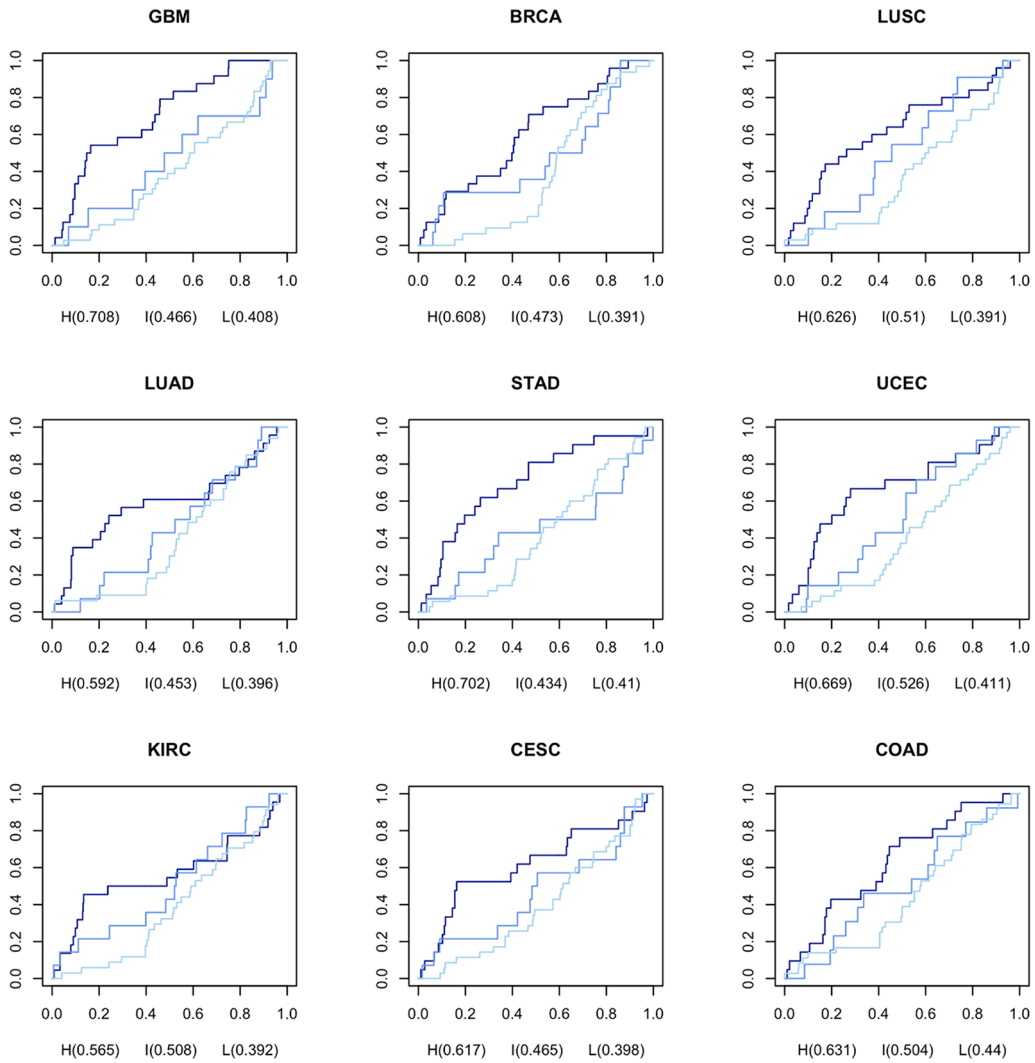

FIGURE 2 | AUC for DRPS of each drug per DRPC using prescribed drugs as gold standard from TCGA The navy, medium blue, and light blue lines represent high, intermediate, and low classes in DRPC, respectively.

most frequent drug class was C (Cardiovascular system, 98 drugs) and N (Nervous system, 64 drugs) (**Figure 4A**). We then selected 31 anti-AD drug candidates that were satisfied with the following criteria (**Supplementary Table 4**): 1) The drugs belonging to the intersection of high DRPCs from transcriptomic and proteomic data. 2) the drugs without low DRPC.

Of these, four drugs belonging to the ATC N class are bupivacaine, topiramate, selegiline and iproniazid (**Figure 4B**). We investigated the binding partners of bupivacaine target using AD-related genes from IPA and PPI relations from the STRING database. *SCN10A* (Sodium channel protein type 10 subunit alpha), a target of bupivacaine, was linked with *MAPT* (tau) and *PSEN1* (presenilin1), which were associated with the pathological hallmarks of AD, *via SCN1A* (sodium channel protein type 1 subunit alpha), a target of topiramate (**Figure 4C**). Bupivacaine and topiramate may inhibit neuronal hyper-excitability in AD by blocking sodium channel (Sheets et al., 2010) (**Figure 5A**). In another way, bupivacaine may act on AMP-activated protein kinase (AMPK), and subsequently activate the downstream of AMPK (Huang et al., 2014). Selegiline and iproniazid are inhibitors of monamine oxidase inhibitors (MAO) that are known to be implicated in the AD pathology (Thomas, 2000; Huang et al., 2012; Quartey et al., 2018) (**Figure 5B**). In this context, this approach can repurpose potential anti-AD drug candidates that may be further investigated.

DISCUSSION

Despite rapid increases in the prevalence of AD, therapeutic agents against AD have not yielded successful results in most clinical trials. Thus, treatment of AD urgently requires the development of novel, rationally designed therapeutic agents. Drug repositioning has attracted great interest, as it may lead to

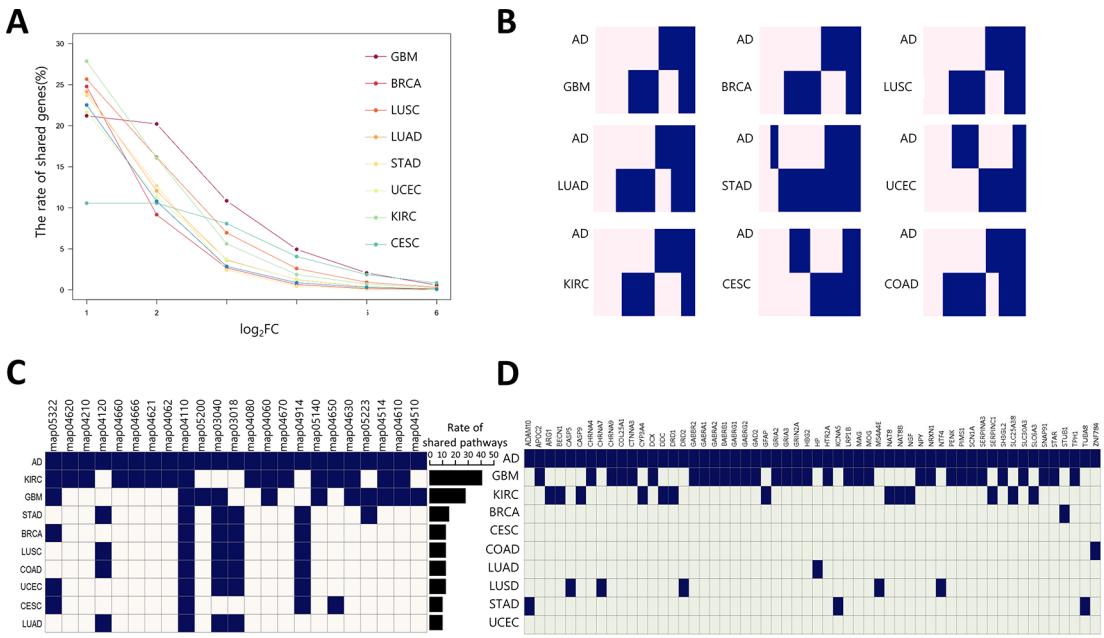

FIGURE 3 | Correlation of gene expression between AD and cancer types **(A)** The ratio of shared DEGs between AD and nine cancer per each fold-change. **(B)** Gene-expression pattern similarity of AD and nine cancers. The pink and blue colors represent over-expressed and under-expressed DEGs, respectively. Coderivative of AD-related pathways **(C)** and genes **(D)** between AD and nine cancers. A navy square denotes an AD-related pathway in each cancer type, and light beige and light green indicates the opposite. The bar chart indicates rate of shared pathways between AD and each cancer type. KEGG pathway number description as follows: map05322, Systemic lupus erythematosus; map04620, Toll-like receptor signaling pathway; map04210, Apoptosis; map04120, Ubiquitin mediated proteolysis; map04660, T cell receptor signaling pathway; map04666, Fc gamma R-mediated phagocytosis; map04062, Chemokine signaling pathway; map04110, Cell cycle; map05200, Pathways in cancer; map03040, Spliceosome; map03018, RNA degradation; map04080, Neuroactive ligand-receptor interaction; map04060, Cytokine-cytokine receptor interaction; map04670, Leukocyte transendothelial migration; map04914, Progesterone-mediated oocyte maturation; map05140, Leishmaniasis; map04650, Natural killer cell mediated cytotoxicity; map04630, *JAK-STAT* signaling pathway; map05223, Non-small cell lung cancer; map04514, Cell adhesion molecules (CAMs); map04610, Complement and coagulation cascades; map04510, Focal adhesion.

the discovery of novel drugs for diseases as well as reducing the risk of new drug development at the clinical stage. The commonly used computational drug repositioning methods started by searching for drugs that had an inverse association of gene expression pattern between disease and drugs. However, most approaches use transcriptomic data (Chen et al., 2017), and there have been few reports of a systematic drug repositioning method based on multi-omics data. Based on the inverse association, we developed a new method, DRPS/C, using public multi-omics data (transcriptomes and proteomes) incorporating pharmacogenomics knowledge. The DRPS/C method was successfully validated using a prescribed drug list in clinical data of cancer patients.

Since GBM outperforms the other cancer types by comparison of AUC values using the DRPS/C method, we further investigated gene expression pattern similarity, shared DEGs, and the related pathways between AD and GBM. GBM showed a consistent strong correlation with AD among nine cancer types. GBM is characterized by a high degree of cellular and molecular heterogeneity both among patients and within the same patient (Skaga et al., 2019). AD is also a heterogeneous disease that is classified into three clinical stages including the preclinical, mild cognitive impairment, and dementia (Jack et al., 2018), and its neuropathology is highly variable (Whitwell et al., 2012). For this reason, there might be far more complexities at the molecular level of these diseases. Advances in the diagnosis and single cell analysis as well as large scale multi-omics data for enough clinical samples may help investigation of the pathophysiological relationship between GBM and AD.

The most widely accepted theory to explain the pathogenic mechanism of AD is the amylod hypothesis, which states that the accumulation of Aß) leads to formation of amylod plaque and NFTs, ultimately, neuronal death (Selkoe and Hardy, 2016). Accumulating studies have shown various features in AD brain such as neuronal hyperexcitability, epileptic seizures, diminished glucose uptake, glutamate excitotoxicity, oxidative stress induced neurotoxicity, cholinergic hypofunction, metal dyshomeostasis, mitochondrial dysfunction, and neuroinflammation. Furthermore, these pathways are found to influence one another in the pathogenesis of AD (De Strooper and Karran, 2016).Although most current therapeutic approaches are focused on Aβ and hyperphosphophorylated tau, such complex features in AD have challenged the conventional paradigm in drug development. Among the anti-AD drug candidates predicted by using our methods, four drugs with the ATC nervous system code are voltage-gated sodium channel blockers (bupivacaine

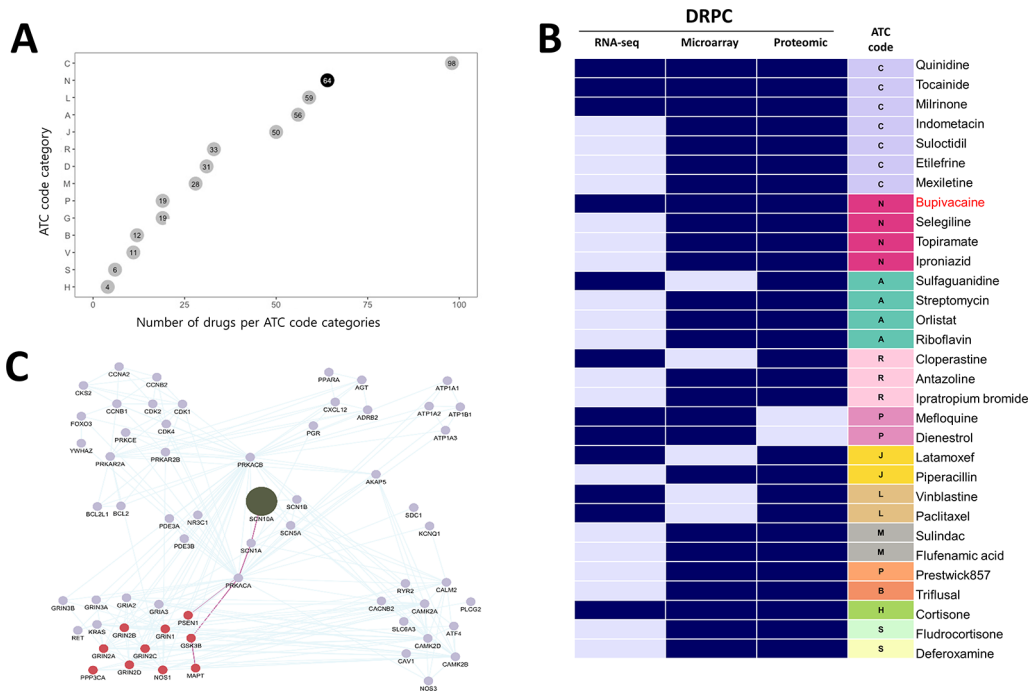

FIGURE 4 | Potential anti-AD drugs with mode of action **(A)** The number of drugs per ATC code categories. **(B)** The DRPC per multi-omics data type with ATC class. Navy and sky-blue represent "high" and "intermediate" DRPCs, respectively. **(C)** The PPI network of *SCN10A* (olive node), the target protein of bupivacaine. Burgundy nodes denote AD-related proteins. The edges highlighted in purple denote the connectivity from SCN10A to PSEN1 or MAPT proteins, which are associated with AD pathological hallmarks.

and topiramate) and MAO inhibitors (selegiline and iproniazid). According to the literature review, we inferred the mechanism of action of the drugs from a potential anti-AD drug perspective.

Bupivacaine, a FDA-approved local anesthetic, is known to block voltage-gated sodium channels by binding to SCN10A, inhibit *ionotropic glutamate receptors*, and activate AMPK (Lu et al., 2011). Topiramate, another sodium channel blocker to bind SCN1A, is approved to treat seizure disorders (Mantegazza et al., 2010). Topiramate has been known to modulate gamma-aminobutyric acid receptor subunit alpha-1(GABRA1) and glutamate receptors, and stimulate insulin-mediated glucose uptake by activation of AMPK (Caricilli et al., 2012). One of the characteristics of AD is neuronal hyper-excitability due to stimulated action potentials, which causes the loss of electrical signal transmission and ultimately neuronal death (Palop and Mucke, 2010). When increasing neuronal excitability, bupivacaine or topiramate may act on sodium channels to suppress neuronal action potentials (Sheets et al., 2010).

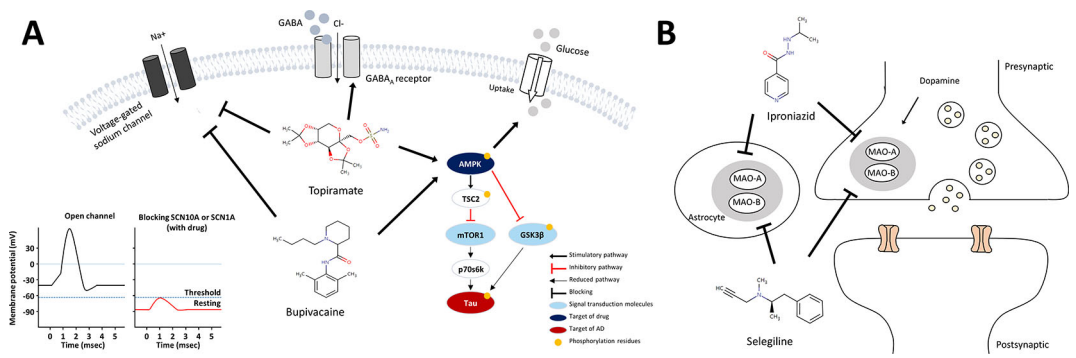

FIGURE 5 | Schematic models for mechanism of action of anti-AD drug candidates in relation to AD pathology. **(A)** mechanism of action of the sodium channel blockers, bupivacaine and topiramate **(B)** mechanism of action of the MAO inhibitors, selegiline and iproniazid.

Moreover, *SCN1A* connecting with *SCN10A* in the PPI network in **Figure 4C** is regulated by *BACE1*, the beta-site amyloid precursor protein cleaving enzyme for generation of Aβ peptides in AD. *PSEN1*, a component of γ-secretase producing Aβ, also mediates proteolytic cleavage of the voltage-gated sodium channel β-subunits (Kim et al., 2011). Glutamate receptor proteins, which are also related to neuronal excitability and affect synaptic plasticity *via JAK-STAT* signaling (**Figure 3C**), were indirectly linked with *SCN10A* and *SCN1A* (**Figure 4C**) (Nicolas et al., 2012). We thus inferred that bupivacaine or topiramate may prevent the neuronal cell damage in AD by regulating neuronal excitability.

In regards to AMPK activation, bupivacaine and topiramate might be associated with insulin-mediated glucose uptake (Caricilli et al., 2012) or tau phosphorylation through *AMPK*/*TSC2*/*mTOR1*/p70s6k pathway. Bupivacaine is known to activate *AMPK* along with T172 phosphorylation, and activated *AMPK* mediates the phosphorylation of S1387 in *TSC2* that initiates strong activation of the *AMPK*/*TSC2* pathway (Dibble et al., 2012; Huang et al., 2014). *mTOR1*, a central regulator of cell growth and metabolism, is inhibited by activated *AMPK*/*TSC2*. The mTOR-dependent *p70s6k* activity is also inhibited (Kickstein et al., 2010) and mediates *tau* phosphorylation, which is crucial in AD pathogenesis (Pei et al., 2006; Taga et al., 2011). Moreover, the activated *AMPK* inhibits activation of *GSK3β*, a major kinase of *tau* (Ryder et al., 2004; Horike et al., 2008). Combining all of these, we proposed the mechanism of action of bupivacaine and topiramate for the treatment of AD as shown in **Figure 5A**.

On the other hand, selegiline and iproniazid are inhibitors of MAO, a family of enzymes catalyzing the oxidation of monoamines. There are two types of MAO: MAO-A and MAO-B, and inhibition of MAO-A and MAO-B proteins increased dopamine in brain. Selegiline is used in the treatment of depression and early-stage Parkinson disease by modulation of dopaminergic transmission though blocking MAO-B (Finberg and Rabey, 2016). It is a selective irreversible MAO-B inhibitor in clinical doses, whereas it also inhibits MAO-A in larger doses (Fowler et al., 2015). Iproniazid, another MAO inhibitor, is used as an antidepressant drug (Yáñez et al., 2012). Several mechanisms have been proposed to account for involvement of MAO in AD pathology such as cognitive dysfunction *via* destroying cholinergic neurons and the formation of Aß aggregation or NFTs (Thomas, 2000; Huang et al., 2012; Mousseau and Baker, 2012; Cai, 2014; Quartey et al., 2018). This is in line with the recent study reporting that selegiline suppressed GABA production from reactive astrocytes, and restores the synaptic plasticity, and learning and memory function in the AD model mice (Park et al., 2019). Indeed, several studies showed beneficial effect of the MAO inhibitor, selegiline in AD (Tariot et al., 1987; Knoll et al., 1989; Sano et al., 1997), and dextroamphetamine, an inhibitor of MAO-A and MAO-B, is in Phase 4 clinical trial as a combination drug together with methylphenidate for AD treatment (Herrmann et al., 2008). Clinical trials have inherent limitations such that results can vary depending on patient population, dosage, duration of administration, and endpoint selection. Accordingly, there are still opportunities of applying these drugs for AD treatment.

However, for repositioning approved drugs in clinical trials, drug toxicity and unfavorable pharmacokinetics should be considered significant. Bupivacaine is primarily metabolized by the liver and should be used cautiously in patients with hepatic disease. There are also serious concerns about the systemic toxicity and cardiotoxicity of bupivacaine (El-Boghdadly et al., 2018). Topiramate is excreted predominantly in the urine as an unmetabolized drug and symptoms of overdose may cause vision problems, dehydration, metabolic acidosis, depression, encephalopathy, and kidney stones (Topiramate from Drugs.com, 2019). Selegiline is primarily metabolized by cytochrome P450 into L-desmethylselegiline, L-amphetamine, and L-methamphetamine in the liver and the intestines; they are excreted together with its metabolites in the urine and feces. However, amphetamine metabolites are also known to be associated with orthostatic hypotension and hallucinations (Romberg et al., 1995; Am et al., 2004). The side effects of selegiline include dizziness, insomnia, nausea, abdominal pain, skin rash, and weight loss (Selegiline from Drugs.com, 2019]. Iproniazid is a prominent mood stimulant for the treatment of debilitated individuals but was withdrawn from most markets because of its hepatotoxicity. The adverse effects of iproniazid also include dizziness, drowsiness, headaches, ataxia, numbness of the feet and hands, and muscular twitching (Lichtenstein and Mizenberg, 1954). Collectively, considering the pharmacokinetics and side effects of the repositioned drug candidates, further investigation of dose-dependent selectivity and interactions or development of specific drug moieties and targeted drug delivery systems must be undertaken.

We suggest expanding utilization of DRPS/C in diverse perspectives as follows. First, DRPS/C was designed for the easy addition of data from other biological signature including personal genomic variants by NGS, metabolomes, post-transcriptional translation, protein kinases, etc. If the other biological signatures are added, the anti-neuronal drugs predicted using our methods will be more reliable. Second, DRPS/C based on diverse biological signatures would be used in the strategic development of novel drug targets or biomarkers. Third, DRPS/C could be utilized in precision medicine. If we use a personal multi-omics expression profile instead of each disease multi-omics expression profile, our method will be able to suggest appropriate drugs for an individual.

In conclusion, DRPS/C method was developed to predict novel potential anti-neuronal drug candidates based on biological multi-omics signatures which reflected the inverse association and pharmacogenomics knowledge. Using the DRPS/C methods, we predicted potential anti-AD drug candidates including bupivacaine, topiramate selegiline, and iproniazid, and inferred their mechanism of action. Our approach suggests a shortcut to discover new drugs for AD. It may be also applicable to not only discovery of drug targets or biomarkers but also precision medicine.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.

cgi?acc=GSE92742, https://portals.broadinstitute.org/cmap/, https://portal.gdc.cancer.gov/, https://www.synapse.org/#!Synapse:syn17010685, https://www.ebi.ac.uk/arrayexpress/experiments/E-TABM-185/, and https://www.ebi.ac.uk/pride/archive/projects/PXD006122.

AUTHOR CONTRIBUTIONS

SL, M-YS, and YK designed the study. SL developed the method algorithm. SL and M-YS performed omics data analyses. DK, CP, DP, and DGK participated in the construction of database, collection of dataset, and standardization of drug names. SL, M-YS, JY, and YK contributed to data interpretation and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01653/full#supplementary-material.

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Genetic Diversity of *HLA* Class I and Class II Alleles in Thai Populations: Contribution to Genotype-Guided Therapeutics

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Human leukocyte antigen (HLA) class I and II are known to have association with severe cutaneous adverse reactions (SCARs) when exposing to certain drug treatment. Due to genetic differences at population level, drug hypersensitivity reactions are varied, and thus common pharmacogenetics markers for one country might be different from another country, for instance, *HLA-A*31:01* is associated with carbamazepine (CBZ)-induced SCARs in European and Japanese while *HLA-B*15:02* is associated with CBZ-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) among Taiwanese and Southeast Asian. Such differences pose a major challenge to prevent drug hypersensitivity when pharmacogenetics cannot be ubiquitously and efficiently translated into clinic. Therefore, a population-wide study of the distribution of HLA-pharmacogenetics markers is needed. This work presents a study of Thai *HLA* alleles on both *HLA* class I and II genes from 470 unrelated Thai individuals by means of polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO) in which oligonucleotide probes along the stretches of *HLA-A, -B, -C, -DRB1, -DQA1, and -DQB1* genes were genotyped. These 470 individuals were selected according to their regional locations, which were from North, Northeast, South, Central, and a capital city, Bangkok. Top ranked *HLA* alleles in Thai population include *HLA-A*11:01* (26.06%), *-B*46:01* (14.04%), *-C* 01:02* (17.13%), *-DRB1*12:02* (15.32%), *-DQA1*01:01* (24.89%), and *-DQB1*05:02* (21.28%). The results revealed that the distribution of HLA-pharmacogenetics alleles from the South had more HLA-B75 family that a typical *HLA-B*15:02* pharmacogenetics test for SJS/TEN screening would not cover. Besides the view across the nation, when compared *HLA*

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alleles from Thai population with *HLA* alleles from both European and Asian countries, the distribution landscape of *HLA*-associated drug hypersensitivity across many countries could be observed. Consequently, this pharmacogenetics database offers a comprehensive view of pharmacogenetics marker distribution in Thailand that could be used as a reference for other Southeast Asian countries to validate the feasibility of their future pharmacogenetics deployment.

Keywords: human leukocyte antigen, *HLA* class I, *HLA* class II, Thai population, pharmacogenetic marker

INTRODUCTION

Human leukocyte antigen (HLA) gene is located on chromosome 6p21, which was considered the most polymorphic of human genetic system (Shiina et al., 2009). *HLA* encodes cell surface molecules that present antigenic peptides to the T-cell receptor (TCR) on T cells (Sette and Sidney, 1998). There are two main classes of HLA allele. *HLA* class I and II encode cell surface heterodimers that play a role in antigen presentation, tolerance, and self/nonself-recognition. *HLA* class I molecules gather peptides that have been synthesized within the individual nucleated cell, three main *HLA* class I genes including *HLA-A*, *HLA-B*, and *HLA-C* (Howell et al., 2010). Whereas *HLA* class II molecules gather exogenously synthesized peptide ligands by endocytic pathway and expressed with antigen-presenting cells (APCs), six main *HLA* class II genes including *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA*, and *HLA-DRB1* (Ulvestad et al., 1994). The *HLA* system plays a critical role in regulating the immune response, tissue or organ transplantation, autoimmunity, vaccine development, susceptibility or resistance disease, and pharmacogenomics (Anania et al., 2017; Illing et al., 2017; Petersdorf, 2017). Over the past decade, there have been reported associations between various *HLA* alleles and different adverse drug reactions, especially severe cutaneous adverse reactions (SCARs).

One prominent report on drug-induced SCARs is the association between *HLA-B*15:02* allele and carbamazepine (CBZ)-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) in Han Chinese, Thai, and Southeast Asians (Ferrell and McLeod, 2008; Locharernkul et al., 2008; Hung et al., 2010; Chang et al., 2011; Nguyen et al., 2015). Conversely, Japanese and European populations were shown to have the association of *HLA-A*31:01* and CBZ-induced hypersensitivity reactions (McCormack et al., 2011; Ozeki et al., 2011). While *HLA-B*58:01* could be used as a pharmacogenetic risk prediction marker for allopurinol-induced SJS/TEN in many populations (Somkrua et al., 2011). In addition, the association between *HLA* class II and adverse drug reactions were reported, such as amoxicillin–clavulanate that induces liver injury was found to be associated with *HLA-DRB1*15:01*, *HLA-DRB5*01:01*, and *HLA-DQB1*06:02* haplotype in European (Hautekeete et al., 1999; Lucena et al., 2011). Thus, the distributions of *HLA* alleles and pharmacogenetic markers that could vary among different populations might affect incidences of adverse drug reactions or drug dosage responses (Donnell PH and Dolan, 2009).

Although there is a clear need to investigate at a population level *HLA* alleles, a study on a distribution of Thai *HLA* alleles was limited. Puangpetch et al. (2015) previously reported only *HLA-B* polymorphisms from 986 Thai individuals. The top five of such *HLA-B* alleles consisted of *HLA-B*46:01* (11.51%), *HLA-B*58:01* (8.62%), *HLA-B*40:01* (8.22%), *HLA-B*15:02* (8.16%), and *HLA-B*13:01* (6.95%). However, from this work, there were no reports of *HLA* class I and II allele in Thai population. There are other *HLA* alleles that play important roles in predicting various adverse drug reactions. Hence, the aim of this study was to comprehensively investigate both *HLA* class I (*HLA-A*, *-B*, and *-C*) and II (*HLA-DRB1*, *-DQA1*, and *-DQB1*) distribution of alleles in Thailand and the potential association with adverse drug reactions of these alleles.

MATERIALS AND METHODS

Subjects

We recruited 470 unrelated healthy Thai individuals from the 4th National Health Examination Survey in Thailand during August 2008 and March 2009, and the information was obtained from National Health Examination Survey Office, Health System Research Institute, Ministry of Public Health, Thailand. The 470 Thai individuals were randomly chosen according to their *self-reported* origins which can be characterized into five regional groups: (n = 70) Bangkok, (n = 100) Central, (n = 100) Northeastern, (n = 100) Northern, and (n = 100) Southern. Since we want this study to represent majority of Thai people, subjects for each group must have lived in the aforementioned regions for more than three generations. Furthermore, these healthy individuals must have no history of cutaneous adverse drug reactions (CADRs). Thailand is a country located at the center of Southeast Asia, sharing boundaries with Myanmar (west), Laos (north east), Cambodia (east), and Malaysia (south). This study was approved by the Ethical Review Committee on Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Written informed consent was obtained from all participants.

HLA Class I and II Genotyping

Recruited genomic DNA samples were isolated from EDTA blood using the MagNAprue Compact Nucleic Acid Isolation kits (Roche Applied Science, Mannheim, Germany). The quality of genomic DNA was measured by NanoDrop® ND-1000

(Thermo Scientific, Wilmington, USA). *HLA* class I alleles, comprising *HLA-A*, *HLA-B*, and *HLA-C*, and *HLA* class II alleles, comprising *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1*, were genotyped using sequence-specific oligonucleotides (PCR-SSOs). Briefly, the DNA samples obtained from patients of the five regions in Thailand were amplified by polymerase chain reaction (PCR). The PCR products were hybridized against a panel of SSO probes on coated polystyrene microspheres that had sequences complementary to the stretches of polymorphism within the target *HLA* class I and II alleles using the Lifecodes HLA SSO typing kits (Immucor, West Avenue, Stamford, USA). The amplicon-probe complex was then visualized using a colorimetric reaction and fluorescence detection technology by the Luminex®IS 100 system (Luminex Corporation, Austin, Texas, USA). Interpretations of *HLA* class I and II alleles from the probe signals were performed using MATCH IT DNA software version 1.2.2 (One Lambda, Canoga Park, CA, USA).

Statistical Analysis

The allele frequency and statistical analyses were performed using the Arlequin program version 3.1 for Hardy–Weinberg equilibrium testing. We used SPSS Compare allele frequencies between each region in Thailand using the SPSS software for Windows version 16.0 (SPSS Inc., Chicago, IL). A given pair of each region in Thai population was determined significant difference if the *p*-value was less than 0.05.

Population Structure Analysis

There were only six *HLA* haplotypes from class I and II to be used in this population structure analysis which were not enough to investigate substructure from these 470 individuals. We employed *HLA* probe polymorphism signals obtained directly from each "stretch" of the six *HLA* haplotypes. The total number of probes used in this experiment was 403 polymorphism probes distributed across six *HLA* haplotypes. The numbers of probes for six *HLA* haplotypes are as follows: *HLA-A* (72 probes), *HLA-B* (92 probes), and *HLA-C* (77 probes) for *HLA* class I and *HLA-DRB1* (91 probes), *HLA-DQA1* (23 probes), and *HLA-DQB1* (48 probes) for *HLA* class II.

We concatenated the raw data containing probe signals for each *HLA*-haplotype into one tab-delimited file (Supplemental text: mergeallHLA.txt) containing a 470 × 403 HLA-probe matrix that was used in both principal component analysis (PCA) (Chaichompoo et al., 2018) and STRUCTURE analysis (Pritchard et al., 2000; Kopelman et al., 2015).

Principal Component Analysis

Before performing PCA, the HLA-probe data entries were normalized based on z-score calculation. In particular, a probe signal X is converted to $X\prime = (X - \overline{X})/\mathrm{SD}$ where SD represents a standard deviation value of each probe column. The normalization step was done to minimize *HLA*-typing batch effects. We used *cal.pc.linear* function with default options from KRIS R package version 1.1.1 (Chaichompoo et al., 2018) to perform PCA. The PCA visualization was done using command *plot3views* from KRIS R package to display three main PCA perspectives, namely, PC1 vs. PC2, PC2 vs. PC3, and PC1 vs. PC3.

STRUCTURE Analysis

The normalization of the raw probe data was done similar to PCA with an extra step to round all X' values into integer. The conversion was done so that STRUCTURE could treat these normalized signals as a type of variation patterns similar to that of microsatellites. For STRUCTURE analysis, we used 30,000 burnin length with 80,000 MCMC iterations. The analyses were done from K = 1 to K = 10 (K represents a number of Bayesian-inferred clusters in a given population) each of which had 30 repeats with the same parameter setting. The total 100 STRUCTURE analysis results were used as the input to CLUMPAK (Kopelman et al., 2015), which helped determine the optimal K based on an *ad hoc* quantity Delta (K) approach (Evanno et al., 2005). The STRUCTURE visualization was done using *pophelper* function from pophelper R package version 2.30 (Francis, 2017).

RESULTS

HLA Class I and II Allele Frequencies in Thai Population

The allele frequency distribution of *HLA* class I and II are shown in **Tables 1**–**6**. We found that the alleles *HLA-A*11:01*, *-A*24:02*, *-A*02:03*, *-A*33:03*, *-A*02:07*, and *-A*02:01* were more common than the others. *HLA-A*11:01* was the most common allele found across five designated regions. For *HLA-B*, *HLA-B*46:01* was the predominant allele commonly found in Northern, Northeastern, Central, and Bangkok regions. *HLA-B* allele profile from the Southern group, however, showed that *HLA-B*15:02* was found more commonly (**Table 2**). Additionally, the allele distribution of *HLA-B* and their frequencies were *HLA-B*46:01* (14.04%), *-B*15:02* (7.66%), *-B*40:01* (6.60%), *-B*58:01* (6.38%), *-B*13:01* (5.96%), *-B*44:03* (4.47%), and *-B*38:02* (4.26%), respectively. For *HLA-C,* the allele distribution and the corresponding frequencies were *HLA-C*01:02* (17.13%), *-C*07:02* (11.91%), *-C*08:01* (10.32%), *-C*03:04* (8.09%), *-C*03:02* (7.77%), *-C*07:01* (6.38%), and *-C*07:04* (7.00%), respectively (**Table 3**). Particularly, *HLA-C*08:01* (14.50%) allele was the highest frequency in the Southern group, but there was not significant difference when compared with the other regions (*p*-value = 0.4764) as shown in **Table 7**.

The frequency of *HLA* class II alleles including *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* alleles were presented in **Tables 4**–**6**. The frequency distributions of *HLA-DRB1* alleles were *HLA-DRB1*12:02* (15.32%), *-DRB1*15:02* (14.47%), *-DRB1*09:01* (9.89%), *-DRB1*07:01* (8.94%), *-DRB1*15:01* (8.09%), *-DRB1*14:01* (5.96%), *-DRB1*16:02* (5.96%), and *-DRB1*03:01* (5.00%). Particularly, *HLA-DRB1*15:02* was the highest allele frequency presented in the Southern, Northern, and Northeastern groups, while Central and Bangkok regions share the top allele frequency of *HLA-DRB1*12:02*. Moreover, frequencies of *HLA-DQA1* alleles were *HLA-DQA1*01:01* (24.89%), *-DQA1*01:02* (22.23%), *-DQA1*03:02* (13.30%), *-DQA1*06:01* (12.02%), *-DQA1*02:01* (8.72%), and *-DQA1*05:01* (5.43%) (**Table 5**). *HLA-DQA1*01:01* allele was

TABLE 1 | The frequency of *HLA* class I alleles in Thai population and five regions (n = 470).

HLA-A	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
01:01	0.0223	0.04468	1.0000	0.0450	0.0900	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0250	0.0500	1.0000	0.0357	0.0714	1.0000
01:03	0.0011	0.00213	1.0000													0.0071	0.0143	1.0000
02:01	0.0553	0.10851	1.0000	0.0450	0.0800	1.0000	0.0400	0.0800	1.0000	0.0350	0.0700	1.0000	0.0700	0.1400	1.0000	0.1000	0.2000	1.0000
02:02	0.0011	0.00213	1.0000				0.0050	0.0100	1.0000									
02:03	0.1117	0.21064	1.0000	0.0400	0.0800	1.0000	0.1450	0.2800	1.0000	0.1400	0.2400	0.6827	0.1350	0.2600	1.0000	0.0929	0.1857	1.0000
02:06	0.0223	0.04468	1.0000	0.0200	0.0400	1.0000	0.0200	0.0400	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0143	0.0286	1.0000
02:07	0.0840	0.14468	0.0556	0.0500	0.0900	1.0000	0.1000	0.1700	1.0000	0.1000	0.1800	1.0000	0.0750	0.1300	1.0000	0.1000	0.1571	0.6193
02:11	0.0043	0.00851	1.0000	0.0100	0.0200	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000						
02:17	0.0043	0.00851	1.0000	0.0050	0.0100	1.0000							0.0100	0.0200	1.0000	0.0071	0.0143	1.0000
02:20	0.0011	0.00213	1.0000							0.0050	0.0100	1.0000						
02:33	0.0032	0.00426	1.0000							0.0050	0.0100	1.0000	0.0100	0.0100	1.0000			
02:58	0.0117	0.02340	1.0000	0.0050	0.0100	1.0000	0.0150	0.0300	1.0000				0.0250	0.0500	1.0000	0.0143	0.0286	1.0000
03:01	0.0064	0.01277	1.0000	0.0150	0.0300	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
03:02	0.0011	0.00213	1.0000							0.0050	0.0100	1.0000						
11:01	0.2606	0.44894	0.8984	0.2300	0.4100	1.0000	0.3550	0.5900	0.8307	0.2500	0.4600	0.7475	0.2600	0.4200	0.6162	0.1857	0.3286	1.0000
11:02	0.0181	0.03617	1.0000	0.0200	0.0400	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
11:03	0.0032	0.00638	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
11:04	0.0053	0.01064	1.0000	0.0150	0.0300	1.0000				0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
11:09	0.0011	0.00213	1.0000	0.0050	0.0100	1.0000												
23:01	0.0011	0.00213	1.0000	0.0050	0.0100	1.0000												
24:01	0.0011	0.00213	1.0000	0.0050	0.0100	1.0000												
24:02	0.1149	0.20213	0.1643	0.1450	0.2200	0.1679	0.1000	0.1900	1.0000	0.1150	0.2100	1.0000	0.1000	0.1900	1.0000	0.1143	0.2000	1.0000
24:03	0.0053	0.01064	1.0000							0.0100	0.0200	1.0000	0.0050	0.0100	1.0000	0.0143	0.0286	1.0000
24:07	0.0426	0.08298	1.0000	0.0700	0.1400	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0350	0.0700	1.0000	0.0571	0.1000	1.0000
24:10	0.0170	0.03404	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0400	0.0800	1.0000	0.0050	0.0100	1.0000	0.0214	0.0429	1.0000
24:17	0.0011	0.00213	1.0000	0.0050	0.0100	1.0000												
24:25	0.0011	0.00213	1.0000	0.0050	0.0100	1.0000												
26:01	0.0128	0.02553	1.0000	0.0150	0.0300	1.0000	0.0200	0.0400	1.0000	0.0050	0.0100	1.0000	0.0150	0.0300	1.0000	0.0071	0.0143	1.0000
29:01	0.0053	0.01064	1.0000				0.0050	0.0100	1.0000	0.0150	0.0300	1.0000	0.0050	0.0100	1.0000			
29:10	0.0011	0.00213	1.0000													0.0071	0.0143	1.0000
30:01	0.0213	0.04255	1.0000	0.0350	0.0700	1.0000	0.0100	0.0200	1.0000	0.0200	0.0400	1.0000	0.0250	0.0500	1.0000	0.0143	0.0286	1.0000
31:01	0.0085	0.01489	1.0000	0.0200	0.0300	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000	0.0143	0.0286	1.0000
32:01	0.0021	0.00426	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000			
33:01	0.0032	0.00638	1.0000				0.0050	0.0100	1.0000				0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
33:03	0.1117	0.21064	1.0000	0.1050	0.1900	1.0000	0.0850	0.1700	1.0000	0.1000	0.1900	1.0000	0.1450	0.2600	1.0000	0.1286	0.2571	1.0000
33:12	0.0021	0.00426	1.0000	0.0100	0.0200	1.0000												
34:01	0.0096	0.01915	1.0000	0.0200	0.0400	1.0000	0.0050	0.0100	1.0000	0.0150	0.0300	1.0000				0.0071	0.0143	1.0000
68:01	0.0096	0.01915	1.0000	0.0200	0.0400	1.0000				0.0150	0.0300	1.0000				0.0143	0.0286	1.0000
68:02	0.0021	0.00426	1.0000	0.0100	0.0200	1.0000												
74:01	0.0064	0.01277	1.0000	0.0050	0.0100	1.0000				0.0200	0.0400	1.0000				0.0071	0.0143	1.0000
74:05	0.0021	0.00426	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000						

HLA-A, human leukocyte antigen-A; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

TABLE 2 | The frequency of *HLA* class I alleles in Thai population and five regions (n = 470).

HLA-B	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
03:01	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
03:02	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
07:02	0.0032	0.0064	1.0000	0.0100	0.0200	1.0000							0.0050	0.0100	1.0000			
07:05	0.0255	0.0511	1.0000	0.0050	0.0100	1.0000	0.0250	0.0500	1.0000	0.0400	0.0800	1.0000	0.0300	0.0600	1.0000	0.0286	0.0571	1.0000
08:01	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
13:01	0.0596	0.1149	1.0000	0.0350	0.0700	1.0000	0.0800	0.1400	0.4980	0.0600	0.1200	1.0000	0.0650	0.1300	1.0000	0.0571	0.1143	1.0000
13:02	0.0213	0.0426	1.0000	0.0350	0.0700	1.0000	0.0150	0.0300	1.0000	0.0150	0.0300	1.0000	0.0150	0.0300	1.0000	0.0286	0.0571	1.0000
13:10	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
14:02	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
15:01	0.0053	0.0106	1.0000	0.0050	0.0100	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000						
15:02	0.0766	0.1511	1.0000	0.0900	0.1800	1.0000	0.0650	0.1300	1.0000	0.0650	0.1200	1.0000	0.0750	0.1500	1.0000	0.0929	0.1857	1.0000
15:04	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
15:07	0.0021	0.0043	1.0000				0.0100	0.0200	1.0000									
15:10	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000						
15:11	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000									
15:12	0.0021	0.0043	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000						
15:13	0.0096	0.0191	1.0000	0.0250	0.0500	1.0000				0.0050	0.0100	1.0000	0.0100	0.0200	1.0000	0.0071	0.0143	1.0000
15:17	0.0043	0.0085	1.0000	0.0100	0.0200	1.0000							0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
15:18	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000										0.0143	0.0286	1.0000
15:20	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
15:21	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000						
15:25	0.0223	0.0426	1.0000	0.0200	0.0400	1.0000	0.0250	0.0500	1.0000	0.0300	0.0600	1.0000	0.0100	0.0200	1.0000	0.0286	0.0429	1.0000
15:31	0.0021	0.0043	1.0000	0.0100	0.0200	1.0000												
15:32	0.0043	0.0085	1.0000	0.0100	0.0200	1.0000				0.0100	0.0200	1.0000						
15:35	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000						
18:01	0.0383	0.0766	1.0000	0.0500	0.1000	1.0000	0.0150	0.0300	1.0000	0.0300	0.0600	1.0000	0.0600	0.1200	1.0000	0.0357	0.0714	1.0000
18:02	0.0160	0.0319	1.0000	0.0200	0.0400	1.0000	0.0100	0.0200	1.0000	0.0400	0.0800	1.0000				0.0071	0.0143	1.0000
18:12	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
18:14	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
27:03	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
27:04	0.0213	0.0404	1.0000	0.0200	0.0400	1.0000	0.0400	0.0700	1.0000	0.0150	0.0300	1.0000	0.0100	0.0200	1.0000	0.0214	0.0429	1.0000
27:06	0.0138	0.0255	1.0000	0.0050	0.0100	1.0000	0.0200	0.0300	1.0000	0.0350	0.0700	1.0000	0.0050	0.0100	1.0000			
27:07	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
35:01	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
35:02	0.0021	0.0043	1.0000										0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
35:03	0.0074	0.0149	1.0000	0.0200	0.0400	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
35:05	0.0191	0.0383	1.0000	0.0300	0.0600	1.0000	0.0200	0.0400	1.0000	0.0200	0.0400	1.0000	0.0100	0.0200	1.0000	0.0143	0.0286	1.0000
35:06	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
35:11	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
35:32	0.0021	0.0043	1.0000	0.0100	0.0200	1.0000												
35:68	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
37:01	0.0085	0.0170	1.0000	0.0150	0.0300	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0214	0.0429	1.0000
38:02	0.0426	0.0830	1.0000	0.0400	0.0800	1.0000	0.0550	0.1100	1.0000	0.0300	0.0600	1.0000	0.0500	0.0900	1.0000	0.0357	0.0714	1.0000
39:01	0.0064	0.0128	0.5000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0143	0.0286	1.0000
39:06	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												

(Continued)

TABLE 2 | Continued

HLA-B	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
39:09	0.0160	0.0319	1.0000				0.0150	0.0300	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0071	0.0143	1.0000
39:15	0.0053	0.0106	1.0000	0.0150	0.0300	1.0000				0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
39:49	0.0011	0.0021	1.0000										0.0050	0.0100	1.0000			
40:01	0.0660	0.1234	0.6870	0.0300	0.0600	1.0000	0.0750	0.1400	1.0000	0.0950	0.1700	1.0000	0.0650	0.1200	1.0000	0.0643	0.1286	1.0000
40:02	0.0074	0.0149	1.0000				0.0100	0.0200	1.0000	0.0150	0.0300	1.0000	0.0100	0.0200	1.0000			
40:06	0.0234	0.0468	1.0000	0.0450	0.0900	1.0000	0.0200	0.0400	1.0000	0.0150	0.0300	1.0000	0.0200	0.0400	1.0000	0.0143	0.0286	1.0000
40:11	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
41:01	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
41:30	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
44:02	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000									
44:03	0.0447	0.0894	1.0000	0.0450	0.0900	1.0000	0.0350	0.0700	1.0000	0.0250	0.0500	1.0000	0.0550	0.1100	1.0000	0.0714	0.1429	1.0000
46:01	0.1404	0.2596	0.8170	0.0650	0.1300	1.0000	0.1850	0.3300	1.0000	0.1300	0.2300	1.0000	0.1900	0.3600	0.6830	0.1286	0.2429	1.0000
46:02	0.0021	0.0043	1.0000							0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
48:01	0.0043	0.0085	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000			
48:03	0.0106	0.0213	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0250	0.0500	1.0000	0.0214	0.0429	1.0000
51:01	0.0426	0.0851	1.0000	0.0550	0.1100	1.0000	0.0500	0.1000	1.0000	0.0350	0.0700	1.0000	0.0350	0.0700	1.0000	0.0357	0.0714	1.0000
51:02	0.0138	0.0277	1.0000	0.0250	0.0500	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0143	0.0286	1.0000
52:01	0.0330	0.0638	1.0000	0.0450	0.0800	1.0000	0.0350	0.0700	1.0000	0.0200	0.0400	1.0000	0.0250	0.0500	1.0000	0.0429	0.0857	1.0000
53:01	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
54:01	0.0085	0.0170	1.0000				0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0150	0.0300	1.0000	0.0071	0.0143	1.0000
55:01	0.0011	0.0021	1.0000							0.0050	0.0100	1.0000						
55:02	0.0213	0.0426	1.0000	0.0100	0.0200	1.0000	0.0300	0.0600	1.0000	0.0200	0.0400	1.0000	0.0150	0.0300	1.0000	0.0357	0.0714	1.0000
56:01	0.0106	0.0191	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0150	0.0200	1.0000	0.0100	0.0200	1.0000	0.0071	0.0143	1.0000
56:02	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
56:04	0.0128	0.0255	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0200	0.0400	1.0000	0.0250	0.0500	1.0000	0.0071	0.0143	1.0000
57:01	0.0117	0.0234	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0150	0.0300	1.0000	0.0143	0.0286	1.0000
57:02	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
58:01	0.0638	0.1213	1.0000	0.0600	0.1100	1.0000	0.0500	0.1000	1.0000	0.0800	0.1400	0.4980	0.0750	0.1500	1.0000	0.0500	0.1000	1.0000
58:51	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												

HLA-B, human leukocyte antigen-B; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

TABLE 3 | The frequency of *HLA* class I alleles in Thai population and five regions (n = 470).

HLA-C	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
01:02	0.1713	0.3043	0.5895	0.0750	0.1400	1.0000	0.2200	0.3800	0.7560	0.1700	0.3100	1.0000	0.2200	0.3900	1.0000	0.1714	0.3000	1.0000
01:08	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
02:02	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
03:02	0.0777	0.1468	1.0000	0.0750	0.1300	1.0000	0.0600	0.1200	1.0000	0.1000	0.1800	1.0000	0.0900	0.1800	1.0000	0.0571	0.1143	1.0000
03:03	0.0202	0.0362	0.4995	0.0200	0.0400	1.0000	0.0200	0.0400	1.0000	0.0250	0.0400	1.0000	0.0050	0.0100	1.0000	0.0357	0.0571	1.0000
03:04	0.0809	0.1404	0.0901	0.0450	0.0900	1.0000	0.1600	0.2500	0.3311	0.0800	0.1400	1.0000	0.0800	0.1500	1.0000	0.0214	0.0429	1.0000
03:08	0.0021	0.0043	1.0000				0.0050	0.0100	1.0000				0.0050	0.0100	1.0000			
03:09	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
03:17	0.0021	0.0043	1.0000							0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
03:94	0.0011	0.0021	1.0000										0.0050	0.0100	1.0000			
04:01	0.0468	0.0936	1.0000	0.0800	0.1600	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0300	0.0600	1.0000	0.0786	0.1571	1.0000
04:03	0.0426	0.0809	1.0000	0.0400	0.0800	1.0000	0.0450	0.0900	1.0000	0.0600	0.1100	1.0000	0.0200	0.0400	1.0000	0.0500	0.0857	1.0000
04:06	0.0117	0.0234	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0250	0.0500	1.0000	0.0200	0.0400	1.0000			
05:01	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000			
06:02	0.0426	0.0851	1.0000	0.0600	0.1200	1.0000	0.0200	0.0400	1.0000	0.0400	0.0800	1.0000	0.0350	0.0700	1.0000	0.0643	0.1286	1.0000
06:89	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
07:01	0.0638	0.1234	1.0000	0.0700	0.1200	1.0000	0.0400	0.0800	1.0000	0.0350	0.0700	1.0000	0.0850	0.1700	1.0000	0.1000	0.2000	1.0000
07:02	0.1191	0.2149	0.4752	0.0900	0.1600	1.0000	0.1000	0.1800	1.0000	0.1450	0.2600	1.0000	0.1400	0.2700	1.0000	0.1214	0.2000	0.6195
07:04	0.0500	0.0979	1.0000	0.0650	0.1300	1.0000	0.0300	0.0600	1.0000	0.0650	0.1200	1.0000	0.0350	0.0700	1.0000	0.0571	0.1143	1.0000
07:27	0.0117	0.0234	1.0000	0.0150	0.0300	1.0000	0.0200	0.0400	1.0000				0.0150	0.0300	1.0000	0.0071	0.0143	1.0000
07:29	0.0085	0.0170	1.0000				0.0050	0.0100	1.0000	0.0200	0.0400	1.0000	0.0100	0.0200	1.0000	0.0071	0.0143	1.0000
08:01	0.1032	0.1915	0.7714	0.1450	0.2700	1.0000	0.0850	0.1700	1.0000	0.0850	0.1500	1.0000	0.1150	0.2200	1.0000	0.0786	0.1286	0.4964
08:02	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000										0.0071	0.0143	1.0000
08:03	0.0032	0.0064	1.0000				0.0050	0.0100	1.0000	0.0100	0.0200	1.0000						
12:02	0.0404	0.0787	1.0000	0.0550	0.1000	1.0000	0.0700	0.1400	1.0000	0.0150	0.0300	1.0000	0.0200	0.0400	1.0000	0.0429	0.0857	1.0000
12:03	0.0138	0.0277	1.0000	0.0200	0.0400	1.0000	0.0100	0.0200	1.0000	0.0200	0.0400	1.0000	0.0050	0.0100	1.0000	0.0143	0.0286	1.0000
12:04	0.0011	0.0021	1.0000							0.0050	0.0100	1.0000						
14:02	0.0287	0.0574	1.0000	0.0350	0.0700	1.0000	0.0300	0.0600	1.0000	0.0300	0.0600	1.0000	0.0250	0.0500	1.0000	0.0214	0.0429	1.0000
15:02	0.0340	0.0681	1.0000	0.0600	0.1200	1.0000	0.0150	0.0300	1.0000	0.0250	0.0500	1.0000	0.0300	0.0600	1.0000	0.0429	0.0857	1.0000
15:05	0.0043	0.0085	1.0000				0.0050	0.0100	1.0000	0.0150	0.0300	1.0000						
16:02	0.0064	0.0128	1.0000	0.0200	0.0400	1.0000							0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
17:01	0.0021	0.0043	1.0000	0.0100	0.0200	1.0000												
18:01	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000

HLA-C, human leukocyte antigen-C; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

TABLE 4 | The frequency of *HLA* class II alleles in Thai population and five regions (n = 470).

HLA-DRB1	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
01:01	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000							0.0100	0.0200	1.0000			
01:02	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
03:01	0.0500	0.0915	0.3737	0.0500	0.0900	1.0000	0.0400	0.0800	1.0000	0.0650	0.1100	1.0000	0.0500	0.1000	1.0000	0.0429	0.0714	1.0000
03:20	0.0032	0.0064	1.0000				0.0050	0.0100	1.0000							0.0143	0.0286	1.0000
04:01	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000							0.0071	0.0143	1.0000
04:02	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
04:03	0.0234	0.0447	1.0000	0.0300	0.0600	1.0000	0.0150	0.0300	1.0000	0.0050	0.0100	1.0000	0.0250	0.0500	1.0000	0.0500	0.0857	1.0000
04:04	0.0032	0.0064	1.0000	0.0150	0.0300	1.0000												
04:05	0.0489	0.0957	1.0000	0.0150	0.0300	1.0000	0.0500	0.1000	1.0000	0.0850	0.1600	1.0000	0.0650	0.1300	1.0000	0.0214	0.0429	1.0000
04:06	0.0032	0.0064	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
05:01	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
07:01	0.0894	0.1766	0.3737	0.0850	0.1600	1.0000	0.0600	0.1200	1.0000	0.0750	0.1500	1.0000	0.1100	0.2200	1.0000	0.1286	0.2571	1.0000
08:01	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
08:02	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000										0.0071	0.0143	1.0000
08:03	0.0160	0.0298	1.0000	0.0300	0.0600	1.0000	0.0050	0.0100	1.0000	0.0150	0.0300	1.0000	0.0050	0.0100	1.0000	0.0286	0.0429	1.0000
08:12	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000			
08:19	0.0011	0.0021	1.0000										0.0050	0.0100	1.0000			
09:01	0.0989	0.1872	1.0000	0.0800	0.1600	1.0000	0.1350	0.2600	1.0000	0.1100	0.2200	1.0000	0.0650	0.1200	1.0000	0.1071	0.1714	0.6195
10:01	0.0213	0.0426	1.0000	0.0300	0.0600	1.0000	0.0200	0.0400	1.0000	0.0200	0.0400	1.0000	0.0150	0.0300	1.0000	0.0214	0.0429	1.0000
11:01	0.0160	0.0319	1.0000	0.0200	0.0400	1.0000	0.0100	0.0200	1.0000	0.0150	0.0300	1.0000	0.0200	0.0400	1.0000	0.0143	0.0286	1.0000
11:02	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
11:04	0.0021	0.0043	1.0000	0.0100	0.0200	1.0000												
11:05	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000							0.0050	0.0100	1.0000			
11:06	0.0138	0.0277	1.0000	0.0100	0.0200	1.0000				0.0200	0.0400	1.0000	0.0250	0.0500	1.0000	0.0143	0.0286	1.0000
12:01	0.0074	0.0149	1.0000	0.0050	0.0100	1.0000	0.0100	0.0200	1.0000				0.0150	0.0300	1.0000	0.0071	0.0143	1.0000
12:02	0.1532	0.2851	0.8253	0.1300	0.2500	1.0000	0.1350	0.2600	1.0000	0.1600	0.3100	0.6212	0.1650	0.2900	1.0000	0.1857	0.3286	1.0000
12:07	0.0032	0.0064	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
12:12	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
12:16	0.0021	0.0043	1.0000	0.0050	0.0100	1.0000										0.0071	0.0143	1.0000
13:01	0.0096	0.0191	1.0000	0.0200	0.0400	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0214	0.0429	1.0000
13:02	0.0138	0.0277	1.0000	0.0300	0.0600	1.0000				0.0150	0.0300	1.0000	0.0100	0.0200	1.0000	0.0143	0.0286	1.0000
13:12	0.0074	0.0149	1.0000				0.0050	0.0100	1.0000	0.0150	0.0300	1.0000				0.0214	0.0429	1.0000
14:01	0.0596	0.1085	1.0000	0.0400	0.0700	1.0000	0.1150	0.2000	0.6195	0.0350	0.0700	1.0000	0.0650	0.1200	1.0000	0.0357	0.0714	1.0000
14:03	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
14:04	0.0234	0.0447	1.0000	0.0250	0.0500	1.0000	0.0150	0.0300	1.0000	0.0300	0.0500	1.0000	0.0300	0.0600	1.0000	0.0143	0.0286	1.0000
14:05	0.0074	0.0149	1.0000				0.0150	0.0300	1.0000	0.0050	0.0100	1.0000				0.0214	0.0429	1.0000
14:07	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
14:22	0.0021	0.0043	1.0000										0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
15:01	0.0809	0.1532	1.0000	0.1050	0.2000	1.0000	0.0750	0.1500	1.0000	0.0650	0.1200	1.0000	0.0950	0.1800	1.0000	0.0571	0.1000	1.0000
15:02	0.1447	0.2638	0.8292	0.1600	0.3100	0.6212	0.1650	0.2900	1.0000	0.1700	0.3100	1.0000	0.1250	0.2200	1.0000	0.0857	0.1571	1.0000
15:03	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
15:06	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
15:24	0.0011	0.0021	1.0000							0.0050	0.0100	1.0000						

(Continued)

TABLE 4 | Continued

HLA-DRB1	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
16:01	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
16:02	0.0596	0.1106	0.6865	0.0350	0.0700	1.0000	0.1000	0.1600	1.0000	0.0700	0.1400	1.0000	0.0650	0.1300	1.0000	0.0143	0.0286	1.0000
16:05	0.0021	0.0043	1.0000							0.0100	0.0200	1.0000						
16:10	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
16:12	0.0064	0.0128	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000				0.0100	0.0200	1.0000	0.0143	0.0286	1.0000

HLA-DRB1, human leukocyte antigen-DRB1; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

the highest allele in the Southern, Northern, and Central groups. On the contrary, *HLA-DQA1*01:02* allele was found more commonly in both Northeastern and Bangkok regions. The frequency distribution of *HLA-DQB1* alleles includes *HLA-DQB1*05:02* (21.28%), *-DQB1*03:01* (17.23%), *-DQB1*05:01* (14.04%), *-DQB1*03:03* (11.28%), *-DQB1*02:02* (7.23%), *-DQB1*06:01* (7.13%), and *-DQB1*02:01* (5.43%), as shown in **Table 6**. *HLA-DQB1*03:01* was the main allele in both Southern and Bangkok regions meanwhile *HLA-DQB1*05:02* was found more common in Northern, Northeastern, and Central. For the statistical analysis, the allele frequency of *HLA-A*, *-B*, *-C*, *-DRB1*, *-DQA1*, and *-DQB1* were tested from Hardy–Weinberg equilibrium (p-value < 0.05). There was no significant differentiation in each *HLA* class I and class II alleles except only *HLA-DQA1*03:02* allele in Thai population.

Distribution of Pharmacogenetics Markers

The allele frequencies across Thailand five regions of *HLA-B*15:02* (IMGT/HLA ID: HLA00165) which induces the risk of having SJS/TEN upon taking aromatic antiepileptic drug [CBZ, oxcarbazepine (OXC), phenytoin (PHT), and lamotrigine (LTG)], had no significant differences (p-value > 0.05) (**Table 7**). The frequency of *HLA-B*15:02* allele was much lower in other populations, namely, African Americans (Cao et al., 2001), Caucasians (Cao et al., 2001), Hispanics (Cao et al., 2001), and North Americans (Cao et al., 2001) (**Table 8A** and **Figure 1**). Additionally, *HLA-B*15:02* allele belongs to the HLA-B75 family, which consists of *HLA-B*15:11* (IMGT/HLA ID: HLA00174) (0.50%) and *HLA-B*15:21* (IMGT/HLA ID: HLA00184) (0.50%) in Southern group, whereas *HLA-B*15:08* (IMGT/HLA ID: HLA00171) was not found in this population. In terms of *HLA-A*31:01* (IMGT/HLA ID: HLA00097) allele which associates with CBZ-induced CADRs, neither the frequencies within Thailand nor among different populations were significantly different (**Tables 7**, **8A**).

*HLA-B*58:01* (IMGT/HLA ID: HLA00386) allele was similarly distributed in Thais (6.38%), African Americans (6.37%), and Asians (7.38%) (Yang et al., 2016) and higher than those reported in Caucasians (Cao et al., 2001), Hispanics (Cao et al., 2001), and North Americans (Cao et al., 2001) (**Tables 7**, **8A**). However, *HLA-B*58:01* allele was not significantly different across different populations. Furthermore, other *HLA* alleles which are associated with allopurinol-induced SJS/TEN such as *HLA-A*33:03* (IMGT/HLA ID: HLA00106), *HLA-C*03:02* (IMGT/HLA ID: HLA00410), and *HLA-DRB1*15:02* (IMGT/HLA ID: HLA00867) alleles were found to have higher frequency in Thai population than others. Note that the high distribution of *HLA-DRB1*13:02* (IMGT/HLA ID: HLA00798) allele was observed in African Americans (8.5%) and Japanese (7.7%) but not so much (1.38%) among Thais (**Tables 8A, B**).

*HLA-B*13:01* (IMGT/HLA ID: HLA00152) allele has been reported to be associated with dapsone and salazosulfapyridine-induced drug reaction with eosinophilia and systemic symptoms (DRESS). The frequencies of *HLA-B*13:01* were similar among Thai population (p-value = 0.7450) and higher than African Americans, Caucasians, Hispanics, and North American

TABLE 5 | The frequency of *HLA* class II alleles in Thai population and five regions (n = 470).

HLA-DQA1	Thailand (n = 470)			Southern (n = 100)			Northern (n = 100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
01:01	0.2489	0.4170	0.3105	0.2350	0.4000	0.7740	0.3250	0.5000	0.5280	0.2450	0.4300	1.0000	0.2400	0.4200	1.0000	0.1786	0.3000	0.6810
01:02	0.2223	0.3894	0.7692	0.2150	0.3900	1.0000	0.2150	0.3700	0.7560	0.2600	0.4300	0.7940	0.2200	0.3900	1.0000	0.1929	0.3571	1.0000
01:03	0.0383	0.0723	1.0000	0.0850	0.1600	1.0000	0.0150	0.0300	1.0000	0.0150	0.0300	1.0000	0.0350	0.0700	1.0000	0.0429	0.0714	1.0000
01:06	0.0011	0.0021	1.0000													0.0071	0.0143	1.0000
02:01	0.0872	0.1723	0.3740	0.0800	0.1500	1.0000	0.0600	0.1200	1.0000	0.0700	0.1400	1.0000	0.1100	0.2200	1.0000	0.1286	0.2571	1.0000
03:01	0.0457	0.0851	0.6240	0.0650	0.1200	1.0000	0.0350	0.0700	1.0000	0.0300	0.0600	1.0000	0.0400	0.0700	1.0000	0.0643	0.1143	1.0000
03:02	0.1330	0.2660	0.0080	0.0850	0.1700	1.0000	0.1700	0.3400	0.2460	0.1700	0.3400	0.2460	0.1250	0.2500	0.4980	0.1071	0.2143	1.0000
04:01	0.0021	0.0043	1.0000				0.0050	0.0100	1.0000							0.0071	0.0143	1.0000
05:01	0.0543	0.1043	1.0000	0.0600	0.1100	1.0000	0.0450	0.0900	1.0000	0.0600	0.1100	1.0000	0.0500	0.1000	1.0000	0.0571	0.1143	1.0000
05:03	0.0117	0.0234	1.0000	0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0150	0.0300	1.0000	0.0150	0.0300	1.0000	0.0214	0.0429	1.0000
05:05	0.0340	0.0660	1.0000	0.0350	0.0600	1.0000	0.0150	0.0300	1.0000	0.0400	0.0800	1.0000	0.0450	0.0900	1.0000	0.0357	0.0714	1.0000
05:08	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									
06:01	0.1202	0.2277	0.7800	0.1350	0.2600	1.0000	0.1050	0.2100	1.0000	0.0950	0.1800	1.0000	0.1200	0.2200	1.0000	0.1571	0.2857	1.0000

HLA-DQA1, human leukocyte antigen-DQA1; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

TABLE 6 | The frequency of *HLA* class II alleles in Thai population and five regions (n = 470).

HLA-DQB1	Thailand (n = 470)			Southern (n = 100)			Northern (n =100)			Northeastern (n = 100)			Central (n = 100)			Bangkok (n = 70)		
	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)	AF	CF	HW (p-value)
02:01	0.0543	0.1000	0.3740	0.0550	0.1000	1.0000	0.0450	0.0900	1.0000	0.0650	0.1100	0.4980	0.0500	0.1000	1.0000	0.0571	0.1000	1.0000
02:02	0.0723	0.1447	0.5000	0.0700	0.1400	1.0000	0.0500	0.1000	1.0000	0.0600	0.1200	1.0000	0.0850	0.1700	1.0000	0.1071	0.2143	1.0000
03:01	0.1723	0.3213	0.6850	0.1850	0.3300	1.0000	0.1400	0.2700	1.0000	0.1500	0.2900	1.0000	0.1800	0.3400	1.0000	0.2214	0.4000	1.0000
03:02	0.0426	0.0787	0.6240	0.0600	0.1100	1.0000	0.0300	0.0600	1.0000	0.0200	0.0400	1.0000	0.0400	0.0700	1.0000	0.0714	0.1286	1.0000
03:03	0.1128	0.2149	0.7620	0.0850	0.1700	1.0000	0.1350	0.2600	1.0000	0.1250	0.2500	1.0000	0.0950	0.1800	1.0000	0.1286	0.2143	0.6200
03:38	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
04:01	0.0383	0.0745	1.0000	0.0150	0.0300	1.0000	0.0400	0.0800	1.0000	0.0600	0.1100	1.0000	0.0550	0.1100	1.0000	0.0143	0.0286	1.0000
04:02	0.0032	0.0064	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000				0.0071	0.0143	1.0000
05:01	0.1404	0.2574	0.8210	0.1350	0.2600	1.0000	0.1700	0.2900	0.7210	0.1600	0.3000	1.0000	0.1350	0.2500	1.0000	0.0857	0.1571	1.0000
05:02	0.2128	0.3872	0.7440	0.1600	0.2800	1.0000	0.2850	0.4900	1.0000	0.2400	0.4500	0.4980	0.2000	0.3900	0.3690	0.1643	0.3000	1.0000
05:03	0.0404	0.0787	1.0000	0.0450	0.0900	1.0000	0.0400	0.0800	1.0000	0.0350	0.0600	1.0000	0.0500	0.1000	1.0000	0.0286	0.0571	1.0000
05:66	0.0011	0.0021	1.0000	0.0050	0.0100	1.0000												
06:01	0.0713	0.1340	0.6870	0.1150	0.2100	1.0000	0.0400	0.0800	1.0000	0.0550	0.1100	1.0000	0.0850	0.1600	1.0000	0.0571	0.1000	1.0000
06:02	0.0149	0.0298	1.0000	0.0150	0.0300	1.0000	0.0150	0.0300	1.0000	0.0100	0.0200	1.0000	0.0100	0.0200	1.0000	0.0286	0.0571	1.0000
06:03	0.0074	0.0149	1.0000	0.0200	0.0400	1.0000							0.0050	0.0100	1.0000	0.0143	0.0286	1.0000
06:04	0.0053	0.0106	1.0000	0.0100	0.0200	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
06:05	0.0043	0.0085	1.0000	0.0050	0.0100	1.0000				0.0050	0.0100	1.0000	0.0050	0.0100	1.0000	0.0071	0.0143	1.0000
06:09	0.0043	0.0085	1.0000	0.0150	0.0300	1.0000				0.0050	0.0100	1.0000						
06:10	0.0011	0.0021	1.0000				0.0050	0.0100	1.0000									

HLA-DQB1, human leukocyte antigen-DQB1; AF, allele frequency; CF, carrier frequency; HW, Hardy–Weinberg equilibrium; Significantly different p-value < 0.05.

TABLE 7 | Distribution of pharmacogenetics markers in five regions and Thai population.

Drug	Pharmacogenetics markers	ADR type	Allele frequency (%)						Comparing all five populations (*p*-value)	Reference
			Thai population (n = 470)	Southern (n = 100)	Northern (n = 100)	Northeastern (n = 100)	Central (n = 100)	Bangkok (n = 70)		
Carbamazepine	*HLA-B*15:02*	SJS/TEN	7.66	9.00	6.50	6.50	7.50	9.29	0.9052	(Nahoko and Yoshiro, 2013; Sukasem et al., 2014; Su et al., 2016)
	*HLA-A*31:01*	CADRs, SJS/TEN, DRESS, MPE	0.85	2.00	0.50	0.00	0.5	1.43	0.8050	(Nahoko and Yoshiro, 2013; Sukasem et al., 2014; Su et al., 2016)
	*HLA-B*15:11*	SJS/TEN	0.21	0.50	0.50	0.00	0.00	0.00	1.0000	(Sukasem et al., 2014; Su et al., 2016)
	*HLA-A*24:02*	SJS/TEN	11.49	14.50	10.00	11.50	10.00	11.43	0.8546	(Shi et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	14.50	8.50	8.50	11.50	7.86	0.4764	(Shi et al., 2017)
	*HLA-DRB1*12:02*	SJS/TEN	15.32	13.00	13.50	16.00	16.50	18.57	0.8070	(Shi et al., 2017)
Oxcarbazepine	*HLA-B*15:02*	MPE, SJS	7.66	9.00	6.50	6.50	7.50	9.29	0.9052	(Su et al., 2016)
	*HLA-B*13:02*	MPE	2.13	3.50	1.50	1.50	1.50	2.86	0.8830	(Lauren et al., 2014)
	*HLA-B*38:02*	MPE	4.26	4.00	5.50	3.00	5.00	3.57	0.3380	(Lv et al., 2013)
Phenytoin	*HLA-B*15:02*	SJS/TEN	7.66	9.00	6.50	6.50	7.50	9.29	0.9052	(Lauren et al., 2014; Su et al., 2016)
	*HLA-A*24:02*	SJS/TEN	11.49	14.50	10.00	11.50	10.00	11.43	0.8546	(Shi et al., 2017)
	*HLA-B*13:01*	SCARs	5.96	3.50	8.00	6.00	6.50	5.71	0.7450	(Yampayon et al., 2017)
	*HLA-B*56:02*	DRESS	0.11	0.00	0.00	0.00	0.00	0.71	1.0000	(Yampayon et al., 2017)
	*HLA-B*15:13*	SJS/TEN, DRESS	0.96	2.50	0.00	0.50	1.00	0.71	0.1330	(Hung et al., 2010; Jaruthamsophon et al., 2016; Chang et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	14.50	8.50	8.50	11.50	7.86	0.4764	(Hung et al., 2010; White et al., 2014)
	HLA-DRB1*16:02	**SJS/TEN**	**5.96**	**3.50**	**10.00**	**7.00**	**6.50**	**1.43**	**0.0470**	(Hung et al., 2010; White et al., 2014)
Lamotrigine	*HLA-A*24:02*	SJS/TEN, MPE	11.49	14.50	10.00	11.50	10.00	11.43	0.8546	(Moon et al., 2015; Shi et al., 2017)
	*HLA-A*31:01*	SCARs	0.85	2.00	0.50	0.00	0.5	1.43	0.8050	(Kim et al., 2017)
	*HLA-A*68:01*	SCARs	0.96	2.00	0.00	1.50	0.00	1.43	0.5730	(Kazeem et al., 2009)
Phenobarbital	*HLA-B*51:01*	SJS/TEN	4.26	5.50	5.00	3.50	3.50	3.57	0.9640	(White et al., 2014)
	*HLA-A*01:01*	SCARs, MPE	2.23	4.50	0.50	0.50	2.50	3.57	0.3250	(Manuyakorn et al., 2016)
	*HLA-B*13:01*	SCARs	5.96	3.50	8.00	6.00	6.50	5.71	0.7450	(Manuyakorn et al., 2016)
Allopurinol	*HLA-B*58:01*	CADRs, SCARs, MPE, SJS/TEN, DRESS	6.38	6.00	5.00	8.00	7.50	5.00	0.8528	(Cristallo et al., 2011; Sukasem et al., 2014; Su et al., 2016; Sukasem et al., 2016)
	*HLA-C*03:02*	SJS/TEN	7.77	7.50	6.00	10.00	9.00	5.71	0.7394	(Cristallo et al., 2011; Li et al., 2017)
	*HLA-A*33:03*	SJS/TEN	11.17	10.50	8.50	10.00	14.50	12.86	0.6808	(Cristallo et al., 2011; Li et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	14.50	8.50	8.50	11.50	7.86	0.4764	(Cristallo et al., 2011)
	*HLA-DRB1*13:02*	SJS/TEN	1.38	3.00	0.00	1.50	1.00	1.43	0.5520	(Cristallo et al., 2011)
	*HLA-DRB1*15:02*	SJS/TEN	14.47	16.00	16.50	17.00	12.50	8.57	0.3745	(Cristallo et al., 2011)
Abacavir	*HLA-B*57:01*	AHS	1.17	1.00	1.00	1.00	1.50	1.43	1.0000	(Sukasem et al., 2014; Dao et al., 2015; Su et al., 2016)
Nevirapine	*HLA-B*35:05*	SJS/TEN, DRESS	1.91	3.00	2.00	2.00	1.00	1.43	0.9400	(Chantarangsu et al., 2009; Sukasem et

(Continued)

TABLE 7 | Continued

Drug	Pharmacogenetics markers	ADR type	Allele frequency (%)						Comparing all five populations (*p*-value)	Reference
			Thai population (n = 470)	Southern (n = 100)	Northern (n = 100)	Northeastern (n = 100)	Central (n = 100)	Bangkok (n = 70)		
										al., 2014; Lauren et al., 2014)
Co-trimoxazole	*HLA-B*38:01*	SJS/TEN	0.00	0.00	0.00	0.00	0.00	0.00	N/A	(Lonjou et al., 2008; White et al., 2014)
	*HLA-B*38:02*	SJS/TEN	4.26	4.00	5.50	3.00	5.00	3.57	0.9230	(Lonjou et al., 2008; White et al., 2014)
	*HLA-B*15:02*	SJS/TEN	7.66	9.00	6.50	6.50	7.50	9.29	0.9052	(Kongpan et al., 2015)
	*HLA-C*06:02*	SJS/TEN	4.26	6.00	2.00	4.00	3.50	6.43	0.5100	(Kongpan et al., 2015)
	*HLA-C*08:01*	SJS/TEN	10.32	14.50	8.50	8.50	11.50	7.86	0.4764	(Kongpan et al., 2015)
Dapsone	*HLA-B*13:01*	SCAR DRESS	5.96	3.50	8.00	6.00	6.50	5.71	0.7450	(Zhang et al., 2013; White et al., 2014; Tempark et al., 2017)
Salazosulfa-Pyridine	*HLA-B*13:01*	DRESS	5.96	3.50	8.00	6.00	6.50	5.71	0.7450	(Yang et al., 2014)
Methazolamide	*HLA-B*59:01*	SJS/TEN	0.00	0.00	0.00	0.00	0.00	0.00	N/A	(White et al., 2014; Yang et al., 2016)
Amoxicillin–Clavulanate	*HLA-A*30:02*	DILI	0.00	0.00	0.00	0.00	0.00	0.00	N/A	(Stephens et al., 2013)
	*HLA-DRB1*15:01*	DILI	8.09	10.50	7.50	6.50	9.50	5.71	0.6862	(Lucena et al., 2011; Stephens et al., 2013)
	*HLA-DQB1*06:02*	DILI	1.49	1.50	1.50	1.00	1.00	2.86	0.9400	(Lucena et al., 2011; Stephens et al., 2013)
Ticlopidine	*HLA-A*33:03*	DILI	11.17	10.50	8.50	10.00	14.50	12.86	0.6808	(Hirata et al., 2008)
Flucloxacillin	*HLA-B*57:01*	DILI	1.17	1.00	1.00	1.00	1.50	1.43	1.0000	(Daly et al., 2009)
Lapatinib	*HLA-DQA1*02:01*	DILI	8.72	8.00	6.00	7.00	11.00	12.86	0.3900	(Spraggs et al., 2011)

ADR, adverse drug reactions; HLA-A, human leukocyte antigen-A; HLA-B, human leukocyte antigen-B; HLA-C, human leukocyte antigen-C; HLA-DRB1, human leukocyte antigen-DRB1; HLA-DQA1, human leukocyte antigen-DQA1; HLA-DQB1, human leukocyte antigen-DQB1; AHS, abacavir hypersensitivity; CADRs, cutaneous adverse drug reactions; DRESS, drug reactions with eosinophilia and systemic symptoms; DILI, drug-induced liver injury; MPE, maculopapular exanthema; SCARs, severe cutaneous adverse reactions; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; N/A, not available; Significant difference p-value < 0.05 by Pearson's chi-square test.
Bolded text: Data analysis result was presented statistical significance (p-value < 0.05).

(**Tables 7**, **8A**). Moreover, co-trimoxazole-induced SJS/TEN associated with *HLA-B*15:02* (7.66%) and *-C*08:01* (10.32%) alleles was higher within Thai population than that of African Americans, Caucasians, Hispanics, and North American. Furthermore, *HLA-A*33:03* allele which is associated with ticlopidine-induced liver injury appeared to be higher (11.17%) than the allele frequencies from Caucasians (Cao et al., 2001), Hispanics (Cao et al., 2001), and North American (Cao et al., 2001). The allele frequencies of *HLA-DQB1*06:02 (IMGT/HLA ID:* HLA00646) allele which is associated with amoxicillin–clavulanate-induced liver injury were found to have higher frequency in African Americans and Caucasians in contrast to the allele frequency from Thai population (**Table 8B**).

Population Structure Analysis

We used PCA to observe potential subgroups within a given 470 Thai individuals. Instead of using only six *HLA* halplotypes (from both class I and II), we extract 403 polymorphism probes and used them in the analysis. PCA revealed three prominent subpopulations in which samples from Northeastern (NE:yellow) and Southern (South:pink) groups were somewhat separated (see plots of PC1 vs. PC2 and PC2 vs. PC3 in **Figure 2**). The third subpopulation—placed in the middle of Northeastern and Southern groups—comprised samples from Central (sky blue), North (green), and Bangkok (BKK:red). The PCA plot between PC1 vs. PC3 did not clearly show subpopulations.

STRUCTURE uses Bayesian to infer/predict a contribution to potential subpopulations (K) from estimated genetic variation frequencies. In our case, 403 *HLA* polymorphism probe signals were used to represent the genetic variations. Since this work recruited volunteers from five regions based on recent demographic information, we set K = 10 to cover these five demographic groups. CLUMPAK reported eight as the best number of genetic groups (subpopulations) for both Evanno's (Δ K) and Pritchard's (likelihood K). **Figure 3** shows patterns of individuals' assignments to K populations. Each row in this figure shows proportion of individuals' contribution to K subpopulations, from K = 2 to K = 9. In particular, K colors on each vertical bar reveal genetic composition (admixture).

TABLE 8A | Comparison of pharmacogenetics markers in Thai and other populations.

Drug	Pharmacogenetics markers	ADR type	Allele frequency (%)						Reference
			Thai population (n = 470)	African Americans (n = 252) (Cao et al., 2001)	Caucasians (n = 265) (Cao et al., 2001)	Hispanics (n = 234) (Cao et al., 2001)	North American (n = 187) (Cao et al., 2001)	Asians (n = 358) (Cao et al., 2001)	
Carbamazepine	*HLA-B*15:02*	SJS/TEN	7.66	0.20	0.00	0.00	0.00	4.87	(Nahoko and Yoshiro, 2013; Sukasem et al., 2014; Su et al., 2016)
	*HLA-A*31:01*	CADRs, SJS/TEN, DRESS, MPE	0.85	0.79	3.21	4.91	7.75	3.06	(Nahoko and Yoshiro, 2013; Sukasem et al., 2014; Su et al., 2016)
	*HLA-B*15:11*	SJS/TEN	0.21	0.00	0.00	0.00	0.00	0.28	(Sukasem et al., 2014; Su et al., 2016)
	*HLA-A*24:02*	SJS/TEN	11.49	2.78	6.60	12.18	22.73	18.94	(Shi et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	0.20	0.00	1.71	2.41	11.28	(Shi et al., 2017)
Oxcarbazepine	*HLA-B*15:02*	MPE, SJS	7.66	0.20	0.00	0.00	0.00	4.87	(Su et al., 2016)
	*HLA-B*13:02*	MPE	2.13	1.20	1.32	1.50	1.87	1.95	(Lauren et al., 2014)
	*HLA-B*38:02*	MPE	4.26	0.00	0.19	0.00	0.00	6.55	(Lv et al., 2013)
Phenytoin	*HLA-B*15:02*	SJS/TEN	7.66	0.20	0.00	0.00	0.00	4.87	(Lauren et al., 2014; Su et al., 2016)
	*HLA-A*24:02*	SJS/TEN	11.49	2.78	6.60	12.18	22.73	18.94	(Shi et al., 2017)
	*HLA-B*13:01*	SCARs	5.96	0.00	0.00	0.00	0.00	3.34	(Yampayon et al., 2017)
	*HLA-B*56:02*	DRESS	0.11	0.00	0.00	0.00	0.00	0.28	(Yampayon et al., 2017)
	*HLA-B*15:13*	SJS/TEN, DRESS	0.96	0.00	0.00	0.00	0.00	0.28	(Hung et al., 2010; Jaruthamsophon et al., 2016; Chang et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	0.20	0.00	1.71	2.41	11.28	(Hung et al., 2010; White et al., 2014)
Lamotrigine	*HLA-A*24:02*	SJS/TEN, MPE	11.49	2.78	6.60	12.18	22.73	18.94	(Moon et al., 2015; Shi et al., 2017)
	*HLA-A*31:01*	SCARs	0.85	0.79	3.21	4.91	7.75	3.06	(Kim et al., 2017)
	*HLA-A*68:01*	SCARs	0.96	2.58	3.02	2.56	5.62	0.28	(Kazeem et al., 2009)
Phenobarbital	*HLA-B*51:01*	SJS/TEN	4.26	1.20	5.66	6.20	11.23	6.69	(White et al., 2014)
	*HLA-A*01:01*	SCARs, MPE	2.23	5.56	15.09	5.98	7.49	1.53	(Manuyakorn et al., 2016)
	*HLA-B*13:01*	DRESS	5.96	0.00	0.00	0.00	0.00	3.34	(Manuyakorn et al., 2016)
Allopurinol	*HLA-B*58:01*	CADRs, SCARs, MPE, SJS/TEN, DRESS	6.38	6.37	1.13	1.07	0.80	7.38	(Cristallo et al., 2011; Sukasem et al., 2014; Su et al., 2016; Sukasem et al., 2016)
	*HLA-C*03:02*	SJS/TEN	7.77	2.78	0.38	1.07	0.27	7.66	(Cristallo et al., 2011; Li et al., 2017)
	*HLA-A*33:03*	SJS/TEN	11.17	3.97	0.57	1.07	0.53	11.70	(Cristallo et al., 2011; Li et al., 2017)
	*HLA-C*08:01*	SJS/TEN	10.32	0.20	0.00	1.71	2.41	11.28	(Cristallo et al., 2011)
Abacavir	*HLA-B*57:01*	AHS	1.17	2.39	4.15	1.92	2.14	0.97	(Sukasem et al., 2014; Dao et al., 2015; Su et al., 2016)
Nevirapine	*HLA-B*35:05*	SJS/TEN, DRESS	1.91	0.00	0.38	0.85	0.00	0.14	(Chantarangsu et al., 2009; Sukasem et al., 2014; Lauren et al., 2014)

(Continued)

TABLE 8A | Continued

Drug	Pharmacogenetics markers	ADR type	Allele frequency (%)						Reference
			Thai population (n = 470)	African Americans (n = 252) (Cao et al., 2001)	Caucasians (n = 265) (Cao et al., 2001)	Hispanics (n = 234) (Cao et al., 2001)	North American (n = 187) (Cao et al., 2001)	Asians (n = 358) (Cao et al., 2001)	
Co-trimoxazole	*HLA-B*38:01*	SJS/TEN	0.00	0.40	2.45	1.71	1.07	0.42	(Lonjou et al., 2008; White et al., 2014)
	*HLA-B*38:02*	SJS/TEN	4.26	0.00	0.19	0.00	0.00	6.55	(Lonjou et al., 2008; White et al., 2014)
	*HLA-B*15:02*	SJS/TEN	7.66	0.20	0.00	0.00	0.00	4.87	(Kongpan et al., 2015)
	*HLA-C*06:02*	SJS/TEN	4.26	11.31	8.68	6.84	5.62	3.62	(Kongpan et al., 2015)
	*HLA-C*08:01*	SJS/TEN	10.32	0.20	0.00	1.71	2.41	11.28	(Kongpan et al., 2015)
Dapsone	*HLA-B*13:01*	DRESS	5.96	0.00	0.00	0.00	0.00	3.34	(Zhang et al., 2013; White et al., 2014; Tempark et al., 2017)
Salazosulfa-Pyridine	*HLA-B*13:01*	DRESS	5.96	0.00	0.00	0.00	0.00	3.34	(Yang et al., 2014)
Methazolamide	*HLA-B*59:01*	SJS/TEN	0.00	0.00	0.00	0.00	0.00	0.56	(Stephens et al., 2013; White et al., 2014; Yang et al., 2016)
Amoxicillin–Clavulanate	*HLA-A*30:02*	DILI	0.00	4.96	0.57	3.42	1.87	0.14	(Stephens et al., 2013; White et al., 2014; Yang et al., 2016)
Ticlopidine	*HLA-A*33:03*	DILI	11.17	3.97	0.57	1.07	0.53	11.70	(Hirata et al., 2008)
Flucloxacillin	*HLA-B*57:01*	DILI	1.17	2.39	4.15	1.92	2.14	0.97	(Daly et al., 2009)

HLA-A, human leukocyte antigen-A; HLA-B, human leukocyte antigen-B; HLA-C, human leukocyte antigen-C; AHS, abacavir hypersensitivity; CADRs, cutaneous adverse drug reactions; DRESS, drug reactions with eosinophilia and systemic symptoms; DILI, drug-induced liver injury; MPE, maculopapular exanthema; SCARs, severe cutaneous adverse reactions; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

TABLE 8B | Comparison of pharmacogenetics markers in Thai and other populations.

Drug	Pharmacogenetics markers	ADR type	Allele frequency (%)					Reference
			Thai population (n = 470)	African Americans (n = 241) (Just et al., 1996)	Caucasians (n = 265) (Begovich et al., 1992)	Japanese (n = 371) (Saito et al., 2000)	Han Chinese (n = 10,689) (Zhou et al., 2016)	
Carbamazepine	*HLA-DRB1*12:02*	SJS/TEN	15.32	0.40	0.00	1.50	7.16	(Shi et al., 2017)
Phenytoin	*HLA-DRB1*16:02*	SJS/TEN	5.96	0.00	0.00	0.90	2.61	(Hung et al., 2010; White et al., 2014)
Allopurinol	*HLA-DRB1*13:02*	SJS/TEN	1.38	8.50	3.40	7.70	3.54	(Cristallo et al., 2011)
	*HLA-DRB1*15:02*	SJS/TEN	14.47	0.00	0.80	10.00	2.91	(Cristallo et al., 2011)
Amoxicillin–Clavulanate	*HLA-DRB1*15:01*	DILI	8.09	16.00	15.80	8.50	12.74	(Lucena et al., 2011; Stephens et al., 2013)
	*HLA-DQB1*06:02*	DILI	1.49	23.20	15.80	8.20	10.69	(Lucena et al., 2011; Stephens et al., 2013)
Lapatinib	*HLA-DQA1*02:01*	DILI	8.72	9.10	13.20	N/A	7.28	(Spraggs et al., 2011)

HLA-DRB1, human leukocyte antigen-DRB1; HLA-DQA1, human leukocyte antigen-DQA1; HLA-DQB1, human leukocyte antigen-DQB1; DILI, drug-induced liver injury; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; N/A, not available.

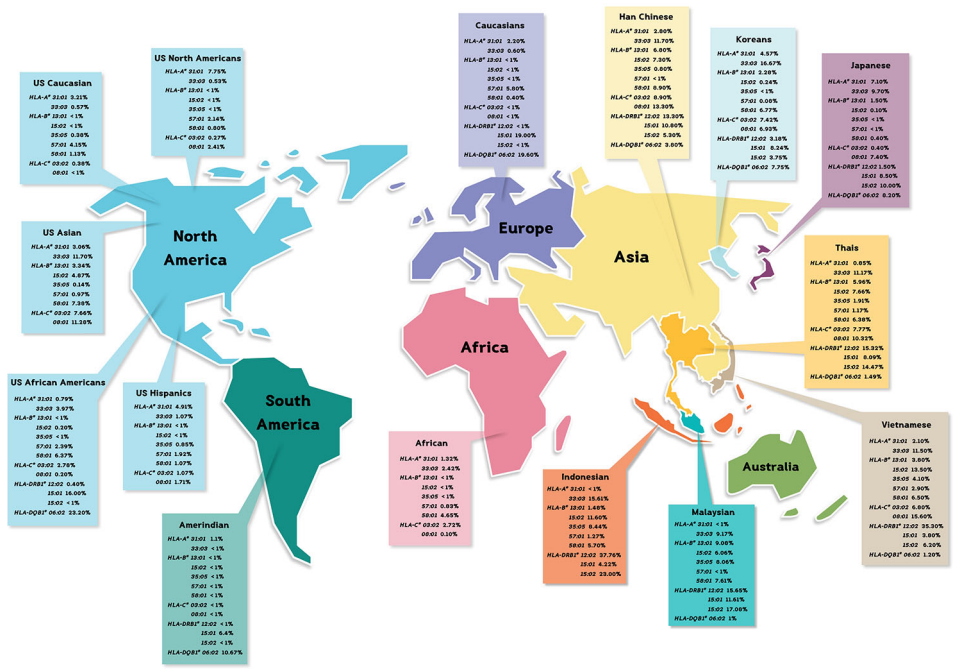

FIGURE 1 | The distribution of pharmacogenetics markers in many populations.

Figure 3 shows the admixture profiles for eight genetic groups (K = 8). All 470 individuals are represented by vertical bars, which are grouped according to their reported five geographical origins. Two distinct admixture patterns of Northeastern and Southern regional groups could be observed, while Bangkok and Central regional groups' admixture patterns tend to be similar.

DISCUSSION

In this study, we examined the frequency distribution of *HLA* class I and II alleles in five regions of Thailand by *HLA*-typing of 470 healthy individuals using PCR-SSOs probe. We found the most prevalent alleles of *HLA* class I and II were *HLA-A*11:01*, *-B*46:01*, *-C* 01:02*, *-DRB1*12:02*, *-DQA1*01:01*, and *-DQB1*05:02*. When compared with the previous study, the allele frequency of *HLA-B,* our frequency report from 470 healthy cohort was similar to the previous one (Puangpetch et al., 2015). Especially, pharmacogenetic markers associated with different ethnic groups such as *HLA-A*33:03*, *-B*15:02*, *-B*13:01*, *-C*03:02*, *-DRB1*12:02*, *-DRB1*15:02*, *-DRB1*16:02*, and *-DQB1*06:02*.

Recent studies confirm that SCARs are caused by certain *HLA* polymorphisms, drugs, peptide, and T cell (Yun et al., 2012; Iasella et al., 2017). There are four phenotypically distinct SCARs including SJS, TEN, DRESS, or drug-induced hypersensitivity syndrome (DIHS) or hypersensitivity syndrome (HSS), and acute generalized exanthematous pustolosis (AGEP) (Marotti, 2012). SJS and TEN are severe life-threatening reactions and are associated with ~5% mortality rate, >30% SJS and >30% TEN (Aihara, 2011). The incidence of SJS/TEN in Southeast Asia (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Vietnam, and Thailand) is largely undetermined. Nevertheless, more and more incidences of culprit drugs have been published, including CBZ (17%), allopurinol (15%), beta-lactam antibiotics (13%), sulfonamide antibiotics (12%), PHT (9%), nonsteroidal anti-inflammatory drugs (8%), LTG (2%), and phenobarbital (1%) (Lee et al., 2013). According to the data from the spontaneous reporting of adverse drug reactions during 1984–2014 by Thailand Health Product and Vigilance Center (HPVC), the culprit drugs causing SJS and TEN in Thailand include sulfamethoxazole and trimethoprim, allopurinol, CBZ, nevirapine containing produce, PHT, amoxicillin, phenobarbital, ibuprofen, tetracycline, piroxicam, diclofenac, rifampicin, ceftriaxone, fluconazole, isoniazid, ciprofloxacin, ethambutol, pyrazinamide, amoxicillin and clavulanic acid, and dapsone. (http://thaihpvc.fda.moph.go.th/thaihvc/Public/News/uploads/hpvc_1_3_4_100538.pdf). DRESS is characterized by fever, cutaneous eruption, internal organ involvement (hepatitis), and hematologic abnormalities (eosinophilia and/or atypical lymphocytosis). The onset of DRESS is approximately 1–8 weeks after drug initiation with the mortality rate of approximately 10% (Criado et al., 2012). From 2004 to 2014, the King Chulalongkorn Memorial Hospital Thailand reported list of drugs that could cause DRESS in Thai patients, including PHT, nevirapine, allopurinol, co-trimoxazole, dapsone, CBZ, isoniazid, ciprofloxacin, clindamycin, dacomitinib, danazol, omeprazole, and sulfadiazine (Hiransuthikul et al., 2016).

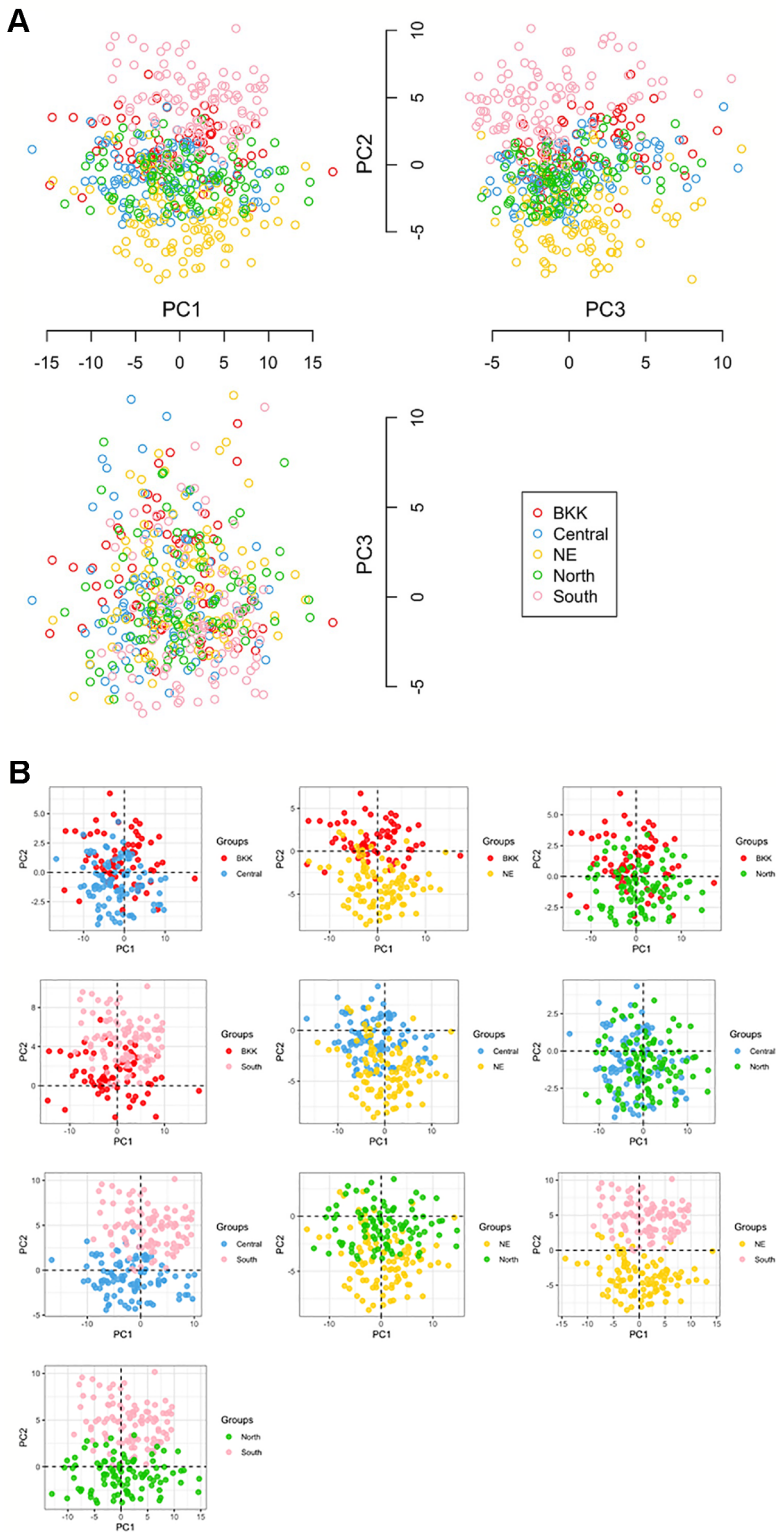

FIGURE 2 | (A) Principal component analysis (PCA) analysis in ggplot library and KRIS library with z-score as the input [five groups: 100 North, 100 NE (Northeastern), 70 BKK (Bangkok), 100 Central, and 100 South]. **(B)** All compare between each population by Principal Component Analysis (PCA).

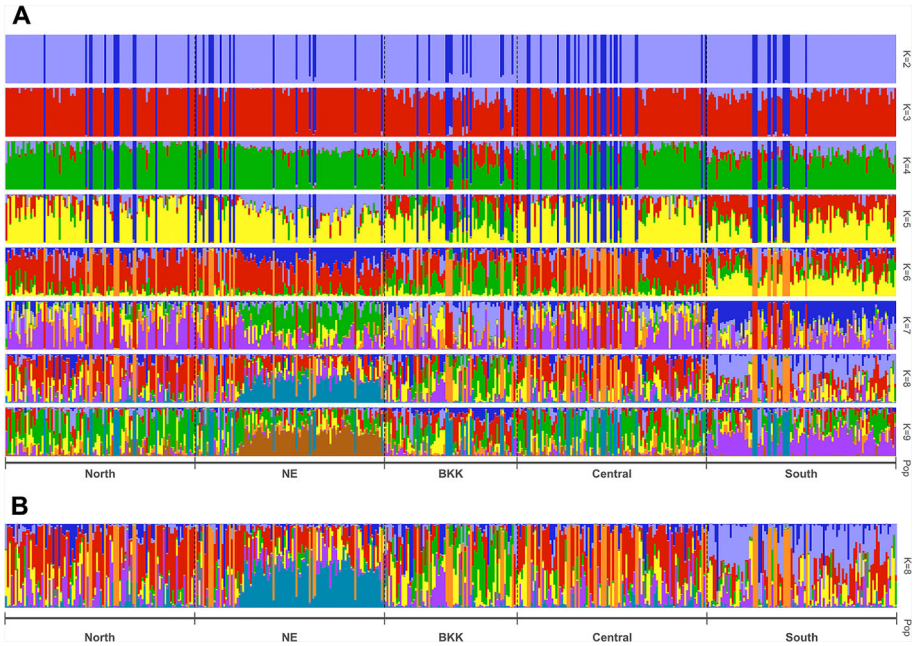

FIGURE 3 | Estimated population structure with z-score as the input based on STRUCTURE analysis with pophelper visualization tool. Bar plot of individual ancestry proportions for the genetic clusters inferred using **(A)** K = 2 to 9, and **(B)** optimal K = 8.

Abacavir is used for treatment of human immunodeficiency virus type I infection (Sukasem et al., 2014; White et al., 2014). Abacavir-hypersensitivity reaction (ABC-HSR) is a major of adverse drug reactions, and its reaction onset occurs during the first 6 weeks of treatment (Chaponda and Pirmohamed, 2011). Previous studies showed the association of *HLA-B*57:01* allele with ABC-HSR in European and African (Mallal et al., 2008; Sukasem et al., 2014; White et al., 2014). The data from 470 Thais showed that the allele frequency of *HLA-B*57:01* was not so common (1.17% of 470): 1.4% Bangkok, 1.5% Central, 1% Northeastern, 1% Northern, and 1% Southern. Our results confirmed the previous study of 986 Thais which revealed 1.5% of *HLA-B*57:01* allele distribution in Thailand (Puangpetch et al., 2015). The range of *HLA-B*57:01* allele distribution was more common (0.97%–4.15%) in African Americans, Caucasians, Hispanics, North American, and Asians (Cao et al., 2001). The distribution of Thai *HLA-B*57:01* allele was closer to Southeast Asian populations: 2.9% of 170 Vietnamese and 1.27% of 237 Indonesian (Hoa et al., 2008; Yuliwulandari et al., 2009). Thus, the allele frequency of *HLA-B*57:01* has confirmed the value of the screening among different ethnicities before initiation of treatment.

CBZ-induced SJS/TEN was strongly associated with *HLA-B*15:02* in Han Chinese, Thais, Vietnamese, Malaysian, and Indian (Ferrell and McLeod, 2008; Locharernkul et al., 2008; Hung et al., 2010; Chang et al., 2011; Nguyen et al., 2015). We found the allele frequency of *HLA-B*15:02* was 0.0766 in Thais, especially, among individuals from Southern Thailand. Particularly, the high expression of HLA-B75 family distribution (*HLA-B*15:02*, *-B*15:11*, and *HLA-B*15:21*) in the Southern group was a genetic factor associated with CBZ-induced SJS/TEN. Nevertheless, the distribution of *HLA-B*15:02* allele in Caucasian and Japanese populations is less than 1% and African Americans is 0.20% (Amstutz et al., 2014; White et al., 2014). Therefore, *HLA-B*15:02* allele is quite ethnic specific as well as having phenotype of SCARs. Furthermore, other studies supported *HLA-A*31:01* associated with CBZ-induced SJS/TEN, DRESS, and maculopapular exanthema (MPE) in European, Japanese, Taiwan Han Chinese, and Korean (McCormack et al., 2011; Ozeki et al., 2011; Amstutz et al., 2014). The allele frequency of *HLA-A*31:01* was higher among North Americans (7.75%), Hispanics (4.91%), and Asians (3.06%), while Thais' and African Americans' were much lower, 0.85% and 0.79%, respectively (Cao et al., 2001). The report also shows that *HLA-A*31:01* is more common in most ethnic groups (Amstutz et al., 2014). The discordant allele frequencies in which Thais harbor less common *HLA-A*31:01* allele supports the need to study more ethnic-specific *HLA* alleles among Southeast Asian populations. Additionally, the cross-reactivity between PHT-induced SJS/TEN and OXC-induced SJS/TEN was associated with *HLA-B*15:02* among Han Chinese (Hu et al., 2011; Su et al., 2016). Previous studies showed that CBZ-, PHT-, and LTG-induced SJS/TEN and MPE were associated with *HLA-A*24:02* (Moon et al., 2015; Shi et al., 2017). We found the distribution of *HLA-A*24:02* allele was 0.1149 in Thailand which was also with similar rate across all five specific regions in Thailand. Although *HLA-A*24:02* allele frequency in African Americans was much lower (0.0278), this *HLA* allele was used as a predictive marker for antiepileptic drug-induced SJS/TEN and MPE (Cao et al., 2001). The frequency distributions of pharmacogenetics markers from the neighboring countries were quite similar to Thais', including Vietnamese: *HLA-B*15:02* (0.135) and *HLA-A*24:02* (0.138), Indonesian: *HLA-B*15:02* (0.116) and *HLA-A*24:02* (0.143), Myanmar: *HLA-B*15:02* (0.088) and *HLA-*

*A*24:02* (0.168), and Malaysian: *HLA-B*15:02* (0.0825) (Hoa et al., 2008; Yuliwulandari et al., 2009; Jinam et al., 2010; Kongmaroeng et al., 2015). Similarly, the association between frequency of specific *HLA* alleles and antiepileptic drug-induced SCARs was investigated in many populations.

Allopurinol-induced SJS/TEN causes disease spectrum, including skin rashes, fever, vasculitis, hepatitis, and epidermal necrosis (Lupton and Odom, 1979; Halevy et al., 2008; Tassaneeyakul et al., 2009). For Thai patients, *HLA-B*58:01* is strongly associated with allopurinol-induced SJS/TEN (odds ratio = 579.0; 95% CI: 29.5–11362.7; p-value < 0.001), DRESS (odds ratio = 430.3; 95% CI: 22.6–8958.9; p-value < 0.001), and MPE (odds ratio = 144.0; 95% CI: 13.9–1497.0; p-value < 0.001) (Sukasem et al., 2016). *HLA-B*58:01* was reported to be present 8%–15% among Han Chinese and Thai population, 0.6% of Japanese, and 0.8% of European population (Hung et al., 2005; Kaniwa et al., 2008; Tassaneeyakul et al., 2009; Somkrua et al., 2011). In this study, the frequency of *HLA-B*58:01* allele was 0.0638 in 470 Thai population, ranging between 0.0500 and 0.0800 when considering at the regional group level. Note that the frequency of *HLA-B*58:01* allele in Thailand was not much different when compared with other populations such as African Americans, Caucasians, Hispanics, North American, Asians, and Southeast Asians (Malaysia, Vietnam, Indonesia, and Myanmar) (Cao et al., 2001; Hoa et al., 2008; Yuliwulandari et al., 2009; Jinam et al., 2010; Kongmaroeng et al., 2015). Therefore, it is likely that the distribution of *HLA-B*58:01* could be used as a universal pharmacogenetic marker for allopurinol-induced CADRs including SJS/TEN, DRESS, and MPE for all ethnicities.

Reports showed the association among Han Chinese leprosy patients and Thai non-leprosy patients between *HLA-B*13:01* and dapsone hypersensitivity reactions (odds ratio 122.1, p-value = 6.038 10^{-12} and odds ratio 20.53, p-value = 6.84 10^{-25}) and dapsone-induced SCARs (odds ratio 54.00, p-value = 0.0001) and dapsone-induced DRESS (odds ratio 60.75, p-value = 0.0001), respectively (Wang et al., 2013; Zhang et al., 2013; Tempark et al., 2017). The distribution of *HLA-B*13:01* allele was absent in Europeans and Africans (Cao et al., 2001; Zhang et al., 2013). Recent publications showed varying distributions of *HLA-B*13:01,* including 1.5% of Japanese, 1%–12% of Indian, 28% of Papuans and Australian aborigines, 2%–20% of Chinese, 2%–4% of Southeast Asians, 1.94% of Koreans, and 6.95% of the previously reported Thai population (Cao et al., 2001; Zhang et al., 2013; Puangpetch et al., 2015; Tempark et al., 2017). This study showed that the frequency of *HLA-B*13:01* allele was 0.0596 which is similar to the previous study in Thailand (Puangpetch et al., 2015). Furthermore, the frequency *of HLA-B*13:01* allele was shown to have a very strong association with many drug-induced DRESS (PHT, phenobarbital, dapsone, and salazosulfapyridine) and Asian populations (Thais, Han Chinese, Malaysian, Vietnamese, Indonesian, and Myanmarese) (Hoa et al., 2008; Yuliwulandari et al., 2009; Jinam et al., 2010; Kongmaroeng et al., 2015). Interestingly, dapsone shows similarity in chemical structure to the sulfonamides; the cross-reactivity of sulfonamide hypersensitivity reactions was also reported (Tomecki and Catalano, 1981). Co-trimoxazole (sulfonamide and trimethoprim) is a sulfonamide antibiotic and the most common culprit drug for SJS/TEN in many countries including Thailand (Barvaliya et al., 2011; Kongpan et al., 2015). The study reported the association of the alleles *HLA-B*38:01* and *HLA-B*38:02* with sulfamethoxazole-induced SJS/TEN in European patients (Lonjou et al., 2008). Furthermore, *HLA-B*15:02* allele was found to be associated with co-trimoxazole-induced SJS/TEN in Thais (odds ratio 3.91, p-value = 0.0037) (Kongpan et al., 2015). In this study, we found the frequencies of *HLA-B*38:01* allele was 0.000, *HLA–B*38:02* allele was 0.043, and *HLA-B*15:02* allele was 0.077. With the presence of these alleles, further investigation should be conducted on other *HLA* alleles and co-trimoxazole-induced SCARs in Thai population.

Drug-induced liver injury (DILI) is rare, but it potentially causes serious idiosyncratic reaction (Kaplowitz, 2005). Previous study reported the association between amoxicillin–clavulanate-induced liver injury and *HLA* haplotypes: *HLA-DRB1*15:01, HLA-DQB1*06:02*, and *HLA-A*30:02* (Stephens et al., 2013). In this study, we found that the frequencies of *HLA-DRB1*15:01* and *HLA-DQB1*06:02* were 0.0809 and 0.0149, respectively. However, *HLA-A**30:02 allele was absent in Thai population. We noticed that the distribution of *HLA-DQB1*06:02* allele in Thai population was much lower than those found in African Americans and Caucasians (Begovich et al., 1992; Just et al., 1996). The distribution of *HLA-DQB1*06:02* was quite similar among Southeast Asian countries (Malaysia, Vietnam, and Myanmar) and was similar to Thais (Hoa et al., 2008; Jinam et al., 2010; Kongmaroeng et al., 2015). Additionally, the frequency of a pharmacogenetic marker associated with ticlopidine-induced hepatotoxicity, *HLA-A*33:03*, was 0.1117 reported by our *HLA* study of Thai cohort. Similarly, the frequencies of this pharmacogenetics marker were 0.1150 for Vietnamese, 0.1516 for Indonesian, 0.1380 for Burmese, and 0.08 for Malaysian (Hoa et al., 2008; Yuliwulandari et al., 2009; Jinam et al., 2010; Kongmaroeng et al., 2015). According to trends of *HLA* prevalence of Southeast Asian, both *HLA-A*33:03* and *HLA-DQB1*06:02* allele should be further investigated in which they can be used as pharmacogenetic markers in Thais and other neighboring populations.

Previous report demonstrated that Thai population contains four prominent substructures using 435,503 single-nucleotide polymorphisms (SNPs) collected from two independent studies comprising 992 Thai individuals (Wangkumhang et al., 2013). The analysis from their work revealed two important concepts: 1) the three main subpopulations are localized in Northern, Northeastern, and Southern parts of Thailand while the members from the fourth group are scattered throughout the country and 2) place of origins of Thai individuals might be discordant with the genetic similarity profile of that place. In other words, people from the north could migrate to the south and stay there for more than three generations. Similar findings were shown in our population genetic analyses in which Northeastern and Southern groups were genetically different while Bangkok and Central groups were mixed and/or scattered to Northern, Northeastern, and Southern groups. In

terms of *HLA*-haplotype distributions over the five regions, we found that the haplotype frequencies in these five regions were slightly different. However, we did not observe novel HLA-haplotypes specific to any subregion. The PCR probes used to call these *HLA*-CLASS I and CLASS II rely on some known collections of *HLA*s and might not be able to predict novel *HLA* haplotypes.

Ethnic-specific genetic variation database is vital to identification of good pharmacogenetic markers in Asian countries such as *CYP2C9*3* associated with PHT-induced SJS/TEN in Taiwanese, Japanese, and Malaysians (Chung et al., 2014). Further studies should be done to confirm pharmacogenetics markers from the ethnic-specific SNP databases. Since there were only six haplotypes and 470 individuals, the challenge of this study was the data analyses obtained from the platform called PCR-SSO probe. To address the lack of genetic polymorphisms in our population genetic study, we extracted probe signals laid across six stretches of *HLA*. Using the probe signals, we observed some distinct as well as cline subpopulations. This discrepancy should be clarified in further study by performing high-resolution DNA typing and recruiting more Thai individuals. In this study, we identified both *HLA* class I and II alleles in Thai population. Furthermore, many *HLA* class I and II alleles were associated with pharmacogenetics markers which might appear exclusively in many populations. Particularly, a database containing distribution of specific *HLA* class I and II alleles will support the development of the pharmacogenetics markers for screening drug hypersensitivity reactions.

DATA AVAILABILITY STATEMENT

The datasets used in this study can be found here https://www.ebi.ac.uk/ipd/imgt/hla using the accession numbers HLA-A*31:01 (IMGT/HLA ID: HLA00097), HLA-A*33:03 (IMGT/HLA ID: HLA00106), HLA-B*13:01 (IMGT/HLA ID: HLA00152), HLA-B*15:02 (IMGT/HLA ID: HLA00165), HLA-B∗15:08 (IMGT/HLA ID: HLA00171), HLA-B∗15:11 (IMGT/HLA ID: HLA00174), HLA-B∗15:21 (IMGT/HLA ID: HLA00184), HLA-B*35:05 (IMGT/HLA ID: HLA00241), HLA-B*57:01(IMGT/HLA ID: HLA00381), HLA-B*58:01 (IMGT/HLA ID: HLA00386), HLA-B*59:01 (IMGT/HLA ID: HLA00389), HLA-C*03:02 (IMGT/HLA ID: HLA00410), HLA-C*06:02 (IMGT/HLA ID: HLA00430), HLA-C*08:01 (IMGT/HLA ID: HLA00445), HLA-DRB1*13:02 (IMGT/HLA ID: HLA00798), HLA-DRB1*15:02 (IMGT/HLA ID: HLA00867) and HLA-DQB1*06:02 (IMGT/HLA ID: HLA00646).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects Faculty of Medicine Ramathibodi Hospital Mahidol University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PS had substantial contributions to the conception, design, analysis, and interpretation of the data, drafting the manuscript, and agrees to be accountable for all aspects of the work. PJ, TJ, NK, CC, JP, CNa, WA, ST, CN and AW had substantial contributions to the conception and analysis of the data and drafting the manuscript. CS had substantial contributions to the conception and design of the work, drafting the work, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and provide approval for publication of the content.

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Risks of Sulpiride-Induced Parkinsonism in Peptic Ulcer and Gastroesophageal Reflux Disease Patients in Taiwan: A Nationwide Population-Based Study

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Background: Sulpiride is a highly selective dopamine D2 receptor antagonist and is commonly used in psychiatric disorders, Tourette syndrome, peptic ulcer disease (PUD), and gastroesophageal reflux disease (GERD). However, sulpiride has been recognized as a potential cause of drug-induced parkinsonism (DIP) for a long time. In this study, we aimed to focus on analysis of sulpiride-induced parkinsonism (SIP) in PUD and GERD patients based on a nationwide population.

Methods: Data were obtained from the Taiwan's National Health Insurance Research Database. The study enrolled 5,275 PUD or GERD patients, of whom were divided into two groups, based on their exposure (1,055 cases) or non-exposure (4,220 cases) to sulpiride.

Results: During the study period (2000–2012), the incidence rate of parkinsonism was 261.5 and 762.2 per 100,000 person-years in the control and sulpiride-treated groups, respectively. For patients with at least 14 days of prescription for sulpiride, the adjusted hazard ratio (aHR) was 2.89, 95% confidence interval (CI): 2.04-4.11. Patients with age more than 65 years (aHR = 4.99, 95% CI = 2.58-9.65), hypertension (aHR = 2.39, 95% CI = 1.49-3.82), depression (aHR = 2.00, 95% CI = 1.38-2.91), and anxiety (aHR = 1.45, 95% CI = 1.01-2.09) had significant higher risk of developing parkinsonism. An average annual cumulative sulpiride dose > 1,103 mg was accompanied by the greatest risk of SIP; sulpiride use for ≥ 9 days is a cut-off point for predicting future SIP.

Conclusion: At the population level, sulpiride may be frequently prescribed and apparently effective for PUD and GERD. SIP is associated with older age, hypertension,

depression or anxiety comorbidities. Physicians should be aware of the neurogenic adverse effects, even when the drug is only used in low-dose or a short duration.

Keywords: sulpiride, drug-induced parkinsonism, peptic ulcer disease, gastroesophageal reflux disease, population-based study

INTRODUCTION

Sulpiride is a substituted benzamide and is classified as a low potent atypical antipsychotics. It is a weak but highly selective dopamine D2 receptor antagonist (Jenner et al., 1982; Caley and Weber, 1995; Mauri et al., 1996). It is used to treat a variety of psychiatric disorders including depression, somatoform disorders, and schizophrenia (Kato, 1993; Mucci et al., 1995; Mauri et al., 1996; Rouillon et al., 2001). Sulpiride is one of the neuroleptics in treating tics for Tourette syndrome (Eddy et al., 2011). In the field of gastroenterology, it is also used as an antiemetic and antidyspeptic drug for peptic ulcer disease (PUD) and gastroesophageal reflux disease (GERD) for more than 50 years (Caldara et al., 1978; Lam et al., 1979; Tatsuta et al., 1986; Trabucchi et al., 1991; Desai and Parmar, 1994).

Sulpiride is commonly used in Asia, Europe, Central America, South America, and South Africa. However, it is not approved in the United States, Canada, or Australia (Caley and Weber, 1995). The safety profile of sulpiride is similar to other typical antipsychotics. Its common adverse effects (1 and <10% by the Council for International Organizations of Medical Sciences (CIOMS) frequency rating) include sedation, drowsiness, insomnia, weight gain, increased hepatic enzyme, constipation, maculo-papular rash, hyperprolactinemia, breast pain, galactorrhoea, and extrapyramidal disorder (Standish-Barry et al., 1983; Gerlach et al., 1985; Lepola et al., 1989; Mauri et al., 1996). The extrapyramidal manifestations caused by sulpiride include dystonia, akathisia, parkinsonism, and tremor (Eapen et al., 1993; Mauri et al., 1996; Lai et al., 2014). Recently, two big data-based studies and one meta-analysis have focused on drug-induced parkinsonism (DIP) (Martino et al., 2018; Byun et al., 2019; Kim et al., 2019). The first population-based study concluded that use of propulsives and antipsychotics including sulpiride had a significant association with the increased risk of DIP, depending on recency and cumulative dose (Kim et al., 2019). Another population-based research found that annual prevalence of DIP has increased, and the usage of specific offending medications is the major cause (Byun et al., 2019). In the meta-analysis study focused on second-generation antipsychotics, the prevalence estimates are of 15.3% for acute dystonia, 16.4% for akathisia, 29.3% for parkinsonism, and 28.2% for tremor induced by sulpiride (Martino et al., 2018).

PUD and GERD are popular gastrointestinal disorders that can cause troublesome symptoms, and have a significant impact on quality of life (Lanas and Chan, 2017; Yamasaki et al., 2018). However, to the best of our knowledge, no population-based analyses have been performed for sulpiride-induced parkinsonism (SIP) in these subjects. This study aimed to investigate the risk factors and the cumulative daily dose associated with SIP.

METHODS

Data Source

Taiwan built a single-payer National Health Insurance program (Taiwan NHI) in 1995, and nearly 99% of Taiwan's citizens were enrolled in the program currently. The database named National Health Insurance Research Database (NHIRD), which included the detailed records of outpatients, hospitalization, treatment, prescription, and other medical services for each patient. In this study, we conducted the analyses by using Longitudinal Health Insurance Database (LHID), which is the subset database and randomly selected 1 million study subjects from NHIRD. The privacy of each patient was protected by encrypting the identification number before the database is released. All diagnoses in Taiwan NHI are coded according to the International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM). The Research Ethics Committee of China Medical University and Hospital in Taiwan approved the study (CMUH-104-REC2-115-R3).

Study Population

To clarify the association between PUD or GERD patients with or without sulpiride and parkinsonism, we defined two cohorts: PUD or GERD patients (ICD-9-CM 533, 530.11, 530.81) with at least 14 days of prescription for sulpiride (ATC code: N05AL01) (case), and PUD or GERD patients without any sulpiride usage record (control). The index date was defined as the starting date of receiving sulpiride therapy, and followed up until patients firstly diagnosed with Parkinson's disease (PD, ICD-9-CM 332) or parkinsonism (ICD-9-CM 333, excluding 333.1-333.8), or withdrawn from NHIRD, or after the date December 31, 2013.

The comorbidities were important confounding factors in NHIRD studies. We defined comorbidities with at least twice outpatients or once hospitalization of diagnoses before index date, including hypertension (ICD-9-CM 401-405), diabetes (ICD-9-CM 250), hyperlipidemia (ICD-9-CM 272), depression (ICD-9-CM 296.2, 296.3, 300.4, 311), anxiety (ICD-9-CM 300.00), and sleep disorder (ICD-9-CM 307.4, 780.5). Patients with PD (ICD-9-CM 332), parkinsonism (ICD-9-CM 333), stroke (ICD-9-CM 430-438), dementia (ICD-9-CM 290, 294, 331.0), head injury (ICD-9-CM 310.2, 800, 801, 803, 804, 850-854, and 959.01), or hydrocephalus (ICD-9-CM 331.3, 331.4, 331.5, 741.0, 742.3) before the index date, those who used antipsychotics (ATC code N05A) during the study period, and patients with age < 20 years or > 90 years were excluded in our study. Each case was propensity matched by age, gender, index year, first sulpiride prescription date, hypertension, diabetes, hyperlipidemia, depression, anxiety, and sleep disorder with four controls (**Figure 1**).

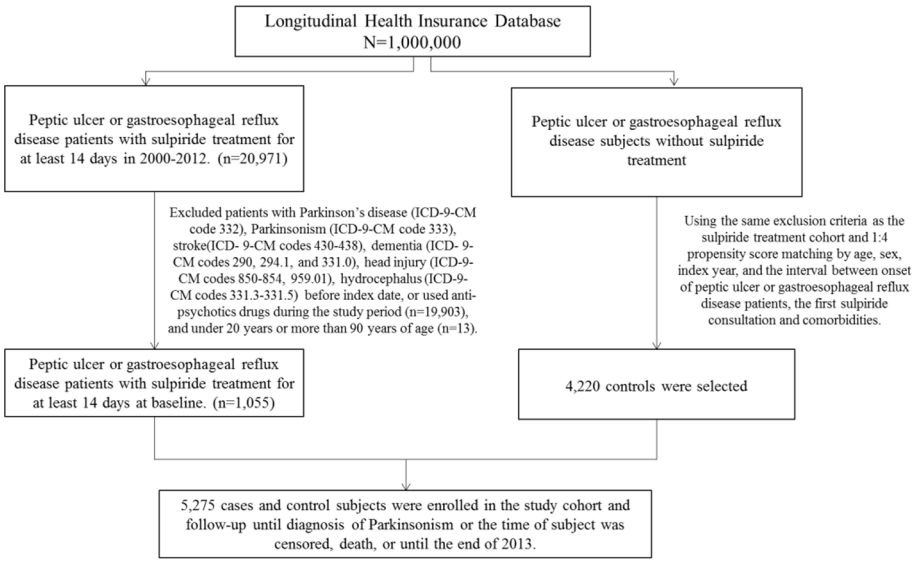

FIGURE 1 | Selection process of patients in the two study cohorts.

Statistical Analysis

To compare the difference between sulpiride and the comparison cohorts, we use two-sample t-test for continuous variable and chi-square test for categorical variable. The incidence rate (per 100,000 person years) of parkinsonism was calculated for both cohorts. The Kaplan-Meier method was used to plot the cumulative incidence curves for each cohort, and log rank test was applied to assess the difference of two survival curves. We estimated hazard ratios (HRs), adjusted hazard ratio (aHR), and 95% confidence intervals (CIs) for risk of parkinsonism in sulpiride, and the comparison cohort by using crude and adjusted Cox proportional hazard models. We also stratified the annual mean sulpiride prescription days, annual mean sulpiride dosage, cumulative defined daily dose (cDDD) of sulpiride into two levels by median, and calculated the risk of parkinsonism in each group.

All statistical analyses were performed using SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC). The figure of cumulative incidence curve was plotted by R software. The significant level was set at less than 0.05 for two-side testing of p-value.

RESULTS

We totally enrolled 5,275 study subjects (**Table 1**), including 1,055 cases and 4,220 controls in this study. Among the patients, about 68.7% were female and the dominant age group was 45 to 65 years. The mean ages were 52.9 and 52.3 years in control and case group, respectively. The distribution of demographic and comorbidities had no significant difference between two groups after propensity score matching ($p > 0.05$).

Table 2 presented the risk factors of parkinsonism among PUD or GERD patients. Patients with at least 14 days of prescription for sulpiride (aHR = 2.89, 95% CI = 2.04-4.11), age more than 65 years (aHR = 4.99, 95% CI = 2.58-9.65), hypertension (aHR = 2.39, 95% CI = 1.49-3.82), depression (aHR = 2.00, 95% CI = 1.38-2.91), and anxiety (aHR = 1.45, 95% CI = 1.01-2.09) had significant higher risk of developing parkinsonism after adjusted by age, gender, and comorbidities.

In our study, the incidence rate of DIP in PUD or GERD patients under sulpiride exposure was 762.2 per 100,000 person-years. **Figure 2** demonstrated significant higher cumulative incidence of parkinsonism in the sulpiride cohort, compared to the non-sulpiride cohort ($p < 0.001$).

The multivariate stratified analysis was conducted and shown in **Table 3**. The incidence rate of parkinsonism was 261.5 and 762.2 per 100,000 person-years in the control and sulpiride-treated group respectively. The sulpiride treatment among PUD or GERD patients increased the risk of parkinsonism; female (aHR = 3.12, 95% CI = 2.02-4.81), male (aHR = 2.53, 95% CI = 1.39-4.60), age less than 45 years (aHR = 8.79, 95% CI = 2.69-28.73), age 45 to 65 years (aHR = 3.85, 95% CI = 2.17-6.84), age more than 65 years (aHR = 1.72, 95% CI = 1.02-2.90), those with hypertension (aHR = 2.24, 95% CI = 1.49-3.39), diabetes (aHR = 2.33, 95% CI = 1.26-4.30), hyperlipidemia (aHR = 2.03, 95% CI = 1.22-3.38), depression (aHR = 2.26, 95% CI = 1.29-3.97), anxiety (aHR = 1.87, 95% CI = 1.07-3.25), and sleep disorder (aHR = 2.41, 95% CI = 1.50-3.87).

The analyses of sulpiride usage were stratified by medication duration (per year), dosage (per year), and cumulative defined daily dose during the study period and classified by median in each group respectively (**Table 4**). Compared to patients without sulpiride, patients with sulpiride have more than 9 days per year (aHR = 4.28, 95% CI = 2.88-6.36), more than 1,103 mg per year

TABLE 1 | Demographic characteristics, comorbidities of PUD or GERD patients with or without sulpiride in Taiwan during 2000-2012.

Variable	PUD or GERD patients			p-value
	Total N=5275 n	Non-Sulpiride n=4220 n (%)/mean ± SD	Sulpiride n=1055 n (%)/mean ± SD	
Gender				0.767
Female	3620	2900 (68.7)	720 (68.2)	
Male	1655	1320 (31.3)	335 (31.8)	
Age at baseline				0.212
<45	1607	1263 (29.9)	344 (32.6)	
45-65	2505	2025 (48)	480 (45.5)	
>65	1163	932 (22.1)	231 (21.9)	
Mean(SD) ‡		52.9 (14.5)	52.3 (14.8)	0.274
Baseline comorbidity				
Hypertension	2315	1857 (44.0)	458 (43.4)	0.729
Diabetes	1334	1072 (25.4)	262 (24.8)	0.704
Hyperlipidemia	2129	1710 (40.5)	419 (39.7)	0.633
Depression	1530	1205 (28.6)	325 (30.8)	0.150
Anxiety	2132	1695 (40.2)	437 (41.4)	0.457
Sleep disorder	3121	2518 (59.7)	603 (57.2)	0.138

Chi-square test, Student's t-test‡.
SD, standard deviation.

TABLE 2 | Cox model measured hazard ratio and 95% confidence intervals of parkinsonism associated with or without sulpiride and covariates among PUD or GERD patients.

Characteristics	Event no. (n=131)	Crude		Adjusted	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Sulpiride					
No	78	Ref.		Ref.	
Yes	53	2.91 (2.06-4.13)	<0.001	2.89 (2.04-4.11)	<0.001
Gender					
Female	85	Ref.		Ref.	
Male	46	1.18 (0.82-1.68)	0.374	1.13 (0.78-1.62)	0.515
Age at baseline					
<45	13	Ref.		Ref.	
45-65	48	2.64 (1.43-4.87)	0.002	1.72 (0.90-3.29)	0.099
>65	70	9.39 (5.19-16.99)	<0.001	4.99 (2.58-9.65)	<0.001
Baseline comorbidity					
Hypertension	102	5.14 (3.40-7.78)	<0.001	2.39 (1.49-3.82)	<0.001
Diabetes	49	2.03 (1.42-2.89)	<0.001	1.10 (0.75-1.61)	0.637
Hyperlipidemia	71	2.02 (1.43-2.85)	<0.001	1.04 (0.72-1.51)	0.842
Depression	53	1.97 (1.39-2.79)	<0.001	2.00 (1.38-2.91)	<0.001
Anxiety	61	1.68 (1.19-2.38)	0.0032	1.45 (1.01-2.09)	0.044
Sleep disorder	76	1.29 (0.91-1.83)	0.1601	0.78 (0.53-1.14)	0.205

HR, hazard ratio; CI, confidence interval;
Adjusted HR: adjusted for age, gender, and comorbidities in Cox proportional hazards regression.

(aHR = 4.63, 95% CI = 3.12-6.86), less than 6.75 cDDD (aHR = 2.50, 95% CI = 1.51-4.13), and more than 6.75 cDDD (aHR = 3.20, 95% CI = 2.13-4.81) during the study period with a significant higher risk of developing parkinsonism.

DISCUSSION

Sulpiride, with molecular formula $C_{15}H_{23}N_3O_4S$, is a selective dopamine D2 receptor antagonist. Because of low lipophilic solubility, it crosses the blood-brain barrier poorly and is mainly excreted unchanged in the urine. Excessive drug accumulation could occur in the elderly or patients with renal dysfunction (Caley and Weber, 1995; Mauri et al., 1996). As other antipsychotics, the main mechanism of SIP is to cause D2 receptor blockade in the striatum, which eventually leads to disinhibition of GABA- and encephalin-containing striatal neurons at the origin of the indirect pathway without alteration of the direct pathway, followed by disinhibition of the subthalamic nucleus (Shin and Chung, 2012). At the same

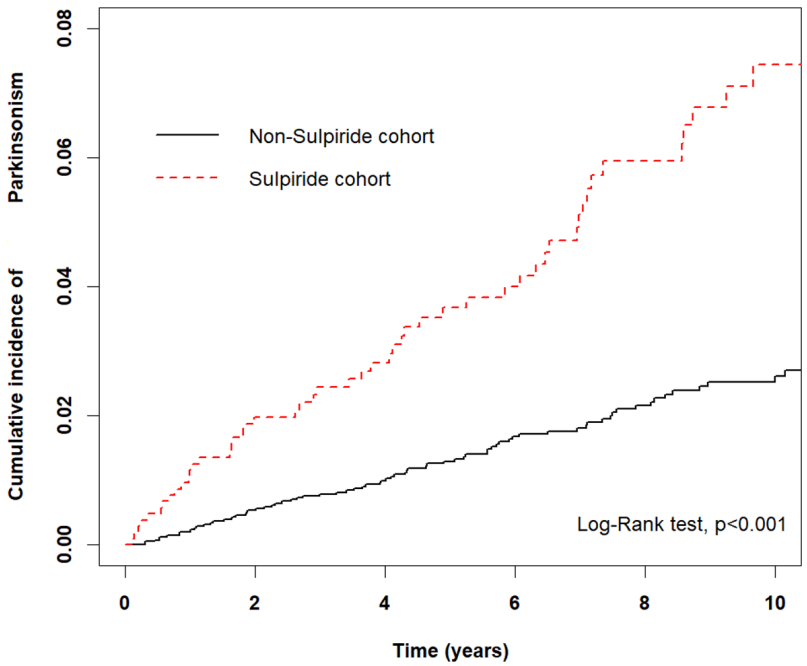

FIGURE 2 | Kaplan-Meir method to determine the cumulative incidence of parkinsonism compared between the sulpiride and non-sulpiride cohort.

TABLE 3 | Incidence rates, hazard ratio and confidence intervals of parkinsonism in different stratification.

Variables	Control N = 4220			Case N = 1055			Case VS. Control			p-value
	Event	Person years	IR	Event	Person years	IR	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)	
Overall	78	29828	261.5	53	6954	762.2	2.91 (2.06-4.13)	< 0.001	2.89 (2.04-4.11)	< 0.001
Gender										
Female	50	20454	244.5	35	4714	742.4	3.04 (1.97-4.68)	< 0.001	3.12 (2.02-4.81)	< 0.001
Male	28	9374	298.7	18	2239	803.9	2.69 (1.49-4.87)	0.001	2.53 (1.39-4.60)	0.002
Age at baseline										
<45	4	9872	40.5	9	2476	363.5	9.11 (2.80-29.60)	< 0.001	8.79 (2.69-28.73)	< 0.001
45-65	24	14120	170	24	3185	753.5	4.39 (2.49-7.74)	< 0.001	3.85 (2.17-6.84)	< 0.001
>65	50	5836	856.7	20	1293	1547.3	1.81 (1.08-3.04)	0.025	1.72 (1.02-2.90)	0.042
Baseline comorbidity										
Hypertension	67	12154	551.3	35	2807	1246.8	2.26 (1.50-3.40)	< 0.001	2.24 (1.49-3.39)	< 0.001
Diabetes	34	6841	497	15	1499	1001.0	1.98 (1.08-3.64)	0.028	2.33 (1.26-4.30)	0.007
Hyperlipidemia	49	11012	445	22	2549	862.9	1.94 (1.17-3.20)	0.010	2.03 (1.22-3.38)	0.006
Depression	33	7386	446.8	20	2029	985.7	2.21 (1.27-3.86)	0.005	2.26 (1.29-3.97)	0.004
Anxiety	43	9936	432.8	18	2590	695.1	1.64 (0.95-2.85)	0.077	1.87 (1.07-3.25)	0.027
Sleep disorder	49	15436	317.4	27	3533	764.1	2.42 (1.51-3.87)	< 0.001	2.41 (1.50-3.87)	< 0.001

IR, incidence rates, per 100,000 person-years; HR, hazard ratio; CI, confidence interval.
Adjusted HR: adjusted for age, gender, and comorbidities in Cox proportional hazards regression.

time, sulpiride has higher 5-HT2A antagonism, with both of the pharmacological characteristics contributing to the risk of developing SIP. Among the second-generation antipsychotics, sulpiride has the highest prevalence of parkinsonism and tremor, even higher than that seen with haloperidol (Martino et al., 2018).

This was the first population-based study, which examined a complete picture of risk of SIP in PUD or GERD patients by using matched cohorts and a long-term follow-up period. The incidence rates of DIP in general population were 3.3 per 100,000 person-years in the United States, and 13.9 per 100,000 person-years in Korea (Savica et al., 2017; Han et al., 2019). The incidence rate of DIP in PUD or GERD patients under sulpiride exposure in Taiwan was reported in our results section, and comparable to that of United States and Korea. Therefore, physicians should be aware for the early signs of

TABLE 4 | Incidence and adjusted hazard ratio of parkinsonism stratified by duration (per year), dosage (per year), and cumulative defined daily dose of sulpiride therapy in PUD or GERD patients.

Medication exposed	Event	Person year	IR	Adjusted HR (95% CI)	p-value
Non-Sulpiride	78	29828	261.5	Ref.	
Sulpiride					
<9 days	15	3988	376.1	1.58 (0.91-2.75)	0.105
≥9 days	38	2904	1274.1	4.28 (2.88-6.36)	<0.001
<1103 mg	15	3997	375.3	1.50 (0.86-2.61)	0.151
≥1103 mg	38	2943	1291.1	4.63 (3.12-6.86)	<0.001
<6.75 cDDD	19	3070	618.8	2.50 (1.51-4.13)	<0.001
≥6.75 cDDD	34	3870	878.6	3.20 (2.13-4.81)	<0.001

IR, incidence rates, per 100,000 person-years; HR, hazard ratio; CI, confidence interval. Adjusted HR: adjusted for age, gender, and comorbidities in Cox proportional hazards regression.

parkinsonism in the PUD or GERD patients treated with sulpiride.

In our study, subjects in all three age levels revealed significant risks of SIP (age less than 45 years, aHR = 8.79, 95% CI = 2.69-28.73; age 45 to 65 years, aHR = 3.85, 95% CI = 2.17-6.84; age more than 65 years, aHR = 1.72, 95% CI = 1.02-2.90); the elder subgroup had the highest risk (aHR =4.99, 95% CI: 2.58–9.65). Age is the most obvious risk factor for DIP because nigral dopaminergic neuronal cells degenerate with age (Shin and Chung, 2012). Female gender is considered to be a risk factor for DIP because estrogen can suppress the expression of dopamine receptors (Bedard et al., 1977; Shin and Chung, 2012). However, in our study, both male and female patients with sulpiride treatment showed a higher tendency to develop parkinsonism when compared to the control subjects.

Based on previous studies, psychological stress had been treated as a risk factor for PUD or GERD patients (Levenstein et al., 2015). Chronic stress may lead to an ulcerogenic effect on corticosterone (Zhang et al., 2012). PUD and GERD was more common among people with anxiety and mood disorders (Lim et al., 2014; Choi et al., 2018). Although sulpiride is reported to be effective to depressive or anxious symptoms (Kato, 1993), this study showed patients with depression or anxiety comorbidity had significant higher risk for developing DIP. In fact, sulpiride is not included in the evidence-based clinical practice guidelines for PUD and GERD at present (Iwakiri et al., 2016; Satoh et al., 2016). For the pharmacologic treatment of depression or anxiety comorbidity, selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs) (Craske and Stein, 2016) is recommended. Therefore, careful considerations should be required for the continue usage of sulpiride for PUD and GERD.

Sulpiride obtained indications from Taiwan FDA to treat schizophrenia (dosage 300–600 mg/day, maximal dosage 1,200 mg/day), depression (dosage 150–300 mg/day, maximal dosage 600 mg/day), and gastric ulcer (dosage 150 mg/day) (Huang et al., 2019). Our study showed that an average annual sulpiride cumulative dosage of >1,103 mg granted the greatest risk of parkinsonism. Sulpiride used for >9 days or ≥6.75 cDDD is a cut-off point for predicting parkinsonism in the future. At the same time, the aHRs changed from 2.50 (95%CI = 1.51–4.13) to 3.20 (95%CI = 2.13–4.81) in cDDD from <6.75 to ≥6.75. It is easy to exceed the risk dosage, so our findings suggested that physicians should prescribe sulpiride in a short term and low dose manner to treat the PUD and GERD patients.

This study has certain limitations that should be considered while interpreting the results. Firstly, NHIRD does not contain the detailed information regarding diet, alcohol consumption, smoking habits, socioeconomic status, living environment, inactivity, or family history, despite the aforementioned factors being the potential risk factors for parkinsonism. Changes in these factors may affect the results. Secondly, although the secondary database research lacks important clinical information such as history, physical evaluation, and clinical course, some of the patients may have been wrongly classified. To eliminate this limitation, we excluded PUD or GERD subjects before sulpiride treatment with a history of PD, parkinsonism, stroke, dementia, head injury, and hydrocephalus. After sulpiride treatment for at least 14 days, we included subjects with PD and parkinsonism. Other limitations in our study that worth to be discussed may include: 1) Depression is recognized to occur as a first sign of parkinsonism, sometimes long before even detectable motor symptoms occur or being diagnosed (Lian et al., 2019). 2) In addition, anxiety can be an accompanying symptom of depression (Koutsimani et al., 2019). 3) There are still large number of patients with depression and anxiety, and their use of antidepressants like SSRIs and SNRIs may influence our conclusion, although our study excluded the individuals with antipsychotics treatment. Finally, all data in the NHIRD are anonymous. Therefore, relevant clinical variables such as body mass index, neuroimaging results, and serum laboratory data were unavailable for the study subjects. However, data related to sulpiride and parkinsonism diagnosis were highly reliable.

CONCLUSION

In conclusion, sulpiride may be frequently prescribed and apparently effective for PUD and GERD. However, it is not included in clinical practice guidelines currently. SIP is associated with older age, and comorbidities of hypertension, depression, or anxiety. Parkinsonism could be induced, even exposing in a low-dose or a short duration. Physicians should be aware of the neurogenic adverse effects.

DATA AVAILABILITY STATEMENT

The dataset used in this study is held by the Taiwan Ministry of Health and Welfare (MOHW). MOHW must approve the application to access this dataset. Any researcher interested in accessing this dataset can submit an application form to MOHW requesting access. Please contact the staff of MOHW (Email:

stcarolwu@mohw.gov.tw) for further assistance. The address of Taiwan Ministry of Health and Welfare is No.488, Sec. 6, Zhongxiao E. Rd., Nangang Dist., Taipei City 115, Taiwan (R.O.C.). Phone: +886-2-8590-6848. All relevant data are detailed in the manuscript.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Research Ethics Committee of China Medical University and Hospital in Taiwan. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

C-YW and W-MK proposed the research idea, wrote the results and discussion, and contributed to the literature review. M-CL performed the analysis. Y-HY and CYH supported the literature review and helped revise the manuscript. Y-HY and CYH provided clinical suggestions. I-ST supported data analysis and prepared the manuscript for submission. All authors read and approved the final manuscript.

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Prevalence and Accuracy of Information on CYP2D6, CYP2C19, and CYP2C9 Related Substrate and Inhibitor Co-Prescriptions in the General Population: A Cross-Sectional Descriptive Study as Part of the PharmLines Initiative

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Background: Drug-drug interaction (DDI) is one of the main contributors to adverse drug reactions and therefore, it is important to study its frequency in the population. We aimed to investigate frequency and concordance on CYP2D6, CYP2C19, and CYP2C9 (CYP2D6/2C19/2C9)-mediated potential DDIs at the Lifelines cohort and linked data from the pharmacy database IADB.nl.

Methods: As part of the University of Groningen PharmLines Initiative, data were collected on CYP2D6/2C19/2C9-related substrate/inhibitors from entry questionnaires of Lifelines participants and linked information from the pharmacy database IADB.nl. CYP2D6/2C19/2C9 related co-prescriptions were divided based on the type of drugs i.e. chronically used medication (CM) or occasionally used medication (OM). This resulted in the combination of two chronically used drugs (CM-CM), chronically and occasionally used medication (CM-OM), and two occasionally used drugs (OM-OM). To measure the agreement level, cohen's kappa statistics and test characteristics were used. Results were stratified by time window, gender, and age.

Results: Among 80,837 medicine users in the Lifelines, about 1–2 per hundred participants were exposed to a CYP2D6/2C19/2C9-mediated potential DDI. Overall, the overlapping time window of three months produced the highest mean kappa values between the databases i.e. 0.545 (95% CI:0.544–0.545), 0.512 (95% CI:0.511–0.512), and 0.374 (95% CI:0.373–0.375), respectively. CM-CM had a better level of agreement

(good) than CM-OM (fair to moderate) and OM-OM combination (poor to moderate). The influence of gender on concordance values was different for different CYPs. Among older persons, agreement levels were higher than for the younger population.

Conclusions: CYP2D6/2C19/2C9-mediated potential DDIs were frequent and concordance of data varied by time window, type of combination, sex and age. Subsequent studies should rather use a combination of self-reported and pharmacy database information.

Keywords: CYP2D6, CYP2C19, CYP2C9, drug-drug-interaction, Lifelines, IADB.nl

INTRODUCTION

Drug-drug interactions (DDIs) are an important contributor to adverse drug reaction leading to hospitalization or mortality (Doucet et al., 1996; Montane et al., 2018). CYP2D6, CYP2C19, and CYP2C9 (CYP2D6/2C19/2C9), subtypes of CYP450 drug metabolizing enzymes, are commonly involved in mediating partly inappropriate DDIs as these enzymes metabolize a wide variety of drugs in clinical practice (Flockhart and Oesterheld, 2000; Bahar et al., 2017b). CYP2D6/2C19/2C9 are highly polymorphic enzymes and the genetic polymorphisms produce inter-individual variabilities in drug metabolisms ranging from poor to accelerated metabolic activities (Zanger and Schwab, 2013). Consequently, the clinical impact of CYP2D6/2C19/2C9-mediated DDIs might be variable from person to person and depends on his/her genetic profile (Bahar et al., 2017a). The information on the clinical relevance and management of DDIs mediated by different CYP2D6/2C19/2C9 genotypes is therefore needed. In order to provide the information, the first step that needs to be done is to generate the data about the burden and type of potential DDIs mediated by these enzymes in the general population.

Estimation of the prevalence rate of a potential DDI is commonly performed using self-reporting methods in which patients are interviewed or filled out a self-administered questionnaire (Van den Brandt et al., 1991; Classen et al., 2007; Secoli et al., 2010). However, this kind of assessment is prone to information bias, because of inaccurate recall, which may influence the validity of results (Rockenbauer et al., 2001; West et al., 2005). Hence, it is important to validate drug information collected with self-reporting methods (Haapea et al., 2010; Hafferty et al., 2018).

The Lifelines cohort is a Dutch three-generation population cohort that provides a wide variety of medical and non-medical data, genomic information, and data on medication use (Stolk et al., 2008; Scholtens et al., 2014). The Lifelines cohort, as a prospective and long-term database, offers possibilities in pharmaco-epidemiological studies, such as assessing the impact of gene polymorphism on the magnitude of DDIs in the population. However, currently not much is known about the frequency, type and validity of potential DDIs in the open population.

This study has both a methodological and an epidemiological aim: we studied the frequency of potentially interacting substrates and inhibitors of the CYP2D6/2C19/2C9 and the concordance level of the information derived by self-reported drug use and an analysis of data from a drug-use database. For the latter aim, information as observed in the Lifelines cohort was compared with data from a prescription database, the University of Groningen prescription database IADB.nl, across type of medications, sex, and age (Visser et al., 2013; Sediq et al., 2018). A prescription database is regarded as an accurate database and not to be influenced by so-called recall bias (Monster et al., 2002; Schneeweiss and Avorn, 2005). Additionally, IADB.nl has been proven a reliable database in many pharmaco-epidemiological studies (Daud et al., 2017; Alfian et al., 2018; Bahar et al., 2018b).

MATERIALS AND METHODS

The PharmLines Initiative is a university wide project in which the data of the Lifelines Cohort study have been linked to the University of Groningen prescription database IADB.nl. The project was started in 2017 by the Groningen Research Institute of Pharmacy, Departments of Epidemiology and Clinical Pharmacy, Department of Pharmacology of the University Medical Center Groningen and the Lifelines Cohort Study (https://www.lifelines.nl/researcher/cohort-and-biobank) (Sediq et al., 2018).

The Lifelines Cohort

The Lifelines cohort covers 167,729 participants from the Northern part of the Netherlands, aged 6 months until 93 years old, which were recruited from 2006 until 2013 (Stolk et al., 2008; Scholtens et al., 2014). It is an observational cohort study intended to facilitate research on the contribution and interaction between environmental, genetic, and phenotypic aspects in the development of chronic diseases and healthy aging (Stolk et al., 2008; Scholtens et al., 2014). The recruited participants will be followed for at least 30 years and are asked to complete a questionnaire every 1.5 years. In addition, once every 5 years, the participants have a comprehensive physical examination (Stolk et al., 2008; Scholtens et al., 2014). Baseline questionnaires included questions about general information, lifestyle and environment, psychosocial aspects, and health (including medication use) (Stolk et al., 2008;

Scholtens et al., 2014; Klijs et al., 2015). The medication use information were collected in two ways i.e. a) patients filled out a questionnaire or b) patients carried the medication at the time of interview (Sediq et al., 2018). The medication data regarding their current prescription and dose were recorded and classified using the Anatomical Therapeutic Chemical (ATC) coding scheme (Stolk et al., 2008; Scholtens et al., 2014). The Lifelines population is multigenerational and generally representative of the Dutch population resided in the Northern part of the Netherlands (Klijs et al., 2015).

University of Groningen IADB.nl Database

The University of Groningen prescription database IADB.nl has recorded prescriptions from community pharmacies in the Netherlands since 1994, and is updated annually (Visser et al., 2013; Sediq et al., 2018). In 2017, it contained prescription data of approximately 700,000 individuals from around 72 pharmacies that are located in most of the area where the Lifelines cohort is also resident. The study population was reported to represent the general population in Netherlands (Visser et al., 2013; Sediq et al., 2018). In the IADB.nl, each patient has a unique and anonymous identifier. Each record contains information about patient's sex, date of birth, and information about his/her prescribed medication such as ATC code, duration, daily dose, amount prescribed, and dispensing date (Visser et al., 2013; Sediq et al., 2018). The IADB.nl has no information about over-the-counter (OTC) drugs and prescriptions from the hospital.

Study Population and Linkage of Databases

The study population consists of all medicine users (≥18 years) in the Lifelines cohort. A Trusted Third Party, Statistics Netherlands (Dutch: *Centraal Bureau voor de Statistiek;* CBS), carried out the linkage of the Lifelines and the IADB.nl records at the patient level based on postal code in combination with sex and date of birth. The unique identifiers from both databases were removed, and once the linkage was completed, each patient was assigned a new unique code that cannot be traced back to their previous identifier. Using the new identifier, the data from both databases could be combined. The complete linking process was described in more detail by Sediq et al. (Sediq et al., 2018).

Exposures

Exposures were defined as substrates and inhibitors of CYP2D6/2C19/2C9. We defined a potential DDI as each combination of a substrate and inhibitor listed in the international standard and local guideline, Flockhart Table for CYP-mediated drug interactions and *the Dutch Commentaren Medicatiebewaking book,* respectively (Borgsteede, 2015; Flockhart, 2018). Based on the main indication according to the official product information, the exposures were classified as: 1) chronically used medication (CM) for example CYP2D6 substrates such as beta-blockers (metoprolol), and 2) occasionally used medication (OM) for example CYP2D6 substrates such as opioids (tramadol). The full list of medications including their classification can be found in **Supplementary Material 1**.

Outcomes

Outcome measures were defined as frequency of potential CYP2D6/2C19/2C9-mediated DDIs as well as the levels of agreement between the self-reported information from the Lifelines cohort and the IADB.nl prescription data on these potential DDIs across type of medications, age, and sex. If one patient was exposed to different types of CYP2C9/2D6/2C19 mediated DDIs, we calculated them as one participant with several incidences of potential DDIs. If the potential DDI was only found in the Lifelines cohort records, it was categorized as over-reporting (false positive). If the potential DDI was only found in the IADB.nl, it was categorized as under-reporting (false negative). We also provided data on sensitivity, specificity, negative predictive value/NPV, and positive predictive value/PPV for the top five potential DDIs detected in the lifelines database. Different overlapping time windows (i.e. 1 month, 3 months, 6 months, 9 months, and 1 year) between baseline date of self-reporting medication in the Lifelines cohort and dispensing date of prescription in the IADB.nl were applied to determine the optimum time window for assessing the agreement of both databases. Subgroup analyses by the type of medication (CM vs OM), age, and sex were performed to observe the potential influence of these factors on the agreement. Additionally, we also presented information about the clinical relevance of the potential DDIs based on the suggested management provided by Epocrates® i.e. "contraindicated, avoid combination/use alternative, modify treatment/monitor and caution". If Epocrates® had no recommendation for the potential DDI, we checked whether Drugs.com, another online drug interactions screening software, provided suggestions for the potential DDI. Both of them were reported to have a high sensitivity for detection of potential DDIs (Perkins et al., 2006; Bossaer and Thomas, 2017).

Statistical Methods

Comparisons of the prevalence of potential CYP2D6/2C19/2C9-mediated DDIs [mean (SD)] and the frequency of participants with the potential DDIs [number (%)] across age groups (18–59 vs >=60 years old) and sex (men vs women) were performed by using independent sample t-test. A p-value which is less than 0.05 (< 0.05) is considered to indicate a statistically significant difference between comparison groups. Multivariate analysis of the influence of age and sex on the risk of having the potential DDIs was conducted by using a binary logistic regression method to obtain the crude and covariate-adjusted odds ratios as a measure of association. A p-value < 0.05 and 95% confidence interval (CI) not including 1 are considered as indicators for significant associations. To determine the agreement values between the databases on the potential DDIs, we used Cohen's kappa statistics and 95% CI. Altman et al. provided some guidelines to define the

TABLE 1 | Characteristics of participants with self-reported medication use at entry in the Lifelines cohort database and overlap with IADB.nl prescription database.

Characteristics	Number of participants (n = 80,837)
Age in year, mean (± SD)	46.13 (± 14.21)
18-59 years old, N (%)	64,807 (80.17%)
>= 60 years old, N (%)	16,030 (19.83%)
Gender, N women (%)	55,352 (68.50%)
Total participants with CYP2D6/2C19/2C9 mediated DDI, N(%)	1,125 (1.40%)
Total participants overlapped with IADB.nl database, N (%)	25,387 (31.41%)
• Age in year, mean (± SD)	45.54 (± 14.62)
• 18-59 years old, N (%)	20,277 (79.90%)
• >= 60 years old, N (%)	5,110 (20.10%)
• Gender, N women (%)	17,416 (68.60%)
• Total participants with CYP2D6/2C19/2C9 mediated DDI, N (%)	366 (1.44%)

SD, Standard Deviation; DDI, drug-drug interaction.

Cohen's kappa values i.e. poor (< 0.20), fair (0.20–0.40), moderate (0.41–0.60), good (0.61–0.80), and very good (0.81–1.00) (Altman, 1990).

RESULTS

Among of 167,729 Lifelines participants, 80,837 adults were recorded with self-reported medicine use (mean age 46 years and 68.5% women) in the cohort at entry (**Table 1**). Among the subjects, there were 1,125 (1.4%) self-reported medicine users exposed to 1,199 potential CYP2D6/2C19/2C9-mediated DDIs (**Figure 1**). The prevalence of potential CYP2D6/2C19/2C9-mediated DDIs was 488, 513, and 198 respectively (**Table 2**). Older women had a significantly higher risk to be exposed to CYP2D6 (OR: 2.159, 95% CI: 1.386–3.363) and CYP2C19 (OR: 1.691, 95% CI: 1.184–2.416) mediated DDIs than older men but the comparable risks were observed among younger group.

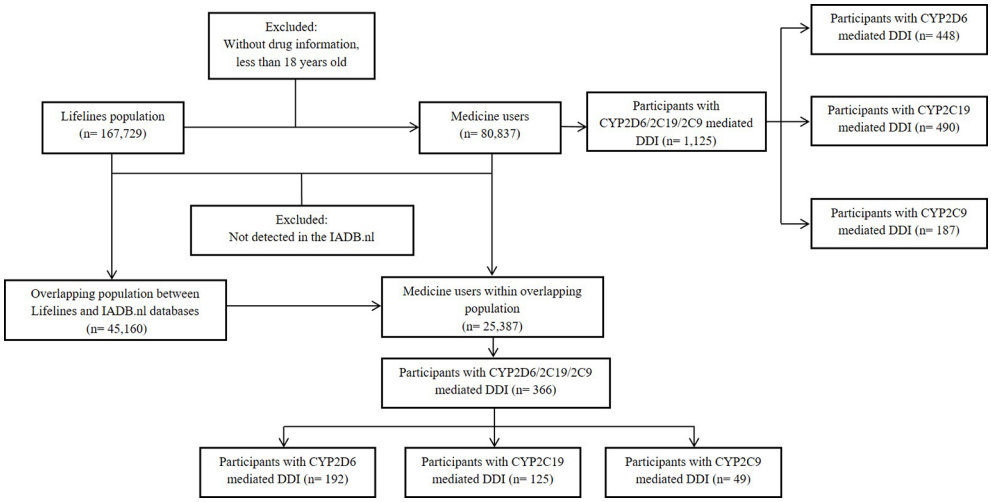

FIGURE 1 | Selection of the population study. DDI, drug-drug interaction.

TABLE 2 | Prevalence and participants with potential DDIs in the Lifelines cohort.

Variables	Prevalence of potential DDIs (*n* = 1,199)						Variables	Participants with potential DDIs (*n* = 1,125)					
	Age in years [mean (SD)]		P-value	Gender [mean (SD)]		P-value		Age in years [n (%)]		P-value	Gender [n (%)]		P-value
	18-59	>=60		Men	Women			18-59	>=60		Men	Women	
CYP2D6	0.006	0.006	0.519	0.005	0.006	0.048	CYP2D6	349	99	0.227	118	330	0.018
(*n* = 488)	(0.09)	(0.01)		(0.08)	(0.08)		(*n* = 448)	(0.54)	(0.62)		(0.46)	(0.59)	
CYP2C19	0.006	0.009	0.0002	0.006	0.007	0.428	CYP2C19	351	139	0.000002	148	342	0.527
(*n* = 513)	(0.08)	(0.09)		(0.08)	(0.08)		(*n* = 490)	(0.54)	(0.87)		(0.58)	(0.62)	
CYP2C9	0.003	0.002	0.178	0.002	0.003	0.037	CYP2C9	156	31	0.264	47	140	0.060
(*n* = 198)	(0.05)	(0.05)		(0.04)	(0.05)		(*n* = 187)	(0.24)	(0.19)		(0.18)	(0.25)	

SD, Standard Deviation; DDI, drug-drug interaction.

TABLE 3 | Multivariate analysis on the influence of age and sex on risk of having potential CYP2C9/2D6/2C19 mediated DDIs.

	Multivariate analysis				Sub-group analysis		
Variables	**Crude OR (95% CI)**	**P-Value**	**Adjusted OR (95% CI)**	**P-Value**	**Variable**	**OR (95% CI)**	**P-Value**
CYP2D6 (n = 448)							
Age					18–59		
18–59	Ref.		Ref.		Men	Ref.	
>=60	1.148 (0.918–1.436)	0.228	1.119 (0.956–1.503)	0.116	Women	1.119 (0.881–1.422)	0.358
Sex					>=60		
Men	Ref.		Ref.		Men	Ref.	
Women	1.289 (1.044–1.592)	0.018	1.319 (1.066–1.632)	0.011	Women	2.159 (1.386–3.363)	0.001
CYP2C19 (n = 490)							
Age					18–59		
18–59	Ref.		Ref.		Men	Ref.	
>=60	1.606 (1.319–1.956)	0.000002	1.640 (1.343–2.002)	0.000001	Women	0.949 (0.754–1.195)	0.659
Sex					>=60		
Men	Ref.		Ref.		Men	Ref.	
Women	1.064 (0.877–1.291)	0.528	1.139 (0.936–1.385)	0.194	Women	1.691 (1.184–2.416)	0.004
CYP2C9 (n = 187)							
Age					18–59		
18–59	Ref.		Ref.		Men	Ref.	
>=60	0.803 (0.546–1.181)	0.265	0.841 (0.570–1.241)	0.383	Women	1.316 (0.906–1.910)	0.149
Sex					>=60		
Men	Ref.		Ref.		Men	Ref.	
Women	1.372 (0.986–1.910)	0.061	1.345 (0.964–1.878)	0.081	Women	1.466 (0.702–3.063)	0.308

SD, Standard Deviation; DDI, drug-drug interaction; OR, Odds Ratio; CI, Confidence Interval.

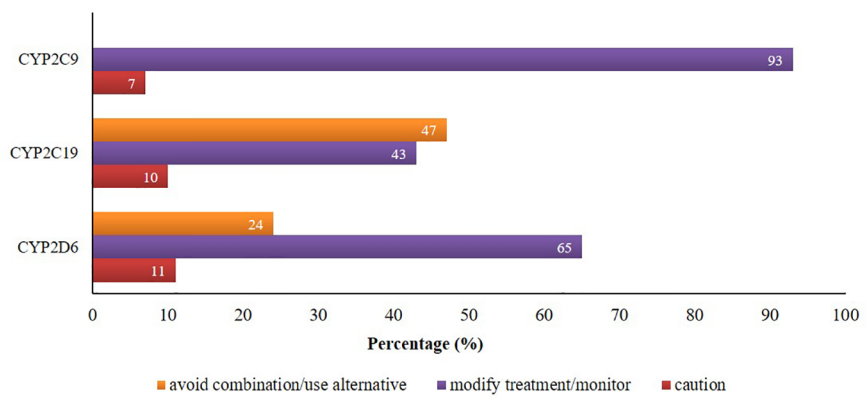

FIGURE 2 | Proportion of potential DDIs based on the suggested managements provided by Epocrates® and Drugs.com.

There was also a tendency that women had an increased risk to be exposed to CYP2C9 mediated DDIs than men (**Table 3**).

There were 24% and 47% of CYP2D6 and CYP2C19 mediated co-prescriptions, respectively, which were in the category of "avoid combination/use alternative". Additionally, about 65%, 43%, and 93% of CYP2D6/2C19/2C9-mediated combinations were in the category of "modify treatment/monitor" according to the knowledgebase (**Figure 2**).

Information from 45,160 Lifelines participants could be linked to the IADB.nl database. Among this linked population, there were 25,387 self-reported medicine users with comparable age and sex distribution (mean age 45.5 years and 68.6% women) as observed in the total medicine users in the Lifelines cohort (**Table 1**). Metoprolol-paroxetine (83 events), citalopram-omeprazole (173 events), and diclofenac-paroxetine (51 events) were the most prevalent potential DDIs mediated by CYP2D6/2C19/2C9, with good, moderate, and fair agreement of questionnaire and prescription data, respectively. Data on kappa, sensitivity, specificity, PPV and PPV values of the top five most frequent potential DDIs in the Lifelines database can be found in **Table 4**. Information on self-reported combinations of chronically used medications such as metoprolol-fluoxetine had very good agreement, high sensitivity and specificity as well as high PPV and NPV. Meanwhile, information on self-reported combinations with occasionally used medication such as ibuprofen-paroxetine tended to have fair kappa, sensitivity, and PPV but high specificity and NPV. The complete list of the potential DDIs in the Lifelines database and their kappa values

TABLE 4 | Top five potential DDIs in the Lifelines cohort detected in the IADB.nl database with their kappa, sensitivity, specificity, PPV, and NPV, as well as 95% CI (time window: 3 months).

	CYP2D6/2C19/2C9 mediated potential DDI										
Potential DDI	**N[a]**	**N1[b]**	**N2[c]**	**Detected in both databases (TP)**	**Over-reporting[d] (FP)**	**Under-reporting[e] (FN)**	**Kappa (95%CI)**	**Sensitivity, % (95%CI)**	**Specificity, % (95%CI)**	**PPV, % (95%CI)**	**NPV, % (95%CI)**
CYP2D6											
metoprolol_paroxetine	83	39	28	22	17	6	0.656 (0.655–0.656)	78.57 (59.05–91.07)	99.93 (99.89–99.96)	56.41 (43.65–63.37)	99.98 (99.95–99.99)
metoprolol_clomipramine	18	15	11	10	5	1	0.769 (0.768–0.770)	90.91 (58.72–99.77)	99.98 (99.95–99.99)	66.67 (44.94–83.05)	100.00 (99.97–100.00)
metoprolol_fluoxetine	17	10	9	9	1	0	0.947 0.946–0.947)	100.00 (66.37–100.00)	99.99 (99.98–100.00)	90.00 (55.90–98.46)	100.00 (-)
metoprolol_amiodarone	14	11	5	5	6	0	0.625 (0.623–0.627)	100.00 (47.82–100.00)	99.98 (99.95–99.99)	45.45 (27.24–64.97)	100.00 (-)
metoprolol_duloxetine	14	3	2	2	1	0	0.800 (0.797–0.802)	100.00 (15.81–100.00)	100.00 (99.98–100)	66.67 (21.98–93.42)	100.00 (-)
CYP2C19											
citalopram_omeprazole	173	37	29	19	18	10	0.575 (0.574–0.576)	65.52 (45.67–82.06)	99.93 (99.89–99.96)	51.35 (38.27–64.25)	99.96 (99.93–99.98)
diazepam_omeprazole	151	40	49	19	21	30	0.425 (0.424–0.426)	38.78 (25.20–53.76)	99.92 (99.87–99.95)	47.50 (34.21–61.15)	99.88 (99.85–99.91)
omeprazole_fluvoxamine	28	5	4	3	2	1	0.667 (0.665–0.669)	75.00 (19.41–99.37)	99.99 (99.97–100.00)	60.00 (25.13–87.02)	100.00 (99.98–100.00)
diazepam_esomeprazole	27	8	10	5	3	5	0.555 (0.553–0.557)	50.00 (18.71–81.29)	99.99 (99.97–100.00)	62.50 (31.45–85.83)	99.98 (99.96–99.99)
clopidogrel_omeprazole	24	10	8	5	5	3	0.555 (0.553–0.557)	62.50 (24.49–91.48)	99.98 (99.95–99.99)	50.00 (26.35–73.65)	99.99 (99.97–100.00)
CYP2C9											
diclofenac_paroxetine	51	9	14	4	5	10	0.347 (0.345–0.348)	28.57 (8.39–58.10)	99.98 (99.95–99.99)	44.44 (19.32–72.77)	99.96 (99.95–99.97)
ibuprofen_paroxetine	22	4	6	1	3	5	0.200 (0.198–0.202)	16.67 (0.42–64.12)	99.99 (99.97–100.00)	25.00 (3.86–73.47)	99.98 (99.97–99.99)
naproxen_paroxetine	20	6	4	3	3	1	0.600 (0.598–0.602)	75.00 (19.41–99.37)	99.99 (99.97–100.00)	50.00 (22.01–77.99)	100.00 (99.98–100.00)
diclofenac_fluoxetine	14	4	3	1	3	2	0.286 (0.283–0.289)	33.33 (0.84–90.57)	99.99 (99.97–100.00)	25.00 (4.48–70.29)	99.99 (99.98–100.00)
diclofenac_fluvoxamine	8	2	3	1	1	2	0.400 (0.397–0.403)	33.33 (0.84–90.57)	100.00 (99.98–100.00)	50.00 (7.38–92.62)	99.99 (99.98–100.00)

[a]N, the number of participants with DDIs in the Lifelines cohort (n = 80,837); [b]N1, the number of participants with DDIs in the Lifelines cohort within overlapping population between the Lifelines and IADB.nl databases (n = 25,387); [c]N2, the number of participants with DDIs in the IADB.nl cohort within overlapping population between the Lifelines and IADB.nl databases (n = 25,387). [d]Over-reporting, detected only in the Lifelines cohort but not on the IADB.nl. [e]Under-reporting, detected only in the IADB.nl but not in the Lifelines cohort.

can be found in the **Supplementary Material 2** and **3**, respectively.

The application of different time windows resulted in different agreement levels of the potential DDIs (**Figure 3**). Overall, the time window of three months produced the highest mean kappa values among potential CYP2D6/2C19/2C9-mediated DDIs i.e. moderate [0.545 (95% CI: 0.544–0.545)], moderate [0.512 (95% CI: 0.511–0.512)], and fair [0.374 (95% CI: 0.373–0.375)], respectively. Extension of the time windows to 6, 9, and 12 months decreased the mean kappa values. The time window of 1 month also produced a low kappa value. For the time window of 3 months, subgroup analysis for the type of medication indicated the potential DDIs in CM-CM had better level of agreements (good) than CM-OM (fair to moderate) and OM-OM (poor to moderate). For the CYP2D6 and CYP2C9 mediated DDIs, CM-OM combination had better kappa values (fair agreement) than OM-OM combination (poor agreement). Meanwhile, for the CYP2C19 mediated DDIs, both the CM-OM and OM-OM combination had comparable agreement level (moderate). The summary of the results can be found in **Supplementary Material 3**.

Subgroup analysis of agreement by sex showed mixed results (**Figure 4**). In CYP2D6 mediated potential DDIs, females appeared to have a better level of agreement than males. The opposite result was observed in CYP2C19 and CYP2C9 mediated potential DDIs where males mostly had a better kappa value compared to females. Stratification by age indicated that people aged 60 years or older had a generally better kappa value than the younger population in CYP2D6/2C19/2C9 mediated potential DDIs (**Figure 5**).

DISCUSSION

In this cross-sectional study, CYP2D6/2C19/2C9-mediated potential DDIs were frequent and concordance of data varied by time window, type of medication, sex, and age. We found that one to two per hundred drug users in the Lifelines cohort were exposed to a potential CYP2D6/2C19/2C9-mediated DDI at a short moment in life time. Some of these potential DDIs are regarded as clinically relevant DDIs such as metoprolol and CYP2D6 inhibitors combinations. The DDIs may lead to bradycardia, hypotension, and atrioventricular block (Walley et al., 1993; Konig et al., 1996; Onalan et al., 2008; Bahar et al., 2018a). Other relevant DDIs were the combination of CYP2C9 inhibitors that consist of selective serotonin inhibitors (SSRIs), and nonsteroidal anti-inflammatory drugs (NSAIDs). The combination of SSRIs and NSAIDs was reported to increase risk of gastrointestinal bleedings (de Abajo et al., 1999; De Jong et al., 2003). Yet, the interaction between SSRIs and NSAIDs might be not solely a pharmacokinetic interaction but also involves a pharmacodynamic interaction (Moore et al., 2015). Our findings on the burden of DDIs might have potential clinical as well as economic implications. A DDI is one of the main contributors of an adverse drug reaction (ADR) which is one of the leading causes of hospitalisation and it can cost at minimum around €200 to €9,000 per hospitalisation (Formica et al., 2018).

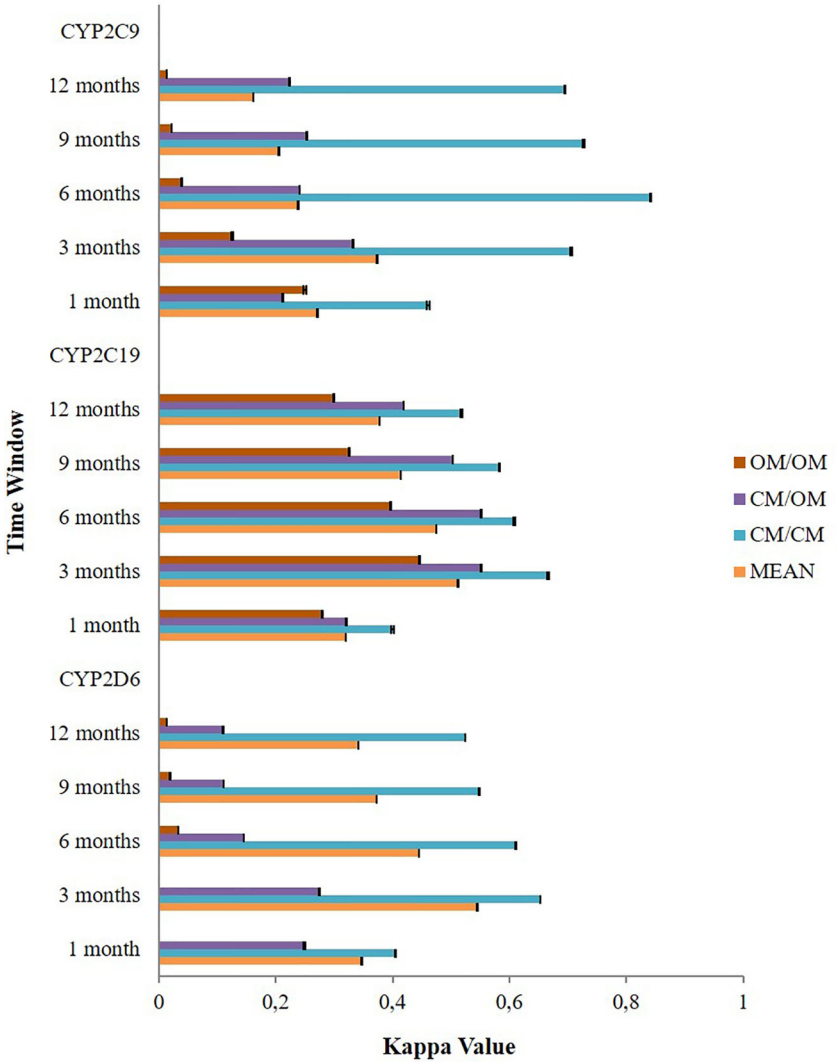

FIGURE 3 | The effect of different time windows on the agreement between the Lifelines cohort and the IADB.nl database.

Older women tended to have a higher risk to be exposed to potential CYP2D6/2C19/2C9-mediated DDIs than older men. A survey study from the United States about the pattern of drug use among adults in the outpatient setting indicated that elderly women (≥65 years old) had the highest burden of medication use in which 23% and 12% of them used at least five and 10 drugs, respectively (Kaufman et al., 2002). The risk of experiencing DDI is increased as the number of drugs taken also increased (Åstrand et al., 2006). Taking five to seven drugs and 10 to 14 drugs enhanced the risk of potentially relevant DDI by about 20%–30% and 40%–60%, respectively (Johnell and Klarin, 2007). Other studies also reported that being women and old are risk factors associated with DDIs (Grattagliano et al., 2010; Magro et al., 2012).

Based on this study, a three-month time window appeared to result in the best agreement level. This is consistent with the previous study by Sediq et al. about the validation of single drug used in the Lifelines (Sediq et al., 2018). Additionally, Lau et al. also found similar finding in their work on the validation of pharmacy records in Amsterdam, Netherlands (Lau et al., 1997). One of the possible reasons for this finding is that the Dutch reimbursement system only allows drugs to be prescribed for a maximum of 3 months period of supply (Lau et al., 1997). A comparable study to validate self-reported medication using a national prescription database in the Danish population also found a fixed 3 month time window was suitable for checking the agreement between the two sources of information on medicine use (Nielsen et al., 2008). Considering the time window is an important aspect, because a long time window may hamper the analysis of drugs used on as needed basis and a short time window may impair the analysis of drugs used chronically (Johnson and Vollmer, 1991; Nielsen et al., 2008).

Sub-analysis by type of medication indicates self-reported information on the CM-CM combination was more reliable than

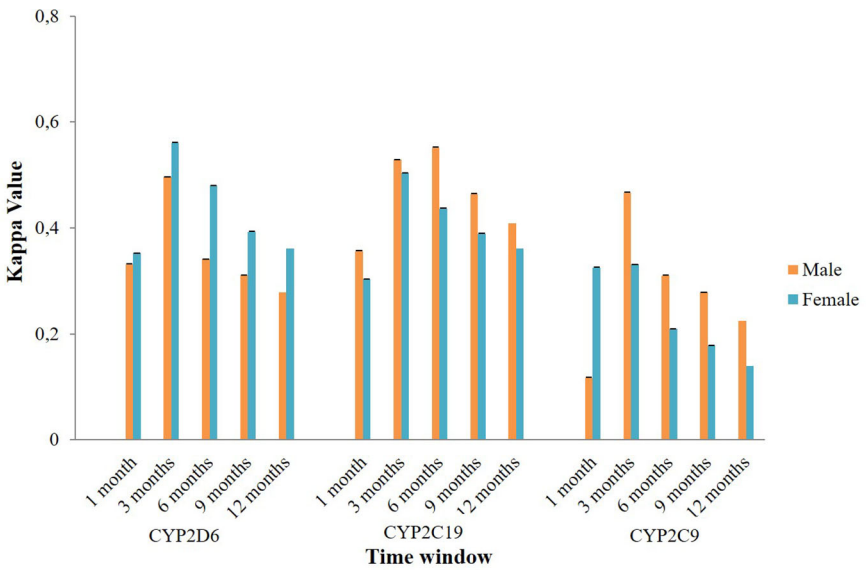

FIGURE 4 | The effect of sex on the agreement between the Lifelines cohort and the IADB.nl database.

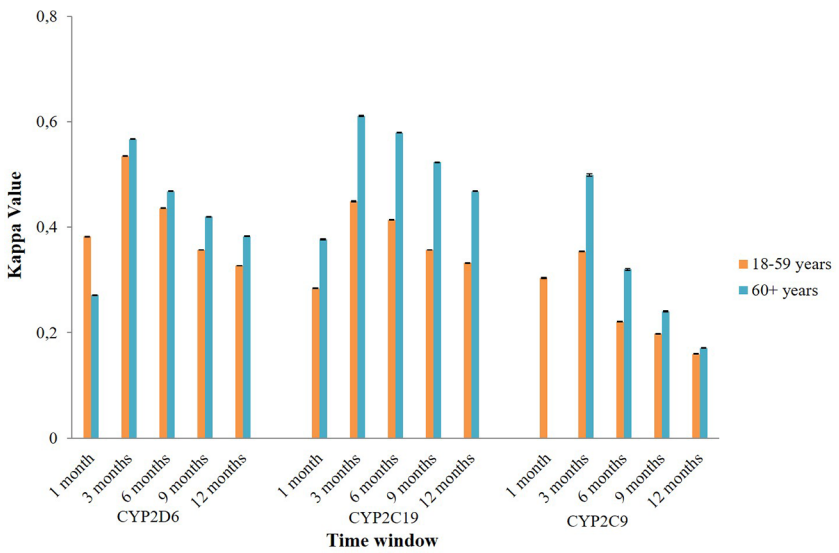

FIGURE 5 | The effect of age on the agreement between the Lifelines cohort and the IADB.nl database.

information on CM-OM and OM-OM combination. It offers the possibility to use co-prescription data of the CM-CM combination from the Lifelines cohort in research. This may because routinely used medication is more easily remembered by patients than drugs used occasionally. These results are consistent with previous studies (Nielsen et al., 2008; Sarangarm et al., 2012; Cohen et al., 2018).

Furthermore, for CYP2D6 and CYP2C9 related co-prescriptions, the kappa value of the OM-CM combination (fair agreement) was higher than OM-OM (poor agreement) except for CYP2C19. For the latest, the agreement level of CYP2C19 mediated OM-CM seems comparable with those of the OM-OM combination (moderate agreement). There are some possible explanations for this finding. One is the inclusion of proton pump inhibitors (PPIs) in the OM groups which are the main drugs in this group. PPIs have wide therapeutic indications and some of these indications need a chronic use of PPIs such as Zollinger-Ellisone syndrome, Barrett's esophagus, and esophagitis (KNMP, 2018b). Another explanation is the

inclusion of diazepam in the OM groups. Diazepam may be used chronically for treating patients with panic disorder and generalized anxiety disorder (KNMP, 2018a). Consequently, OM groups not solely consisted of drugs used 'as needed' but also may include chronically used drugs.

We found that the effect of sex on agreement is not consistent. Previous studies also reported mixed results. Some reports showed that men had a better recall accuracy than women (Linet et al., 1989; Haapea et al., 2010). Meanwhile, other studies indicated that sex had no influence on the agreement between self-reported medication use and prescription database (Van den Brandt et al., 1991; West et al., 1995). Therefore, more research is needed to determine the effect of sex on the recall accuracy, and concordance between self-reported medication use and information from a prescription database.

Our study found the agreement between the Lifelines and the IADB.nl database records is better in the older population (aged 60 years and older) than in younger adults. This result is in contrast to previous reports which found aging led to a low agreement between self-reported medication use and information from a drug database (Van den Brandt et al., 1991; West et al., 1995). A decrease in cognitive function and polypharmacy may cause poor recall information by old patients (Van den Brandt et al., 1991). However, other studies reported that age did not influence the agreement level (Sjahid et al., 1998; Lamiae et al., 2010). The method used to collect drug information may determine the influence of aging in recall bias. If the interviewers visit the patient's house to ascertain the consumed drugs or if the patients are helped by their family in completing the questionnaire, the impact of self-reporting bias in the old participants (≥ 60 years old) can be reduced (Johnson and Vollmer, 1991; Lau et al., 1997; Richardson et al., 2013). In the Lifelines cohort, some participants filled out the questionnaire at home before visiting the premises. Therefore, the participants were potentially assisted by their relatives or may directly check their medication while completing the questionnaire. Meanwhile, some patients brought their medication at the time of interview so that interviewers could ascertain their medication list in the questionnaire.

Another possible culprit of conflicting reports is the type of medication. Most of the drugs related to CYP2D6/219/2C9 are mainly groups of drugs used by the old population chronically and were reported to be associated with a good recall such as cardiac therapy, antidiabetic agents, anti-thrombotic drugs, anticancer agents, antidepressant, and antipsychotic agents (Van den Brandt et al., 1991; Haukka et al., 2007; Gupta et al., 2011; Hafferty et al., 2018; Sediq et al., 2018). For the last two agents, Haukka et al. reported a good recall because patients brought their medication at the time of interview (Haukka et al., 2007). Lastly, the other possible explanation was the differential distribution of the population in each subgroup of age which may give a wrong impression about the influence of different age in the agreement (West et al., 1997). In our study, about 80% of the population is in the 18–59 years old subgroup. Therefore, a larger pharmaco-epidemiological study with a sub-group analysis is needed to elucidate the impact of age in the concordance of self-reported medication use and data from a prescription database.

Some strengths of our study are worth to be mentioned. Firstly, the linkage process between both databases is reliable since it was performed by CBS on individual level. Secondly, the population in our cohort is large and not limited to a certain group of population with diseases or using specific medications. Some other studies were conducted by using a limited sample and only in some particular groups of patients such as in elderly, pregnant women, patients with specific medical conditions or using certain drugs (West et al., 1995; Sjahid et al., 1998; Rockenbauer et al., 2001; Richardson et al., 2013). Thirdly, we included all types of drugs which may potentially trigger CYP2D6/219/2C9-mediated DDIs. However, there are also some limitations from our study. Firstly, we only checked the agreement of prescribed medication but not OTC drugs since the IADB.nl database has no information on OTC drugs. For example, ibuprofen is also available over the counter which may explain lower kappa values in potential DDI combinations. Next, we only had drug information from community pharmacies and, therefore, if the drugs recorded in the self-reported questionnaire were obtained from a hospital, it will not be detected in the IADB.nl and will be categorized as over-reporting information. Further, some potential DDIs included in our study were recommended to manage either by dose adjustments or monitoring of the possible potential side effects which have been possibly done by the responsible clinicians. Meanwhile, some other potential DDIs might not produce important side effects and only need caution on their use. However, we still kept them in our analysis because the influence of genetic polymorphisms on CYP2D6/2C19/2C9 may enhance the magnitude of the clinical impact of those interactions (Bahar et al., 2017a). Therefore, it would be valuable to research the interaction of CYP2D6/2C19/2C9 polymorphisms and CYP2D6/2C19/2C9-mediated DDIs in the next study. Additionally, we did not include the combination of substrates and inducers since the prevalence was too low to allow further analysis. Next, we only limited the focus of our study on the contribution of the three main phase I drug metabolizing enzymes (CYP2D6/2C19/2C9) since they cumulatively metabolize about 42% of drugs currently used in the clinical practice and mounting evidence has shown that clinical consequences of genetic polymorphisms are different among the CYP450 subfamily with CYP2D6/2C19/2C9 polymorphisms reported to have the most important clinically relevant implications (Zanger et al., 2008; Zanger and Schwab, 2013; Bahar et al., 2017a). Therefore, we assume that the CYP2D6/2C19/2C9 mediated DDIs will be the most frequent and relevant DDIs which will be found to be modified by genetic polymorphism in clinical practice. However, we would like to emphasize that drug interactions might also be facilitated by phase II drug metabolizing enzymes and drug transporters which are also subject to genetic polymorphisms and still are not optimally investigated (Board et al., 1998; Kerb, 2006). Furthermore, we only limited the analysis of the potential DDIs to the pairwise combination of medications since it reflects current guidelines and practice related to the management of DDIs in the Netherlands (van Roon et al., 2005; Heringa et al., 2016). However, the DDI might occur not only in the form of bimodal (involving two drugs) interaction but also in multimodal (involving more than two drugs) interactions especially in drugs metabolized by multiple

metabolic pathways (Grönlund et al., 2011). Multimodal drug interactions were reported to produce more severe outcomes than bimodal interaction since all the metabolic pathways of the drugs are impaired (Niemi et al., 2003; Niemi et al., 2006). Lastly, our study had no clinical outcomes of the potential DDIs since in the current study our focus was limited to study the prevalence of the potential DDIs and the agreement of drug information between both databases. This study is pivotal in order to design valid follow-up studies with the aim to determine the clinical impact of the observed potential DDIs especially for chronically used medications.

CONCLUSION

In conclusion, CYP2D6/2C19/2C9-mediated potential DDIs were frequent and the agreement between the Lifelines cohort and the IADB.nl differed between time windows. The best concordance level was achieved at a 3-month time window. CM-CM co-prescription had a better agreement than CM-OM and OM-OM combinations. Sex had no consistent influence on the discordance between the databases. Meanwhile, the older population had a better kappa value than the younger population. For the next drug study, the self-reporting data should be complemented with the pharmacy data in order to achieve a better accuracy in capturing the real word information on medication use.

DATA AVAILABILITY STATEMENT

The datasets for this manuscript are not publicly available, and used under license for the current study, in order to protect information that could potentially compromise the privacy of research participants. Requests to access the datasets should be directed to the Pharmlines Initiatives [email: research@lifelines.nl]. Some data such as frequency, type and agreements (kappa values) of drug-drug-interactions are available as **Supplementary Materials**.

ETHICS STATEMENT

Ethics approval was not needed according to institutional guidelines and national legislation, since the data generated in this manuscript relied exclusively on the research database with pseudonymized information and informed consent was obtained at the time of original data collection

AUTHOR CONTRIBUTIONS

Conceptualization and design (MB, JB, SB, BW, EH), data curation (MB, JB), investigation (MB, JB), resources (JB, AD, RA), statistical analysis (MB), interpretation of data (MB, SB, AD, RA, BW, EH), drafting manuscript (MB), critical evaluation of the manuscript, editing, and supervision (JB, SB, AD, RA, BW, EH). All authors read and agreed on the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00624/full#supplementary-material

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Use of Opioids Increases With Age in Older Adults: An Observational Study (2005–2017)

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Aim: Pain is increasingly treated with opioids. Potential harms of opioid therapy disproportionally affect older patients. This study aims to provide information on trends, nature and duration of opioid prescribing to older adults, in primary care and to explore differences between older patients from different ages.

Methods: Primary care data (2005–2017) were derived from routine electronic medical records of patients in Nivel Primary Care Database. All opioid prescriptions with Anatomical Therapeutic Chemical Classification (ATC) code N02A were selected (except for codeine). Diagnoses were recorded using the International Classification of Primary Care (ICPC). Patients were categorized in three age groups (65–74, 75–84, and ≥85 years). Descriptive analyses were used to describe the trend of opioid prescriptions for specific opioids, the duration of use and underlying diagnoses.

Results: 283,600 patients were included of which 32,287 had at least one opioid prescription in 2017. An increase in the number of older adults who received at least one opioid was seen between 2005 and 2017. The oldest patients were more likely to be prescribed an opioid, especially when it comes to strong opioids, the increase in the volume of prescribing was highest in this group. Moreover, over 40% of the oldest patients used strong opioids chronically. Strong opioids were mostly prescribed for musculoskeletal diagnoses. Cancer was the second most common diagnosis for strong opioids in the younger subgroups, whereas less specified diagnoses were as second in the oldest subgroup.

Conclusion: Opioid prescription changes with increasing age in frequency, nature, and duration, despite higher harm risks among older patients. Because of the high prevalence of chronic use, it is important to monitor the patient throughout the treatment and to critically evaluate the initiation and continuation of opioid prescriptions.

Keywords: opioids, older adults, trend, fentanyl, oxycodone

INTRODUCTION

One out of five adults in Europe experience moderate to severe pain which seriously affects their daily life (Breivik et al., 2006). In the United States, an overall reported experience of pain in the general population of 56% and 10% experiencing severe pain was found (Nahin, 2015). Chronic pain—defined as pain persistent for a period of 3 months or longer—prevalence increases with age, affecting up to 62% of the population over the age of 75 (Fayaz et al., 2016). Increasingly, opioids are being prescribed for pain. However, while strong opioids are very effective pain-relieving medicines, the evidence for their benefits in long term use is limited (Kissin, 2013; Sites et al., 2014; Chou et al., 2015), and several risks have been reported. These include increased risk for side effects (such as constipation, nausea, and sedation), addiction, hospitalization, and even mortality (Calcaterra et al., 2013; Franklin, 2014; Currow et al., 2016). Still, many Western countries are faced with an increase in opioid prescribing. The sharpest increase is seen for strong opioids (Zin et al., 2014; van Amsterdam and van den Brink, 2015) which are often used long-term (Olsen et al., 2006; Foy et al., 2016).

Steinmann (Steinman et al., 2015) argues that there is evidence that the potential harms of opioid therapy disproportionally affect older patients, such as falling and constipation. The higher risk of side effects and negative outcomes (Chau et al., 2008; Buckeridge et al., 2010; Steinman et al., 2015) is a result of physiological changes that come naturally with age, higher risk of polypharmacy and more comorbidities (Huang and Mallet, 2013).

Globally, countries are faced with the aging of their population. Usually elderly are defined as people who are 65 years or older. But does this cutoff point provide sufficiently detailed information for physicians to appropriately treat all older patients? In general, older people are healthier now compared to two decades ago, however, these changes are more visible in people within their sixties than in patients aged 70 years and older (Crimmins, 2004). Particularly patients over the age of 85 can be frail. Therefore, it is important to learn more about the trends and patterns in which opioids are currently prescribed to older patients of different age groups.

Most patients who experience chronic pain are managed by primary care physicians (PCP) (Breivik et al., 2006) and a large proportion of opioids are prescribed by PCPs (Breivik et al., 2006; Chen et al., 2016). Insight in how opioids are prescribed to older adults can help to improve treatment in the future. Therefore, the aim of this study is to provide information on trends in frequency, nature and duration of opioid prescriptions for older adults of different age, in a primary care setting.

METHODS

Source

Data used in this study were derived from the Nivel Primary Care Database (Nivel-PCD), which includes routine care data originating from electronic medical records from PCPs across the Netherlands. The participating PCPs constitute a representative sample of the total population of Dutch PCPs (Gijsen and Poos, 2006; Biermans et al., 2008). Within the Dutch health care system all residents are mandatorily registered with one PCP, who keeps track of the patient's complete medical record and fulfills a gatekeeper role for access to medical specialists. The database consists of longitudinal information of patient characteristics (age, sex), PCP consultations, diagnoses, and drug prescriptions. Diagnoses are recorded by the PCP using the International Classification of Primary Care version 1 (ICPC-1). Prescriptions are coded using the Anatomical Therapeutic Chemical Classification system (ATC). We used data from the years 2005 to 2017.

Dutch law allows the use of these data for research purposes under certain conditions. According to this legislation, neither obtaining informed consent from patients nor approval by a medical ethics committee is obligatory for observational studies containing no directly identifiable data. (Dutch Civil Law, Article 7:458). This study has been approved by the applicable governance bodies of Nivel-PCD under nr. NZR00316.022.

Study Population

For each year, a selection of primary care practices was made in which drug prescriptions were recorded during at least 46 weeks and at least 85% of the prescriptions were coded with a valid ATC code. For each year, we selected patients with the minimum age of 65 years old and classified them in three age groups (65–74 years, 75–84 years, and 85 years and older). Then patients with at least one opioid prescription in the relevant year were identified for the analyses.

Measures

Opioids were selected using the ATC coding "N02A" (analgesics). Codeine (N02AA59, N02AA79, and N02AJ06) was excluded from the analyses because this drug is usually prescribed for other indications than pain in the Netherlands. The Dutch PCP guidelines also discourage the prescription of codeine in the treatment of pain (NHG-Werkgroep Pijn, 2015). The remaining opioids were categorized as strong or weak opioids. When the number of prescriptions of an opioid was very low, it was categorized as "other." (**Table 1**). Individual results of opioid trends are only reported for opioids that are prescribed for at least 1 per 1,000 registered older adults within their age category.

Chronic use and number of prescriptions prescribed were calculated for the year 2017. For chronic use patients were included when they did not receive an opioid in 2016 and had at least one opioid prescription in 2017. The number of opioid prescriptions were calculated for patients who received at least one opioid in 2017. Also the following assumptions were made for the duration of the prescribed opioid. Patients are only included in the analyses of chronic use and number of prescriptions if the patient was registered in 2016 as well as 2017. If only one prescription was recorded, a duration of 30 days for weak opioids and 15 days for strong opioids was assigned. These assumptions are based on the mean duration between two prescriptions. When a patient has two or more prescriptions that were less than 90 days apart, we considered that the prescriptions

TABLE 1 | Opioids included in the study.

Name opioid	ATC code
Weak opioids	
Tramadol	N02AX02
Tramadol and paracetamol combination	N02AX52
Tramadol and paracetamol combination (previous N02AX52)	N02AJ13
Strong opioids	
Tapentadol	N02AX06
Morphine	N02AA01
Fentanyl	N02AB03
Oxycodone	N02AA05
Hydromorphine	N02AA03
Buprenorphine	N02AE01
Other opioids	
Nicomorphine	N02AA04
Diamorphine	N02AA09
Pethidine	N02AB02
Dextromoramide	N02AC01
Piritramide	N02AC03
Dextroproxyphene	N02AC04
Pentazocine	N02AD01

ATC, Anatomical Therapeutic Chemical Classification.

were part of one prescription episode. In case of multiple prescriptions, the duration of the last prescription is determined by the mean duration between the previous prescriptions (see **Figure 1**). Chronic use of opioids was defined as receiving prescriptions for 3 months or longer, per calendar year.

Analyses

For each year in the period of 2005 to 2017 the number of patients in each age group who were prescribed at least one opioid within that year was divided by the total number of registered patients in this age group. This was done to establish the general trend in prescriptions for all opioids and for specific opioids.

Analyses regarding diagnoses were performed only for the most recent year (2017) both on general ICPC-chapter level and for specific diagnoses. Patients are included when they receive at least one opioid prescription in the year of analyses with known diagnoses, missing diagnoses are not included. Because there is no separate chapter for cancer related diagnoses in the primary care database, this chapter was constructed on the basis of the respective ICPC codes. Per person a specific ICPC chapter only counted once; yet, it is possible that one person had a diagnosis in more than one chapter. The same was done for specific diagnoses.

There has been a large increase in the number of participating PCPs in Nivel-PCD over the years (from 18 in 2005 to 384 in 2017). Because of this large increase a sensitivity analysis was carried out to compare outcomes of the 15 practices that participated in both 2005 and 2015 to outcomes including all practices. To take into account the possibility that the opioids were prescribed in palliative care, separate analyses were performed excluding patients who died in the year of analyses and for those patients who died within a year of the opioid prescription. Reported results are based on the study population excluding patients who died in the year of analysis, unless otherwise indicated.

RESULTS

Table 2 shows the characteristics of the study population. Over half of the included patients were females (ranging between 54% and 57% over the years 2005–2017) and the mean age of patients remained constant over the observational period at about 74 years old. There was an increase over the years in the number of patients who received at least one opioid prescription and of patients with at least one prescription of a strong opioid see **Table A1** in the **Appendix**.

Figure 2 shows an overview of trends of prescriptions for specific opioids per 1,000 registered patients within each age group. Overall, the increase in prescriptions of strong opioids was larger in patients aged 85 years or older compared to the two younger age groups. The number of prescriptions for fentanyl and buprenorphine mostly increased in the oldest age group, whereas the increase in oxycodone prescriptions seems to increase more equally in all age groups. A decrease is visible in the oldest age group when it comes to prescriptions for tramadol, alone and in combination with paracetamol. The decrease of prescriptions for tramadol is visible in both oldest groups. Also the use of combination is similar in the two oldest groups, being more similar to a plateau, followed by a slight increase. Morphine is prescribed in a relatively stable frequency over the years.

Indications for opioid prescriptions in 2017 are displayed in **Table 3**. More than half of all opioids were prescribed for a musculoskeletal condition in all age groups. For all age groups

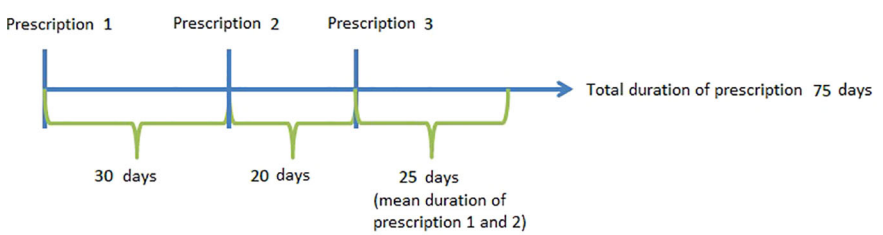

FIGURE 1 | Example of the calculation of duration time.

TABLE 2 | Characteristics of the study population.

Year	Number of practices	Number of patients aged 65+	Number of female patients (%)	Mean age of the population	Percentage of patients with at least 1 opioid prescription (CI 95%)	Percentage of patients with at least 1 stronger opioid (CI 95%)
2005	18	12,065	6,878 (57.0)	74.8	4.2 (3.8–4.6)	1.3 (1.1–1.5)
2006	27	15,356	8,738 (56.9)	74.9	4.9 (4.6–5.2)	1.5 (1.3–1.7)
2007	43	24,029	13,550 (56.4)	74.8	5.0 (4.7–5.3)	1.5 (1.3–1.7)
2008	58	32,244	18,093 (56.1)	74.7	5.7 (5.4–6.0)	2.0 (1.8–2.2)
2009	60	35,288	19,739 (55.9)	74.7	6.4 (6.1–6.7)	2.4 (2.2–2.6)
2010	166	98,772	55,082 (55.8)	74.8	8.0 (7.8–8.2)	3.0 (2.9–3.1)
2011	284	181,557	100,281 (55.2)	74.5	8.8 (8.7–8.9)	3.5 (3.4–3.6)
2012	320	209,280	114,927 (54.9)	74.4	9.1 (9.0–9.2)	3.8 (3.7–3.9)
2013	387	270,165	147,475 (54.6)	74.3	9.6 (9.5–9.7)	4.1 (4.0–4.2)
2014	390	278,427	150,667 (54.1)	74.2	9.9 (9.8–10.0)	4.7 (4.6–4.8)
2015	402	289,636	156,279 (54.0)	74.3	10.8 (10.7–10.9)	5.7 (5.6–5.8)
2016	323	242,594	130,475 (53.8)	74.4	11.3 (11.2–11.4)	6.5 (6.4–6.6)
2017	384	283,600	152,132 (53.6)	74.3	11.4 (11.3–11.5)	7.0 (6.9–7.1)

CI, confidence interval.

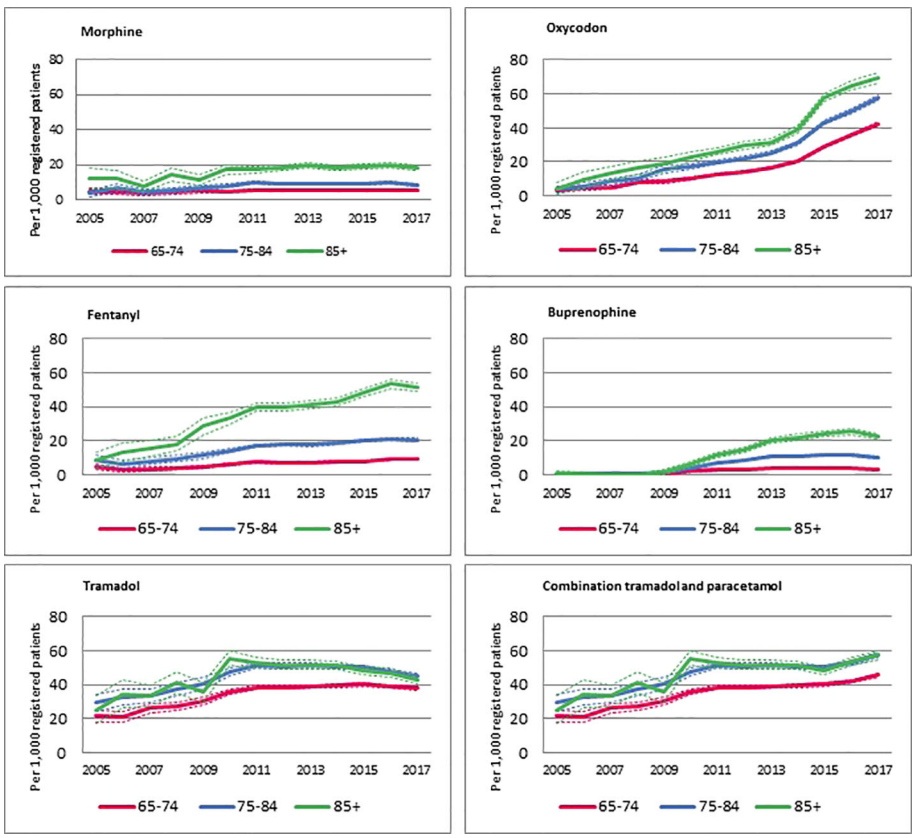

FIGURE 2 | Trend of patients with an opioid, by age categorie.

the top 3 of specific diagnoses were back symptoms, low back symptoms, and back syndrome with radiating pain (not shown in the table). Cancer is more frequently associated with the prescription of strong opioids than weak opioids. In the age groups 65–74 and 75–84, cancer is the second most recorded diagnosis when it comes to the prescription of strong opioids. For the oldest age group the diagnosis "general and unspecified complaints" ranks second, after musculoskeletal conditions. Over the years the indications for opioid prescriptions stayed relatively stable, only the oldest age group showed a decrease in

TABLE 3 | Characteristics of opioid prescriptions to older adults in 2017.

	Age category 65–74		Age category 75–84		Age category 85+	
	Weak (%) (n = 6,404)	Strong (%) (n = 4,733)	Weak (%) (n = 4,464)	Strong (%) (n = 4,615)	Weak (%) (n = 1,728)	Strong (%) (n = 3,095)
Chapter diagnoses*						
Musculoskeletal	4,542 (75.2)	3,274 (69.2)	3,370 (75.5)	3,203 (69.4)	1,258 (72.8)	2,027 (65.5)
General and unspecified	232 (3.8)	285 (6.0)	181 (4.1)	375 (8.1)	108 (6.3)	360 (11.6)
Circulatory	162 (2.7)	200 (4.2)	144 (3.2)	264 (5.7)	62 (3.6)	229 (7.4)
Cancer	118 (2.0)	662 (14.0)	71 (1.6)	478 (10.4)	38 (2.2)	197 (6.4)
Skin	192 (3.2)	170 (3.6)	162 (3.6)	180 (3.9)	97 (5.6)	128 (4.1)
Digestive	247 (4.1)	244 (5.2)	132 (3.0)	214 (4.6)	38 (2.2)	119 (3.8)
Respiratory	85 (1.4)	154 (3.3)	55 (1.2)	145 (3.1)	15 (0.9)	82 (2.6)
Nervous system	219 (3.6)	197 (4.2)	169 (3.8)	171 (3.7)	44 (2.5)	81 (2.6)
Number of prescriptions**						
Only 1 prescription	3,161 (56.8)	2,665 (46,4)	2,057 (46.8)	2,133 (40.3)	846 (52.4)	948 (31.9)
2 or more prescriptions	2,401 (43.2)	3,074 (53.6)	2,341 (53.2)	3,154 (59.7)	767 (47.6)	2,025 (68.1)
Chronic use**						
Yes	1,404 (23.6)	1,685 (28.0)	1,231 (26.4)	1,888 (34.1)	457 (26.5)	1,315 (41.7)

Missing values are excluded from the analyses.

***Only patient who are registered in 2016 and 2017 are included in the calculation.*

strong opioid prescriptions for the cancer diagnoses in the period from 2012 to 2017 (not shown in the table). The oldest age group has the highest percentage of patients receiving two or more prescriptions and has a higher chance of long-term opioid use. In all age groups more than half of the patients with an opioid prescription receive two or more prescriptions of strong opioids, and this is more than 70% of the patients aged 85 years or older with an opioid prescription.

Table 4 shows the most common diagnoses for opioids prescriptions for the subgroup analyses in patients within their last year of life. In the age groups 65–74 and 75–84, the most common diagnoses for an opioid prescription within their last year of life are cancer related. In the oldest age group, more general diagnoses are recorded in patients who died within a year after receiving an opioid prescription.

Results of the sensitivity analysis comparing data from the 15 practices with data for both in 2005 and 2015 showed similar results.

TABLE 4 | Specific diagnoses by prescription opioids by age category and being in their last year of life in 2017.

	N	%
65- to 74-year-old patients in their last year of life (n = 694)		
Malignant neoplasm bronchus/lung	89	12.8
Malignant digestive neoplasm, other/NOS	47	6.8
Malignant neoplasm colon/rectum	39	5.6
Malignancy NOS	27	3.9
Malignant neoplasm prostate	23	3.3
75- to 84-year-old patients in their last year of life (n = 1,091)		
Malignant neoplasm bronchus/lung	92	8.4
Malignant neoplasm colon/rectum	53	4.9
Malignant digestive neoplasm, other/NOS	45	4.1
Malignant neoplasm prostate	41	3.8
Back symptom/complaint	38	3.5
85-year-and-older patients in their last year of life (n = 1,378)		
Feeling ill	112	8.1
Heart failure	103	7.5
Pain general/multiple sites	51	3.7
Low back symptom/complaint	41	3.0
Malignant neoplasm colon/rectum	42	3.0

NOS, not otherwise specified.

DISCUSSION

The aim of this study was to show the patterns of opioid prescriptions among subgroups of older patients. The results show that the prescription rates of strong opioids increased in the last decade and that there are differences between age groups when it comes to the prescription of opioids. Patients in the oldest age group are more likely to be prescribed an opioid compared to the other age groups, especially when it comes to strong opioids. Moreover, over 70% of the patients aged 85 years and older who get an opioid prescription, receive more than one prescription for a strong opioid and more than 30% of all the older adults with an opioid prescription use strong opioids chronically (longer than 3 months). The vast majority of opioids (strong and weak) are prescribed for musculoskeletal diagnoses in all age groups.

The differences between age groups are in line with other studies that found that older patients are more likely to receive opioids than younger patients (Campbell et al., 2010; Thielke et al., 2010; Zin et al., 2014; Foy et al., 2016). The general increase in prescriptions of oxycodone in this study resembles previous research (Leong et al., 2009; Kenan et al., 2012; Zin et al., 2014; van Amsterdam and van den Brink, 2015). The increase in fentanyl and buprenorphine prescriptions was larger for the oldest age group in our study which suggests a preference for these opioids for the oldest olds. One explanation for this could be that fentanyl and buprenorphine are both opioids that can be administered through a patch, which can be preferred in case patients have difficulties with swallowing (Pergolizzi et al., 2008).

The decrease we found in the use of tramadol for the oldest old is in line with the caution that is mentioned when treating vulnerable older adults (Chau et al., 2008), as it may cause mental confusion.

The majority of the diagnoses recorded with opioid prescriptions was for musculoskeletal problems. Other studies also reported that (chronic) non-cancer related diagnoses represent the majority of indications for opioid prescriptions (Olsen et al., 2006; Zin et al., 2014). While cancer was the number two diagnosis in 65- to 84-year-old patients to prescribe a strong opioid, for the oldest age group general or unspecified complaints were the second most common reason to prescribe opioids, which could indicate palliative care.

Strengths and Limitations

This study is based on a representative sample of the Dutch primary care population. Because of their gatekeeper function, the PCP holds a complete record of a patient's medical history, including information on diagnoses recorded with prescriptions. The longitudinal aspect of the data allows us to generate a trend in prescribing opioids to older patients, where the categorization of the older adults in three age groups provides more detailed insight in the prescription of opioids to older patients, a vulnerable, vastly growing patient group.

Over the years the electronic medical records from PCPs have improved and the amount of participating GPs has increased. The electronic health records of the participating GP practices were representative compared to GP practices who did not use electronic health records (ref). Geographic representativeness grew over the years by the increasing amount of GPs, from 2010 on worth the geographic representativeness was steady. In the Dutch healthcare system patients are obligatory to be registered by one GP, and therefore the GP has a representative population. In some PCP registration systems specialist prescriptions are included in their electronic medical records since recent years, which means that opioid use in primary care might be overestimated. We do not expect this to affect results on diagnoses but it may have affected the trend. Because we use the recorded data of the PCP, we only look at community dwelling older patients. But our findings are in line with trends found in the literature.

Clinical Implications

When treating an older patient with opioids it is important to monitor the patient during the entire treatment and periodically evaluate the indication for which the opioid is prescribed. We saw that a majority of the older adults receive more than one prescription and 30% uses the opioids chronically. Having a clear treatment plan which is composed with the patient in the beginning of the treatment might help to reduce the long-term use of opioids. In order to optimize pain relieving treatment, there is a need for more information on safety and efficacy of opioids in older patients including patient-reported outcomes in this regard.

CONCLUSION

This study shows that there are meaningful differences in the prescription of opioids within the group of older patients. The oldest olds faced the highest increase in prescription of opioids over the last decade, while this vulnerable group may experience more side effects. Because of the higher risks of opioids for older patients and the high prevalence of chronic use, it is important to monitor the patient throughout the treatment and to critically evaluate the initiation and continuation of an opioid prescription.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study does not fall within the scope of the Medical Research Involving Human Subjects Act and therefore does not require ethical approval. General practices that participate in Nivel Primary Care Database are contractually obliged to: (1) inform their patients about their participation in Nivel Primary Care Database, and (2) to inform patients about the option to opt-out if patients object to inclusion of their data in the database. Dutch law allows the use of electronic health records data for research purposes under certain conditions. According to Dutch legislation, and under certain conditions, neither obtaining informed consent nor approval by a medical ethics committee is obligatory for this kind of observational studies [Dutch Civil Law (BW), Article 7:458; http://www.dutchcivillaw.com/civilcodebook077.htm, Medical Research Involving Human Subject Act (WMO); http://www.ccmo.nl/en/nonwmo-research), and General Data Protection Regulation (AVG) Article 24 (GDPR)]. This study has been approved by the applicable governance bodies of Nivel Primary Care Database under no. NZR-00316.022.

AUTHOR CONTRIBUTIONS

YW, KH, and LD designed the study. YW and KH did the analyses and wrote the manuscript. TS, FS, HL, ER, and LD critically revised the manuscript. LD supervised the project.

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APPENDIX

TABLE A1 | Trend of patients with at least one (strong) opioid, by age category.

Year	Age 65–74		Age 75–84		Age 85+	
	% Patients with at least one opioid (CI 95%)	% Patients with at least a strong opioid (CI 95%)	% Patients with at least one opioid (CI 95%)	% Patients with at least a strong opioid (CI 95%)	% Patients with at least one opioid (CI 95%)	% Patients with at least a strong opioid (CI 95%)
2005	3.5 (2.1–4.9)	1.1 (0.3–0.9)	5.0 (3.3–6.7)	1.3 (0.5–2.2)	5.0 (3.3–6.7)	2.0 (0.9–3.1)
2006	3.6 (2.3–4.9)	1.1 (0.4–1.8)	5.9 (4.2–7.5)	1.7 (0.8–2.6)	7.8 (5.9–9.6)	2.9 (1.7–4.0)
2007	4.0 (2.9–5.1)	1.0 (0.5–1.5)	6.0 (4.7–7.3)	1.8 (1.1–2.6)	7.1 (5.6–8.5)	3.2 (2.2–4.1)
2008	4.5 (3.5–5.5)	1.4 (0.9–2.0)	6.5 (5.3–7.7)	2.3 (1.6–3.0)	9.2 (7.8–10.6)	4.1 (3.2–5.1)
2009	4.9 (3.9–5.9)	1.6 (1.0–2.1)	7.8 (6.5–9.0)	2.9 (2.1–3.7)	9.0 (7.7–10.4)	4.7 (3.7–5.7)
2010	6.2 (5.5–6.9)	1.9 (1.5–2.2)	9.3 (8.5–10.1)	3.5 (3.0–4.0)	12.9 (12.0–13.9)	6.5 (5.8–7.2)
2011	6.8 (6.3–7.3)	2.3 (2.0–2.6)	10.4 (9.8–11.0)	4.2 (3.8–4.6)	13.7 (13.0–14.4)	7.5 (7.0–8.1)
2012	7.0 (6.6–7.5)	2.4 (2.1–2.7)	10.9 (10.3–11.5)	4.6 (4.2–5.0)	14.5 (13.8–15.1)	8.0 (7.5–8.5)
2013	7.4 (7.0–7.8)	2.7 (2.4–3.0)	11.5 (10.9–12.0)	5.1 (4.7–5.4)	15.5 (14.9–16.1)	8.9 (8.5–9.4)
2014	7.7 (7.3–8.1)	3.1 (2.9–3.4)	11.8 (11.3–12.3)	5.7 (5.4–6.1)	16.2 (15.6–16.8)	9.7 (9.2–10.1)
2015	8.5 (8.1–9.5)	3.9 (3.6–4.2)	12.9 (12.3–13.4)	6.8 (6.4–7.2)	17.4 (16.7–18.0)	11.7 (11.2–12.3)
2016	9.0 (8.5–9.5)	4.7 (4.4–5.1)	13.1 (12.5–13.7)	7.6 (7.1–8.1)	18.4 (17.7–19.1)	12.9 (12.3–13.5)
2017	9.2 (8.8–9.7)	5.3 (4.9–5.6)	13.1 (12.6–13.7)	8.2 (7.7–8.6)	17.8 (17.2–18.4)	12.9 (12.4–13.5)

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Genetic Variations and Frequencies of the Two Functional Single Nucleotide Polymorphisms of *SLCO1B1* in the Thai Population

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Aim: To investigate the variations and the frequencies of the *SLCO1B1* gene in the Thai population.

Methods: Collected samples were categorized into five regions of Thailand. DNA samples were genotyped for two variants, c.388A>G and c.521T>C of the *SLCO1B1,* using TaqMan® real-time PCR.

Results: The minor allele frequencies (MAFs) of two single nucleotide polymorphisms (SNPs) were not significantly different among the five regions. The most frequent haplotype was *SLCO1B1*1b* (frequency: 0.654), followed by **1a* (frequency: 0.217), **15* (frequency: 0.128), and **5* (frequency: 0.001). We observed a similar frequency of OATP1B1 transporter phenotypes compared to other populations. 75.85% of the Thai subjects showed normal OATP1B1 activity, 22.5% showed intermediate OATP1B1 activity, and 1.58% showed low OATP1B1 activity.

Conclusion: This study reported the frequencies of the *SLCO1B1* variants and the subsequent OATP1B1 activity in a large cohort of Thais that can provide important information for the guidance of personalized drug therapy.

Keywords: pharmacogenomics, single nucleotide polymorphisms, *SLCO1B1*, organic anion transporting polypeptides, OATP1B1, frequencies, haplotypes, Thai population

INTRODUCTION

The transmembrane protein transporters can be divided into two groups, the solute-linked carrier (SLC) superfamily or known as influx transporters, which uptake the substrate through the cells, and the ATP-binding cassette superfamily (ABC) or efflux transporters which pump the substrates out of the cells (Gong and Kim, 2013). Several groups of the influx transporters uptake a variety of drugs and organic compounds from the blood into the cell, especially, the organic anion

transporting polypeptides (OATPs) which are expressed in many organs such as the intestine, liver, and kidneys. OATPs have shown an important role in clinical implications for the pharmacokinetics of many drugs, including drug absorption, distribution, and elimination (Gong and Kim, 2013; Maeda, 2015; Alam et al., 2018). Evidence of changes in OATP transport function has been found to affect the efficacy and safety of many drugs and leading to instability in drug disposition and response (Gong and Kim, 2013; Shitara et al., 2013).

The genetic variations in the *SLCO1B1* gene, located on chromosome 12p12.1 and encoding the OATP1B1 influx hepatic transporter, have been widely studied in diverse populations (Ghatak et al., 2010; Mastaglia, 2010; Hu et al., 2012; Mastaglia and Needham, 2012; Sirtori et al., 2012; Shitara et al., 2013; Ramsey et al., 2014; Muntean et al., 2017; Alam et al., 2018). OATP1B1 is a transmembrane protein expressed on the basal side of human liver cells, which not only regulates numerous endogenous compounds, including bilirubin, estradiol, and leukotriene C4, but it also removes many drugs, such as HMG-CoA reductase inhibitors (statins), angiotensin II receptor antagonists (ARBs), angiotensin-converting enzyme inhibitors (ACEIs), rifampicin, some antidiabetic drugs, protease inhibitors (PIs), and some chemotherapies from the blood into the hepatocytes, then metabolizes and removes out of the body (Hu et al., 2012; Gong and Kim, 2013; Maeda, 2015; Alam et al., 2018). The polymorphisms of the *SLCO1B1* gene not only affect the expression, localization, and function of the OATP1B1, but also the drug disposition (Gong and Kim, 2013; Shitara et al., 2013).

Various studies have revealed the single nucleotide polymorphisms (SNPs) of the *SLCO1B1* gene reducing the functional transport activity of OATP1B1 and causing some adverse drug reactions (ADRs) (Mastaglia, 2010; Mastaglia and Needham, 2012; Gong and Kim, 2013; Shitara et al., 2013; Maeda, 2015; Alam et al., 2018). *SLCO1B1* variants, including *SLCO1B1*1a*, *SLCO1B1*1b* (c.388A>G, N130D), *SLCO1B1*5* (c.521T>C, V174A), and *SLCO1B1*15* (c.388A>G and c.521T>C) have been reported in affecting the transport activity and drug disposition (Kim et al., 2007; Ho et al., 2008; Gong and Kim, 2013; Ramsey et al., 2014; Namgoong et al., 2015). Statin-taking patients with *SLCO1B1* polymorphisms showed the area under the plasma-time curve (AUC) up to 130% higher than the patients without the *SLCO1B1* polymorphisms (Ghatak et al., 2010; Sirtori et al., 2012). Also, a reduced transporter activity caused more susceptible to statin-induced myotoxicity in the group of patients carrying *SLCO1B1* polymorphisms than those without polymorphisms (Sakamoto and Kimura, 2013; Hamann et al., 2013; Ramsey et al., 2014). *SLCO1B1*5* has been strongly associated with myopathy among the simvastatin users with an odds ratio (OR) ranging from 4.5 in heterozygotes to 16.9 in homozygotes (Link et al., 2008; Ghatak et al., 2010; Mammen and Amato, 2010; Sirtori et al., 2012; Hu et al., 2012; Rallidis et al., 2012; Sathasivam, 2012; Gong and Kim, 2013; Bhardwaj et al., 2013; Dandona, 2014; Albayda and Christopher-Stine, 2014; Maeda, 2015). Also, *SLCO1B1*15* showed over 70% reduction in the transport activity compared with wild type and showed the association with myopathy in patients taking pravastatin and atorvastatin (Ghatak et al., 2010; Sirtori et al., 2012; Gong and Kim, 2013; Shitara et al., 2013; Maeda, 2015).

Currently, there is a lack of studies reporting the frequency of the SNPs of *SLCO1B1* in the Thai population. The translational decision in the clinical practices has always used the data from the reports in other populations, the Han Chinese population, for instance. The objective of this study was to investigate the regional frequencies of the two functional SNPs of *SLCO1B1* in the Thai population. The findings of this study will serve as the Thai pharmacogenetic data source for decision in drug therapy in a specific group of patients, especially, in patients who will be treated with statins or other medications that are affected by these genetic variants.

MATERIALS AND METHODS

Samples

In the present study, we enrolled 1,205 samples from the previous cohort of Wongkittichote et al. (2013), which were collected from August 2008 to March 2009 by the Health System Research Institute. The selected samples were then categorized into five regions of Thailand, including Northern, Northeastern, Central, Southern, and Bangkok.

Genotyping Analysis

Genotyping of *SLCO1B1* polymorphisms was performed using allele-specific TaqMan® MGB probe 5' nuclease assay with real-time polymerase chain reaction (PCR) ViiA7™ system (Applied Biosystems, Life Technologies). The allele-specific TaqMan® MGB probe 5' nuclease chain reaction assay was performed with primers of *SLCO1B1* c.388A>G (rs2306283; on reference sequence NM_006446.4, assay ID: C:_1901697_20) and c.521T>C (rs4149056; on reference sequence NM_006446.4, assay ID: C:30633906_10). Each 6 µl of PCR mixture contained 2 µl of genomic DNA in a concentration of 5 ng/µl, 2.5 µl of TaqMan® Genotyping Mastermix, 0.25 µl of allele-specific TaqMan® MGB probe and sequence-specific primer kit, and 1.25 µl of DNase-free water. The thermal cycler program started with 10 min at 95°C, followed by 50 cycles of 15 s at 92°C and 90 s at 60°C. The allelic discrimination plot was analyzed by ViiA7™ software (Applied Biosystems, Life Technologies). Allele 1 was labeled with VIC® dye fluorescence, and allele 2 was labeled with FAM™ dye fluorescence.

OATP1B1 Phenotypes Based on *SLCO1B1* Genotypes

The Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *SLCO1B1* and simvastatin-induced myopathy 2014 update was used to assign the likely OATP1B1 phenotype (normal function, intermediate function, and low function) and * allele nomenclature (Ramsey et al., 2014).

Statistical Analysis

The frequencies of two SNPs of *SLCO1B1*, c.388A>G and c.521T>C, were checked for Hardy-Weinberg equilibrium using the R statistic version 3.6.1, the R Foundation for Statistical Computing. Fisher's Exact and Chi-square tests were used to determine the statistical difference between the minor alleles and haplotype frequencies between the geographical regions of Thailand using SPSS version 18.0 for Window, SPSS Inc., Chicago, IL, United States. A *p*-value of less than 0.05 was considered significant.

RESULTS

Allele and Haplotype Frequencies of *SLCO1B1* in Thai Population

The allele frequencies of the non-synonymous polymorphic variants in the coding region c.388A>G (N130D) and c.521T>C (V174A) of *SLCO1B1* gene and haplotype frequencies of *SLCO1B1*1a*, **1b*, **5*, and **15* in 1,205 healthy Thai samples distributed over five regions of Thailand are shown in **Table 1**. All detected variations were in Hardy-Weinberg equilibrium ($p>0.05$). The allele frequencies of c.388A>G were similar among five regions. At the same time, the SNP c.521T>C showed the most frequency in Bangkok and the least in the Southern region, however, there were no significant differences in minor allele frequencies of these two SNPs among the five regions of Thailand. The most frequent haplotype was *SLCO1B1*1b* (frequency: 0.654), followed by **1a* (frequency: 0.217), **15* (frequency: 0.128), and **5* (frequency: 0.001). We did not observe significant differences in haplotype frequencies among the five regions (**Table 1**).

The Phenotypes of OATP1B1 Transporter Based on *SLCO1B1* Diplotypes in Thai Population

The phenotypes of the OATP1B1 transporter have been assigned based on the diplotype at c.388A>G and c.521T>C of the *SLCO1B1* gene. The phenotype frequencies distributed over the five regions of Thailand and worldwide are shown in **Table 2**.

DISCUSSION

Numerous data have reported the genetic variations of the *SLCO1B1* gene for the determination of clinical drug response.

TABLE 1 | The minor allele frequencies of *SLCO1B1* c.388A>G (rs2306283) and c.521T>C (rs4149056) and the observed frequencies for selected *SLCO1B1* haplotypes in Thais distributed among the five regions of Thailand.

SLCO1B1	Total	Northern	Central	Northeastern	Southern	Bangkok	*p*-value
Minor allele frequencies							
N	**1,205**	**279**	**318**	**379**	**159**	**70**	
c.388A>G	0.782	0.805	0.769	0.792	0.755	0.757	0.328
c.521T>C	0.129	0.120	0.143	0.137	0.085	0.150	0.090
Haplotype frequencies (%)							
N	**2,410**	**558**	**636**	**758**	**318**	**140**	
**1a*[a]	21.74	19.53	22.96	20.84	24.21	24.29	0.382
**1b*[b]	65.39	68.46	62.74	65.44	67.30	60.71	0.187
**5*[c]	0.08	0.00	0.16	0.00	0.31	0.00	0.450
**15*[d]	12.78	12.01	14.15	13.72	8.18	15.00	0.071

*[a], *1a = wild type at all loci; [b], *1b =rs2306283 G allele (A ancestral) (c.388A>G, p.N130D); [c], *5 = rs4149056 Callele (T ancestral) (c.521T>C, p.V174A); [d], *15 = rs2306283 G allele (A ancestral) and rs4149056 C allele (T ancestral).*

TABLE 2 | OATP1B1 phenotypes based on *SLCO1B1* diplotypes in Thais distributed among the five regions of Thailand.

OATP1B1 phenotypes	*SLCO1B1* diplotypes	Phenotype frequencies (%)						
		Worldwide[a]	Total	Northern	Central	North eastern	Southern	Bangkok
Normal function	**1a/*1a* **1a/*1b* **1b/*1b*	55 – 88	75.85	76.70	73.27	74.14	84.91	72.86
Intermediate function	**1a/*5* **1a/*15* **1b/*5* **1b/*15*	11 – 36	22.57	22.58	24.84	24.27	13.21	24.29
Low function	**5/*5* **5/*15* **15/*15*	0 – 6	1.58	0.72	1.89	1.58	1.89	2.86

[a], Frequency of the polymorphism varies by ancestral group.

The allele frequencies of the polymorphic variations and haplotype frequencies of the *SLCO1B1* gene, which are *SLCO1B1*1a*, *SLCO1B1*1b*, *SLCO1B1*5*, and *SLCO1B1*15,* have been studied in the various population groups. Among the published reports, *SLCO1B1* c.388A>G (N130D) and *SLCO1B1* c.521T>C (V174A) are the most commonly investigated SNPs in the various ethnic groups. This present study investigated the frequencies of these two common polymorphic variations in the Thai population distributed across five regions of Thailand. We found that allele frequencies of the c.388A>G and c.521T>C variants were close to the frequencies reported in other Asian populations, including Han Chinese, Japanese, Korean, and Vietnamese (Kim et al., 2008; Namgoong et al., 2015). When compared separately, the frequencies of c.388A>G were similar in Asian ancestry but showed differently in Asian Indians and Caucasians, while the frequencies of c.521T>C showed similarity in all ethnicity except Asian Indian and African ancestry (**Table 3** and **Figure 1**).

The most remarkable haplotypes of the *SLCO1B1* gene, *SLCO1B1*5* and *SLCO1B1*15*, have been reported to reduce the number and function of the OATP1B1 transporter. These haplotypes not only alter the disposition of many therapeutic drugs, including various statins, but also affect the adverse events, especially statin-induced myotoxicity (0.3% in Thai population). Many reports have confirmed that the *SLCO1B1*5* allele is associated with statin-induced myotoxicity in several populations (Hamann et al., 2013; Sakamoto and Kimura, 2013; Ramsey et al., 2014). Nevertheless, the frequency of *SLCO1B1*5* haplotype in Asian population was very low when compared with other geographical groups (**Figure 1**). Published research on haplotype frequencies of the *SLCO1B1*5* have shown the prevalence of 1.2% in a Chinese population, 0.7% in Japanese, and absent in Korean, and Vietnamese. We observed the *SLCO1B1*5* haplotype having a frequency of 0.08% in the Thai population. *SLCO1B1*1b* is the most abundant haplotype in Asian populations (approximately 55% to 70%). The frequency of *SLCO1B1*15* in our study showed a similar range compared to the Asian populations but lower when compared with South American populations (Nozawa et al., 2002; Kim et al., 2008).

We observed similar diplotype frequencies of the OATP1B1 transporter in the Thai population compared to previous reports in Asian populations, including Han Chinese, Japanese, Korean,

TABLE 3 | The observed frequencies for selected *SLCO1B1* haplotypes in the Thai population and other geographical groups.

***SLCO1B1* haplotypes**	**Haplotype frequencies (%)**							
	Thai	**Asian[a]**	**SW Asian[b]**	**Middle Eastern**	**Oceania**	**Caucasian[c]**	**South/ Central American**	**African[d]**
**1a*	22	27	47	49	34	50	37	17
**1b*	65	60	46	31	66	22	39	78
**5*	0	0	0	5	0	1	0	0
**15*	13	13	6	15	0	14	24	3

[a], Asian = East Asian, Chinese, Japanese, Korean, Malays, and Vietnamese; [b], SW Asian = South/Central Asian and Indian; [c], Caucasian = American, European, Canadian, German, Finnish, French, Dutch, and Turkish; [d], African = American, North African, Sub-Saharan Africa, Ugandan, and Tanzanian.

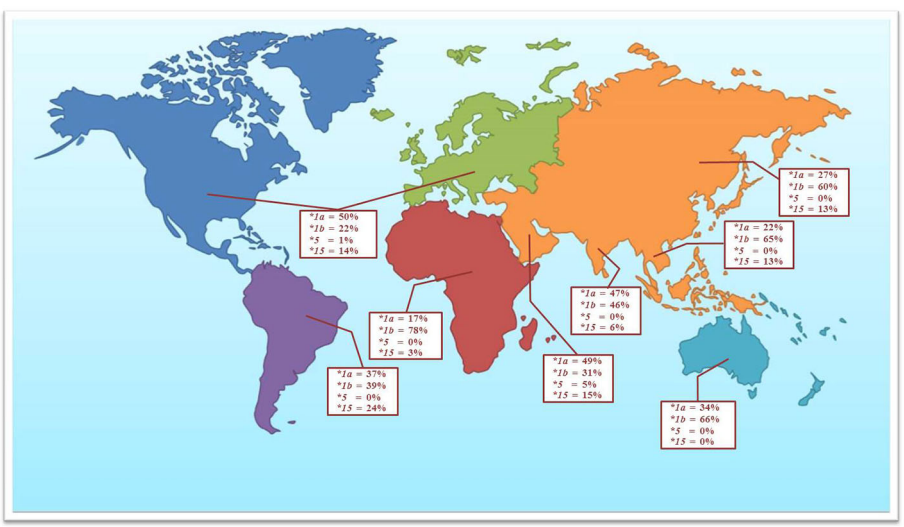

FIGURE 1 | The observed frequencies for selected *SLCO1B1* haplotypes in the Thai population and other geographical groups (data adapted from Ramsey et al., 2014).

and Vietnamese (Nozawa et al., 2002; Kim et al., 2008). The *SLCO1B1* phenotypes based on diplotypes in the Thai population were in agreement with the comprehensive data of the function of the OATP1B1 transporter (**Table 3**).

In conclusion, frequencies of the *SLCO1B1* variants and the subsequent OATP1B1 activity in a large cohort of Thais can provide important information for the guidance of personalized drug therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Committee on Human Rights Related to Reseach Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CN and CS were responsible for analysis, interpretation of data, and final approval of the manuscript. JW and CD were responsible for concept and design. PS was responsible for the analysis of data. SW and CS were responsible for supervising the overall conduct of the study.

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