AUTOIMMUNE THYROID PATHOLOGY - SPECIFICITY OF THE PEDIATRIC AGE

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EDITORIAL
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Editorial: Autoimmune Thyroid Pathology—Specificity of the Pediatric Age

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Editorial on the Research Topic

Autoimmune Thyroid Pathology—Specificity of the Pediatric Age

Autoimmune thyroid diseases (AITDs) are the most common organ-specific autoimmune disorders and the most frequent cause of acquired thyroid dysfunction in adulthood as well as in childhood. AITDs include two main clinical presentations: Hashimoto's thyroiditis (HT) and Graves' disease (GD), both characterized by lymphocytic infiltration of the thyroid parenchyma. Clinical and biochemical features of HT and GD are related to hypothyroidism and thyrotoxicosis, respectively (1). In the pediatric population, the prevalence of AITDs is lower than in adults (1.2–1.3% for HT and 1‰ for GD), but increases in presence of chromosomopathies such as Down and Turner syndromes (2). The most common age of presentation of AITDs is adolescence but they may develop at any time, rarely however in infants. Sexual dimorphism, with a female predominance, is similar to adult population.

Although the pathogenic mechanism of AITDs is still under investigation, the evidence in favor of a genetic basis for AITDs is abundant and the factors that might influence the development of autoimmune disease can be related to genetic susceptibility, chromosomal differences or epigenetics (1).

Aim of this Research Topic was to report the most updated views on epidemiology, pathophysiology, diagnosis, long-term prognosis, treatment and management of AITDs in childhood and adolescence. This Research Topic put together eight original articles, two narrative reviews and one very particular case report, summarizing current knowledge on the pathogenesis, clinical long-term experience, clustering and interrelation with other autoimmune diseases, also on the peculiarity of AITDs in genetic syndromes in pediatric populations.

In the narrative review "Hashimoto Thyroiditis and Dyslipidemia in Childhood", Vukovic et al. discussed recent findings regarding the effects of AITDs on lipid metabolism and cardiovascular risk (CVR), including the beneficial impact of L-T4 treatment on dyslipidemia and potential usefulness of novel lipid biomarkers, such as proprotein convertase subtilisin/kexin type 9 (PCSK9), non-cholesterol sterols, low-density lipoprotein particle size and number and high-density lipoprotein structure and functionality in pediatric patients with AITDs.

In the second review "Autoimmune Thyroid Disease in Specific Genetic Syndromes in Childhood and Adolescence", Kyritsi and Kanaka-Gantenbein presented the state of the art

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on autoimmune and non-autoimmune thyroid pathology in chromosomal and other genetic origin syndromes, in a very detailed and updated way. We want to emphasize the didactic usefulness of this paper for pediatricians and pediatric endocrinologists.

The description, published by Bruns et al., of a rare case of polyautoimmunity including autoimmune thyroiditis, Sjögren's syndrome, vitiligo and celiac disease, entitled "Unusual presentation of polyautoimmunity and renal tubular acidosis in an adolescent with Hashimoto's thyroiditis and central pontine myelinolysis", highlighted the need for pediatricians to be aware of rare accompanying diseases and their complications in "common" pediatric autoimmune diseases like Hashimoto's thyroiditis and celiac disease.

In the context of this Research Topic, there are also four original retrospective studies that allowed us to increase our clinical knowledge based on the wide experience of the Authors.

Calcaterra et al. analyzed a pediatric population with onset of AITDs to assess gender differences with regard to onset age, disease subtype, pubertal status, autoimmune co-morbidity, family history, and treatment, focusing on the interaction between gender and pubertal stage. In the Authors' opinion, the gender specific characteristics of disease across puberty might help early diagnosis and clinical management of the thyroid pathology.

Admoni et al., in their study of "Long-Term Follow-Up and Outcomes of Autoimmune Thyroiditis in Childhood," tried to assess which factors at presentation can predict evolution over time of AITDs in pediatric age.

Kucharska et al. dealt with the topic "Clinical and Biochemical Characteristics of Severe Hypothyroidism Due to Autoimmune Thyroiditis in Children". Authors concluded that in children with severe hypothyroidism, above all without goiter, the most sensitive clinical symptoms were growth arrest and weight gain. Moreover, the specific biochemical profile closely correlated to the condition involved mostly erythropoiesis, liver function, and kidney function. Furthermore, pituitary enlargement should be considered in every child with severe hypothyroidism.

In a single-center retrospective study, over a 9-year observation period, Glowinska-Olszewska et al. revealed an increasing rate of new diagnosis of type 1 diabetes (T1D) together with a growing overall prevalence of additional autoimmune diseases (AITDs and coeliac) at T1D onset.

Again Glowinska-Olszewska et al. prospectively investigated whether HT could increase CVR in young patients with T1D. The crucial finding of their study was that young patients with T1D and coexisting additional HT had a much more unfavorable profile of classical CVR factors compared to T1D peers without any additional disease. Furtak et al. in their clinical study suggested the protective role of infliximab therapy in the development of AITD and the usefulness of thyroid ultrasound to identify the probable risk for AITD in pediatric patients with Crohn's disease.

Finally, two very innovative studies on genetic predisposition for autoimmune diseases development have been published on this Research Topic.

Borysewicz-Sanczyk et al. performed a genetic association study of IL2RA, IFIH1, and CTLA-4 polymorphisms with AITD and T1D. Authors confirmed that in children and adolescents the investigated genes loci had a role in T1D, but not in AITD susceptibility.

Furthermore, Sawicka et al. performed an analysis of polymorphisms rs709369-*IL-2RA*, rs7138803-*FAIM2* and rs1748033-*PADI4* in a cohort of adolescents with AITDs followed in two European centers of pediatric endocrinology and demonstrated that these polymorphisms confer a genetic predisposition to develop autoimmune thyroid disorders, especially Graves' disease.

In conclusion, this Research Topic provides an important and updated contribution on the peculiarity of AITDs in childhood. Several papers highlighted the need for prospective studies to clarify certain pathogenetic, clinical, and also therapeutic aspects of AITDs in childhood.

Finally, AITDs in pediatric age is confirmed as an active and still growing area of research. Increasing knowledge of pathogenetic mechanisms will allow to better determine predisposition to AITD development, to earlier diagnose thyroid gland dysfunction, and to improve treatment.

AUTHOR CONTRIBUTIONS

MW, AG, and TA conceptualized, designed, wrote and approved the Editorial. All authors contributed to the article and approved the submitted version.

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REVIEW
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Hashimoto Thyroiditis and Dyslipidemia in Childhood: A Review

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Hashimoto autoimmune thyroiditis (AIT) is the most common cause of acquired hypothyroidism in the pediatric population. Development of AIT is mediated mainly by cellular immune response directed toward thyroid autoantigens, leading to inflammation and impaired function of thyroid gland. Both thyroid dysfunction and inflammation affect the metabolism of plasma lipoproteins. The alterations in lipid profile worsen with the advancement of hypothyroidism, ranging from discrete changes in euthyroid AIT patients, to atherogenic dyslipidemia in the overt hypothyroidism. In this review, characteristics of dyslipidemia in pediatric AIT patients, and the consequences in respect to the risk for cardiovascular disease (CVD) development are discussed. Additionally, benefit of L-thyroxine treatment on serum lipid profile in pediatric AIT patients is addressed. Finally, potential usefulness of novel lipid biomarkers, such as proprotein convertase subtilisin/kexin type 9 (PCSK9), non-cholesterol sterols, low-density lipoprotein particle size and number, and high-density lipoprotein structure and functionality in AIT patients is also covered. Further longitudinal studies are needed in order to elucidate the long-term cardiovascular outcomes of dyslipidemia in pediatric patients with Hashimoto AIT.

Keywords: Hashimoto autoimmune thyroiditis, children, dyslipidemia, novel lipid biomarkers, L-thyroxine treatment

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INTRODUCTION

Hashimoto autoimmune thyroiditis (AIT) is the most common cause of acquired hypothyroidism in childhood and adolescence. The prevalence of AIT in childhood peaks at early to mid- puberty. Presentation of the disease is rare before the age of 3 years, but there are described cases in infancy, too (1). Female strong preponderance has been reported with female to male ratio up to 3.4:1 (1–3), with high prevalence in patients with Down and Turner syndrome (4). Clinical manifestations of AIT in childhood are extremely diverse, ranging from completely normal, asymptomatic state, to pronounced symptoms of severe thyroid dysfunction.

Thyroid hormones have a broad spectrum of physiological effects on lipoprotein metabolism. As a result, plasma lipid and lipoprotein levels are sensitive to changes in the thyroid hormones concentrations. The alterations in lipid profile accompanying AIT worsen along with the advancement of hypothyroidism, ranging from discrete pro-atherogenic markers in euthyroid AIT, to full-blown dyslipidemia in many patients with the overt hypothyroidism (5–7). Furthermore, autoimmune disease itself has significant impact on lipid profile, as evidenced by a high prevalence of dyslipidemia in patients with autoimmune diseases (8–10), which may account, at least in part,

to the increased cardiovascular disease (CVD) risk. Thus, it could be regarded as convenient that the efficacy of L-thyroxine (L-T4) treatment in the normalization of lipid status is directly proportionate to the degree of thyroid dysfunction, being highest in the overt hypothyroidism (5, 7, 11, 12). However, the waist majority of data linking autoimmune thyroid disease with dyslipidemia were gained from the studies in adults (13), whereas data in pediatric populations are limited. Also, data is scarce regarding the effects of L-T4 treatment on lipid profile in pediatric hypothyroidism, with or without thyroid autoimmunity (14–18).

In this narrative review, we will discuss recent findings regarding the effects of AIT on lipid metabolism and CVD risk, including the impact of L-T4 treatment on dyslipidemia and potential use of novel lipid biomarkers in pediatric patients with AIT.

DEVELOPMENT AND CLINICAL MANIFESTATIONS OF HASHIMOTO'S THYROIDITIS IN CHILDHOOD

Like other autoimmune diseases, AIT is multifactorial disease caused by complex interplay of genetic (1, 19–26), environmental (21, 27–29), and hormonal factors (19, 21, 30), that provoke the inappropriate immune response against thyroid gland. HT is mainly mediated by cellular immune response directed toward thyroid autoantigens, leading to inflammation, fibrosis, and impaired function of thyroid gland (4, 26). The first step in pathogenesis is believed to be activation of autoreactive CD4+ T cells i.e., T helper (Th) cells specific for thyroid autoantigens. Th cells type 1 (Th1) activate cytotoxic T lymphocytes (CD8+ lymphocytes) and macrophages, which directly destroy thyroid follicular cells (31). Another subset of Th cells with a role in development and progress of chronic inflammation and tissue damage in HT are Th17 cells. Higher proportion of Th17 cells, as well as higher levels of cytokines produced by these cells were found in peripheral blood and thyroid tissue in HT patients compared with healthy controls (32–34). It is also observed that T regulatory (Treg) cells, cells with immunosuppressive function, accumulate in thyroid tissue of HT patients. However, in these patients Treg cells were found to be dysfunctional (35, 36). B lymphocytes, although representing humoral immunity, are also activated in AIT, producing antibodies against thyroid autoantigens (26). These cells are part of thyroid lymphocyte infiltrate (37) and exert antibody synthesis in the gland (31, 38). Autoantibodies are crucial component in AIT pathogenesis, since antibody-dependent cell-mediated cytotoxicity is another and important factor responsible for apoptosis of thyroid follicular cells in this disease (26, 31).

Clinical presentation of AIT is best reviewed with respect to the thyroid status, since children with AIT can present as completely euthyroid, with mild subclinical hypothyroidism, severe overt hypothyroidism, or in the state of subclinical or overt hyperthyroidism (Hashitoxicosis) (39–43). Majority of children with AIT are either euthyroid or subclinically hypothyroid at the time of diagnosis (41, 42). Euthyroid state, defined by thyroid function tests within normal range, is usually asymptomatic, besides the frequent finding of a goiter (41, 42, 44, 45). Subclinical hypothyroidism in AIT, defined by elevated TSH with normal levels of serum thyroid hormones (fT4 and fT3), is usually classified as mild (TSH 4.5–10 mIU/l) or severe (TSH > 10 mIU/l) (7, 41, 46–49). Although the very name—"subclinical hypothyroidism" implies that this form of thyroid dysfunction presents merely as a laboratory finding without any signs or symptoms of clinical hypothyroidism besides goiter, these patients actually may present with other clinical and laboratory findings (7, 45, 47, 50, 51). Typical clinical signs and symptoms of hypothyroidism have been reported in some children with subclinical hypothyroidism, as well as the improvement of hypothyroidism symptoms scores with L-T4 treatment (7, 47, 51). Also, untreated long-lasting subclinical hypothyroidism in children has been firmly associated with subtle pro-atherogenic alterations in lipid profile (7, 17, 52). On the other hand, currently available data indicate that children with untreated longstanding subclinical hypothyroidism have normal linear growth, neurocognitive and behavioral outcomes, and bone health status (7, 47, 48, 53). It should be noted that although the association of obesity and subclinical hypothyroidism is well-documented, abnormal thyroid function in obese patients seems to be a consequence of obesity, rather than a cause (47, 48).

Overt hypothyroidism, defined by elevated TSH with low level of serum fT4, is present in ∼20% of all children with AIT at the time of diagnosis, and the onset of clinical manifestations is usually subtle (41, 42, 46). Classical signs and symptoms of overt hypothyroidism which may be seen in these children are: goiter, constipation, weight gain, poor growth velocity or short stature, fatigue and somnolence, poor school performance, cold intolerance, dry skin, bradycardia, yellowish-pale skin tone with facial puffiness (myxedema), with frequent laboratory findings of anemia and dyslipidemia (41, 42, 46). Adolescents with overt hypothyroidism can also present with delayed or arrested pubertal development, irregular menstrual periods, menometrorrhagia, or amenorrhea in girls (41, 46). Rarely, girls with longstanding severe overt hypothyroidism can present with precocious puberty and menstrual bleeding with hyperprolactinaemia and delayed bone age (Van Wyk-Grumbach syndrome) (42, 46, 54).

Hashitoxicosis, the initial hyperthyroid phase of AIT caused by the release of preformed thyroid hormones from the gland, can be detected in ∼10%, and subclinical hypothyroidism in up to 3% of children with AIT (39–43, 55–57). Clinical signs and symptoms of children with hashitoxicosis are those of hyperthyroidism, and cannot be distinguishable can be indistinguishable from Grave's disease: goiter, tachycardia, tremor, weight loss, restlessness, warm moist skin, ophthalmopathy, growth acceleration, delayed, or precocious puberty (39, 41, 58). Fortunately, this hyperthyroid phase of AIT is transient, usually resolving within several months into euthyroid state, or progressing to permanent hypothyroidism (41, 55, 56).

Apart from the described symptoms caused by the thyroid dysfunction itself, children with AIT may also have other autoimmune diseases or syndromes, such as celiac disease,

type 1 diabetes (T1DM), Down's or Turner's syndrome, with corresponding symptoms adding to the overall clinical picture (42, 46).

HASHIMOTO THYROIDITIS AND DYSLIPIDEMIA

In general, subclinical hypothyroidism can slow-down metabolic pathways of cholesterol uptake, synthesis, and secretion, as well as reverse cholesterol transport process and catabolism of triglyceride (TG)-rich lipoproteins (**Figure 1**) (12, 59, 60). As compared to healthy children, total and low-density lipoprotein cholesterol (LDL-C) levels are commonly elevated, while the level of high-density lipoprotein cholesterol (HDL-C) can be normal or decreased in patients with subclinical hypothyroidism (**Table 1**). Although some authors reported no differences in serum lipid profile between pediatric patients with subclinical hypothyroidism and controls, the frequency of dyslipidemic children was significantly higher in the patients group (51, 61). The study evaluating children, adolescents and adults with subclinical hypothyroidism suggested that abnormalities in lipid profile are more pronounced in adult patients, as well as in those with severe form of the disease (62). Yet, the impact of the disease severity on lipid profile was not confirmed in later studies in pediatric patients (61, 63).

The hallmark of subclinical hypothyroidism-related dyslipidemia is reduced synthesis of liver LDL receptors and the mechanisms behind this effect have been extensively studied and explained. Emerging evidence suggests that the levels of circulating proprotein convertase subtilisin/kexin type 9 (PCSK9), a serin-protease responsible for downregulation of liver LDL receptors, is also increased in subclinical hypothyroidism (64), paving the way for innovative lipid-lowering therapy in this category of patients (5). However, data on PCSK9 in children are sparse and the studies examining efficacy, safety, and tolerability of PCSK9 inhibitors in pediatric patients are underway (65).

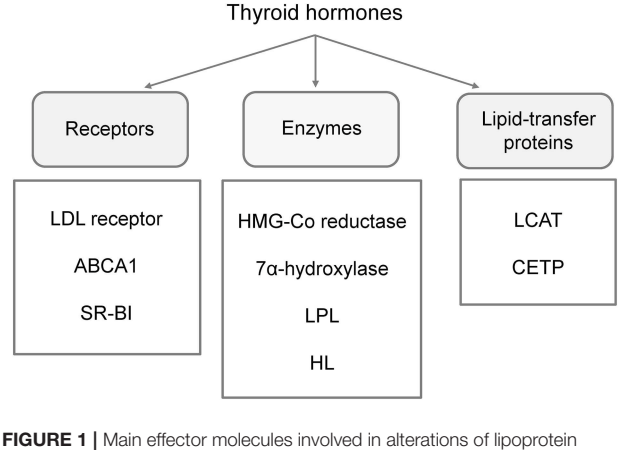

FIGURE 1 | Main effector molecules involved in alterations of lipoprotein metabolism driven by thyroid hormones. LDL, low-density lipoprotein; ABCA1, ATP binding cassette subfamily A member 1; SR-BI, scavenger receptor class B type 1; LPL, lipoprotein-lipase; HL, hepatic lipase; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl-ester transfer protein.

Body cholesterol pool is maintained by delicate balance between the processes of cholesterol synthesis, absorption, and biliary secretion, all of which can be affected even by subtle alterations in thyroid hormones levels (60). Plasma non-cholesterol sterols, including cholesterol precursors and plant sterols, are validated biomarkers of cholesterol biosynthesis and intestinal absorption efficiency (66). The results of a small study by Matysik et al. (67) suggested that the levels of plasma non-cholesterol sterols could serve as indicators of disrupted cholesterol homeostasis in patients with hyper- and hypothyroidism. Recently, plasma profile of non-cholesterol sterols from birth to 15 years of age was characterized in pediatric population without dyslipidemia (68). These data could form a solid base for future evaluation of the extent of cholesterol synthesis and absorption alterations in children and adolescents with AIT.

The lack of thyroid hormones is associated with reduced clearance of TG-rich particles, due to attenuated lipoprotein-lipase (LPL) and hepatic lipase (HL) activities, and increased production of very-low density lipoprotein (VLDL) particles (5). Hence, apart from the impact on LDL-C level, thyroid dysfunction may affect qualitative characteristics and functional properties of LDL particles. Namely, hypertriglyceridemia is intimately linked to the increased production of small, dense LDL particles (69). Bearing in mind that thyroid hormones may protect LDL particles from oxidation (70), and the fact that small, dense LDL particles are prone to oxidative modifications (69), there is increased potential for adverse modification of LDL particles in hypothyroid state. Indeed, increased small, dense LDL, and oxidized LDL particles were recently reported in normolipidemic adult patients with hypothyroidism (71). These data clearly demonstrated the usefulness of advanced lipid testing for identification of the patients with high CVD risk, which should be further confirmed in pediatric patients with both subclinical and overt thyroid dysfunction.

In contrast to firm scientific and clinical evidence which consistently points to elevated LDL-C concentrations in patients with hypothyroidism, data regarding HDL-C are not homogenous. As it has been presented in **Table 1**, HDL-C levels were decreased or unchanged in hypothyroid states. Interpretation of data and drawing of conclusions is particularly complicated in pediatric population, since most of the data regarding the association of HDL and thyroid status is derived from studies in adults. It is also noteworthy that studies analyzing HDL-C concentration in children with hypothyroidism were conducted in smaller cohorts, which might affect the reliability of the obtained results. Therefore, larger studies with prospective design are needed to resolve this issue.

However, a contemporary approach to HDL's clinical significance might put aside these conflicting results concerning HDL-C levels in hypothyroid subjects, since another question is considered as even more important in modern research and clinical practice. Namely, due to complex structure and numerous functions of HDL, it is nowadays accepted that quality of this lipoprotein's particles is more significant than their

TABLE 1 | Lipid profile of pediatric patients with subclinical hypothyroidism.

Authors	*N*	Age, years	Alterations of lipid profile	References
Paoli-Valeri et al. (2005)	17	4.3 ± 1.0	↓HDL-C; ↔TC; ↔ LDL-C; ↔ TG; ↔ TC/HDL-C; ↔ LDL-C/HDL-C	(14)
Cerbone et al. (2014)	49	8.5 ± 0.5	↓HDL-C; ↑TC/HDL-C; ↑TG/HDL-C; ↔TC; ↔ LDL-C; ↔ TG; ↔ non-HDL-C	(17)
Dahl et al. (2018)	228	13.3 ± 4.2	↑TC; ↑non-HDL-C; ↔ HDL-C	(18)
Catli et al. (2014)	27	10 (6.9)*	↔TC; ↔LDL-C; ↔HDL-C; ↔TG	(51)
Cerbone et al. (2016)	39	9.2 ± 3.6	↓HDL-C; ↑TC-/HDL-C; ↑TG/HDL-C; ↔TC; ↔ TG; ↔ LDL-C; ↔ non-HDL-C	(52)
Unal et al. (2017)	38	8.1 ± 3.6	↑TC; ↑LDL-C; ↑LDL-C/HDL-C; ↑TC/HDL-C; ↔ HDL-C; ↔ TG	(61)
Marwaha et al. (2011)	280/35#	12.8 ± 2.8	↔/↓HDL-C; ↔/↔TC; ↔/↑ TG; ↔/↑ LDL-C	(62)
Isguven et al. (2016)	66	14.4 ± 2.4	↑TC; ↑LDL-C; ↔ TG; ↔ HDL-C	(63)

*In relation to euthyroid or control group: ↑, increased; ↓, reduced; ↔, unchanged. *Median (interquartile range) was reported. #280 patients with TSH ≤ 10 mIU/L and 35 patients with TSH > 10 mIU/L were studied. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; non-HDL-C, non-high-density lipoprotein cholesterol.*

cholesterol content (72). As precisely summarized in a review by Triolo et al. (73), functional properties of HDL could be grouped in four essential categories: reverse cholesterol transport, antioxidative, anti-inflammatory, and vasodilatatory activities. It has also been demonstrated that alterations in HDL structure affect its functionality. In addition, it is now clear that both HDL structure and functions can be easily modified if changes occur in their vascular environment (74). Therefore, several new aspects should be considered regarding HDL in patients with AIT, including the impact of subclinical or overt hypothyroidism on HDL's quality, as well as the influence of pro-inflammatory and pro-oxidative environment on these particles. Providing answers on these questions might have significant implications for interpretation of CVD risk in AIT patients, especially those of young age, considering their long-term exposure to possible detrimental factors (**Figure 2**).

Changes of HDL's structure and function in relation to thyroid hormones status are largely unexplored. However, a recent study has shown that both cholesterol efflux and activity of HDL-associated enzyme paraoxonase 1 (PON1) are decreased in patients with overt hypothyroidism (75), thus implicating diminishing of HDL functionality. Several reasons could be responsible for such findings. Namely, studies involving human subjects and animal models have demonstrated that hypothyroidism is associated with decreased activities of cholesteryl-ester transfer protein (CETP), lecithin:cholesterol acyltransferase (LCAT), and HL (76, 77). Having in mind that these enzymes are key regulators of HDL metabolism, changes in HDL structure and consequently function should be expected. Indeed, a higher prevalence of larger HDL 2 subclasses was found in subjects with hypothyroidism in a study by Tan et al. (76). Larger HDL particles are generally considered as highly atheroprotective, but, as it has been already mentioned, novel data (75) suggest that functionality of HDL particles in hypothyroidism is compromised.

The next aspect that should be considered is the impact of inflammation on HDL particles. So far, changes in both HDL-C level and HDL structure have been reported in several autoimmune diseases (78). It is also noteworthy that AIT is the most frequent co-morbidity of pediatric patients with T1DM (79). In our recent study, we analyzed lipid and lipoprotein subclasses profile in pediatric T1DM patients with and without co-existing autoimmune diseases and found that those with associated Hashimoto AIT had more profound dyslipidemia (80). Generally speaking, HDL possesses strong anti-inflammatory properties, but it has been demonstrated that pro-inflammatory environment can diminish its protective capacity (78). Being an autoimmune disease, Hashimoto thyroiditis is characterized by chronic inflammation (81). Many components of inflammation are shown to affect HDL particles. Previous researches pointed toward serum amyloid A (SAA), which is abundantly produced in inflammatory states and is capable to replace apolipoprotein AI (apo-AI) on HDL particles, thereby diminishing their anti-inflammatory properties and, paradoxically, turning them into inflammatory agents (78). Moreover, it has been shown that activity of LCAT is reduced (82) as a consequence of inflammation and this can compromise maturation and normal function of HDL particles. In addition, a decrease in CETP mass and activity was also observed in pro-inflammatory conditions (82, 83), although, it was suggested that this could be an adaptive mechanism aimed to prevent massive HDL-C reduction, which is driven by other factors (83). It should also be mentioned that PON1 levels and activity are decreased during inflammation, thereby diminishing antioxidative properties of HDL (84). As for Hashimoto AIT, it has been shown that PON1 level is decreased in these patients (85, 86).

Finally, HDL is a major carrier of sphingosine-1-phosphate (S1P) and evidence suggests that the interaction of S1P with HDL has significant impact on S1P activity (87, 88). S1P is well-known mediator of immune response (89) and recently it has been demonstrated that S1P participates in the development of Hashimoto AIT through its interaction with S1P receptor 1 (90). Yet, whether structural changes of HDL participate in modification of S1P activity in AIT is still to be revealed. Furthermore, the impact of these interactions on increase of CVD risk in Hashimoto AIT, especially in pediatric population, needs to be evaluated.

Before making any conclusions regarding lipid status in AIT, one should be aware that autoimmune disorders frequently aggregate in the same patient. A recent review (91) has shown

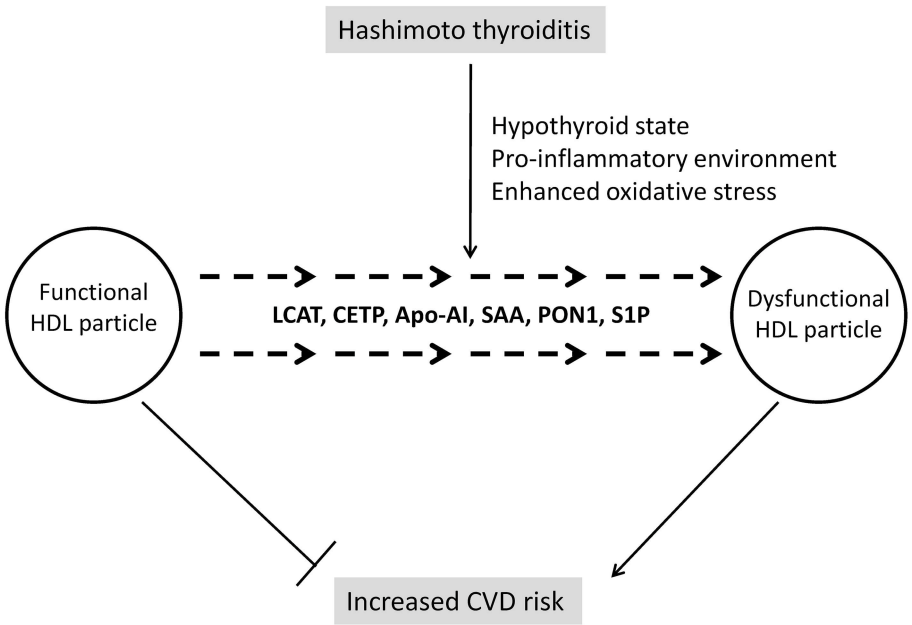

FIGURE 2 | Changes of HDL structure and functionality in Hashimoto thyroiditis. LCAT, lecithin:cholesterol acyltransferase; Apo-AI, apolipoprotein A-I; SAA, serum amyloid A; PON1, paraoxonase 1; S1P, sphingosine-1-phosphate.

that various autoimmune comorbidities are associated with AIT in an age-dependent manner. Namely, studies reported that frequency of co-existing autoimmune diseases in children with AIT ranges between 6.6 and 58.2%, wherein celiac disease and T1DM are the most frequent comorbidities (91). Thus, possible contribution of other co-existing autoimmune diseases on lipid profile in AIT should not be neglected.

It is known that T1DM is associated with alterations of serum lipid profile. Semova et al. (92) recently demonstrated decreased cholesterol synthesis and increased cholesterol absorption, with concomitant changes in TC, LDL-C, and HDL-C levels in young patients with T1DM, when compared to age-matched healthy individuals. Moreover, it has been suggested that both structural and functional alterations affect HDL particles in T1DM (93). Similarly, it has been shown that patients with celiac disease exhibit changes in lipid profile, especially decreased HDL-C concentration (94). Yet, it is noteworthy that independent effects of co-existing autoimmune diseases on serum lipids are rarely evaluated. However, it has been reported that LDL-C and TG levels are higher in children with concomitant presence of T1DM and celiac disease, when compared to children with T1DM alone (95). Moreover, lower HDL-C levels were found in children with T1DM if celiac disease was co-existing (96). Also, our own results demonstrated that the prevalence of dyslipidemia was higher in pediatric T1DM patients if celiac disease or AIT were concomitantly present (80). Summarizing all mentioned findings, eventual co-existence of other autoimmune diseases should be taken into account for comprehensive evaluation of dyslipidemia in AIT. Further studies are needed to fulfill a gap in current understanding of mechanisms by which polyautoimmunity is involved in development of lipid disorders.

EFFECTS OF L-THYROXINE TREATMENT ON LIPID STATUS IN HASHIMOTO'S AIT

Most of the known data regarding the beneficial effects of L-T4 treatment on lipid profile is derived from studies in adults with overt or subclinical hypothyroidism (12, 75, 97–102). Among the recent studies, Minarikova et al. demonstrated significant improvements of total cholesterol (TC), LDL-C, TG, apoB, atherogenic index of plasma, and LDL subclasses following L-T4 substitution treatment in 40 newly-diagnosed overt hypothyroidism patients with AIT (103). Uniquely designed study of 27 adult patients who underwent total thyroidectomy and radioactive iodine treatment for differentiated thyroid carcinoma, revealed that dynamic changes in thyroid function are associated with corresponding dynamic changes of the lipid profile and HDL function (75). Compared to the baseline levels (on L-T4 treatment), when patients entered the overt hypothyroid state (TSH > 30 mU/L after 4 weeks of L-T4 withdrawal), the levels of TC, TG, LDL-C, apoA-I, and apoB significantly increased, and then again recovered to the baseline levels after 3 months, following the reinstitution of L-T4 treatment. The levels of HDL-C increased during the overt hypothyroid state, with impairment of function (evaluated by cholesterol efflux capacity and PON1 activity), and these changes persisted despite restoration of thyroid hormone levels. It could not be concluded if impaired HDL-C function would also improve after a longer period of follow-up (75).

Results from studies in the adult population also indicate that L-T4 treatment could result in lowering of TPO-Ab levels in patients with AIT (104, 105). This could also potentially lead to the improvement of the lipid profile, having in mind that thyroid

autoimmunity with higher TPO-Ab levels is associated with an unfavorable lipid profile irrespective of thyroid function, in both children and adults (13, 63, 80, 106, 107).

Subclinical hypothyroidism in children, with a prevalence of 2–9%, is generally considered a benign condition with a significant chance of remission, however, findings of subtle pro-atherogenic abnormalities in these children highlights the lack of consensus regarding treatment criteria (7, 17, 49, 52, 53, 108–110). Treatment with L-T4, which was usually recommended only in children with goiter, hypothyroidism symptoms or TSH levels > 10 mU/L, is now being recommended by some experts for treatment of mild subclinical hypothyroidism in cases of: positive TPO-Ab, concomitant celiac disease, TSH > 8 mU/L in two repeated measurements, gradually increasing TSH levels, hyperlipidemia and younger patient age (7, 47, 49, 52, 53, 109).

Although the association of thyroid function with atherogenic alterations in lipid profile has been well-documented in children, data is scarce regarding the effects of L-T4 treatment in pediatric hypothyroidism, with or without thyroid autoimmunity (14–18). Dorr et al. observed a decrease of thyroid volume in 25 euthyroid children with Hashimoto's thyroiditis with L-T4 treatment, while changes in lipids were not evaluated (44). Another study also showed reduction of goiter with L-T4 treatment especially in cases of overt hypothyroid, but also in SH and euthyroid children with AIT (45).

Among the very few studies evaluating the effects of L-T4 treatment in children with subclinical hypothyroidism, only two studies investigated changes in the lipid profile (51, 52, 108, 111, 112). In a prospective study by Catli et al., 27 children (median age of 10 years) with subclinical hypothyroidism were treated with L-T4 until achievement of euthyroid state plus 6 months and then compared with euthyroid healthy control group. Seven (26%) of these patients with SH had AIT. Although improvement in the hypothyroidism symptoms score was associated with L-T4 treatment, no significant differences were observed regarding TC, TG, HDL-C, and LDL-C (51). The absence of significant findings could be attributed to the relatively short duration of follow-up as well as low number of patients enrolled. On the other hand, a prospective case-control study by Cerbone et al. discovered significant effects of 2 years of L-T4 treatment on pro-atherogenic markers in children with mild (TSH 4.5–10.0 mU/L) idiopathic subclinical hypothyroidism (52). A total of 39 children (mean age 9.2 years) with mild idiopathic subclinical hypothyroidism were compared with healthy controls. However, in this study patients with detectable TPO-Ab, Tg-Ab, or abnormal thyroid echogenicity on ultrasound were excluded from the study. Mean HDL-C levels were lower, with higher TG/HDL-C ratio and atherogenic index (TC/HDL-C), in subclinical hypothyroidism subjects compared with controls. After 2 years of L-T4 treatment, these parameters improved significantly in the SH group, so no more significant differences between subclinical hypothyroidism subjects and controls were observed. It was concluded that 2 years of L-T4 treatment resulted in an improvement of many lipid abnormalities in children with mild idiopathic subclinical hypothyroidism (52). However, having in mind that patients with AIT were excluded from this study, results should be interpreted with caution in the context of pediatric hypothyroidism caused by AIT. Further studies are needed to evaluate the metabolic effects of L-T4 treatment in hypothyroid children with AIT, compared with treated non-autoimmune hypothyroid children and not treated healthy controls.

IMPLICATIONS FOR CARDIOVASCULAR PREVENTION AND FUTURE DIRECTIONS

Dyslipidemia and increased carotid intima media thickness (cIMT), a reliable indicator of subclinical atherosclerosis, was recently documented in pediatric patients with Hashimoto thyroiditis (61, 63), suggesting the importance of regular evaluation of cardiovascular risk factors in children with AIT (**Figure 3**). In recent years, the role of cholesterol in the development of CVD has been challenged. In particular, the main controversy was centered on the impact of dietary fats on cardiovascular risk. According to a recent meta-analysis by de Souza et al. (113), the intake of saturated fatty acids was not associated with a higher cardiovascular risk. Similarly, data from randomized controlled trials shows that the replacement of saturated with polyunsaturated fatty acids might reduce serum cholesterol levels, but does not decrease the risk from all-cause or mortality due to coronary heart disease (114, 115). Nevertheless, a panel of experts from the European Atherosclerosis Society recently issued a Consensus Statement, supported by the evidence from genetic, prospective epidemiologic studies, Mendelian randomization studies and randomized trials evaluating lipid-lowering therapies, that high LDL-C is a causal factor in the pathophysiology of CVD and should remain the main therapeutic target (116).

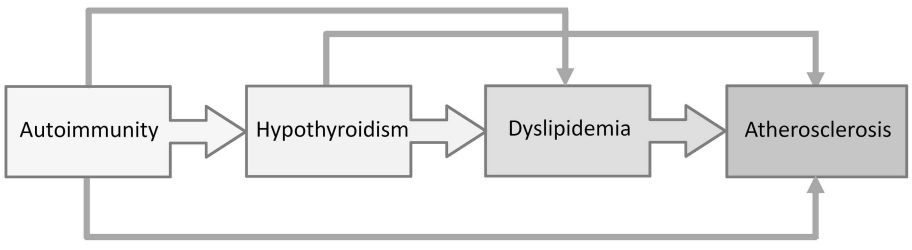

FIGURE 3 | The interplay between Hashimoto thyroiditis, dyslipidemia, and atherosclerosis.

Although LDL-C level remains primary target for CVD prevention, advanced lipid testing could be advised to reveal hidden cardiovascular risk. In this context, lipoprotein particle size, number and subclasses distribution, HDL lipidome, proteome, and functionality testing may provide valuable information beyond LDL and HDL cholesterol levels. Regarding alterations in metabolism of TG-rich lipoproteins, it can be easily assessed by calculation of non-HDL-C level, as it was recently recommended by expert lipidologists (117). To date, increased PCSK9 levels and its impact on dyslipidema has been reported in the most common autoimmune disease in childhood, T1DM (118), and future studies in pediatric Hashimoto thyroiditis are warranted.

Another important aspect in addressing the link between subclinical hypothyroidism and dyslipidemia is related to the laboratory assessment and interpretation of TSH levels. As stated previously, the definition of subclinical hypothyroidism relies on a mildly elevated serum TSH concentration, which is associated with normal T4 or fT4 levels. According to the guidelines of European Thyroid Association for the management of subclinical hypothyroidism in children, appropriate diagnosis of subclinical hypothyroidism in pediatric patients requires adequate age-adjusted reference ranges for TSH and thyroid hormones is mandatory (119). To date, numerous studies have been performed to define reference range of thyroid hormones in pediatric population, and some of them stratified results according to sex (120–122). Yet, there is still no consensus regarding this issue. Onsesveren et al. (123) recently performed a systematic review of published reference ranges for TSH and fT4 in children and demonstrated substantial differences among studies. For instance, the upper reference limit of TSH ranged between 2.36 and 6.57 mU/L (123). The difference among published reference values could be a consequence of age, gender, and demographic characteristics of included populations, including lifestyle, iodine, and selenium status (120) and/or different assays employed for the laboratory measurements of thyroid hormones. As previously acknowledged by the National Academy of Clinical Biochemistry, serum TSH level has high biological variability, due to short half-life and diurnal variation (124). Also, as a consequence of improved sensitivity and specificity of the methods for TSH determination, the upper reference limit for TSH decreased over time. Finally, it should not be neglected that thyroid hormones circulate bound to plasma proteins, and their biological action is exerted by the fraction (0.02–0.1%) of unbound or "free" form (4). Hence, the direct fT4 assays may be inaccurate in patients with severe systemic illness or abnormalities of protein binding, so caution is needed when interpreting such tests in this setting (4).

Unlike other forms of subclinical hypothyroidism in childhood, Hashimoto AIT is characterized by an increased likelihood for progression to overt hypothyroidism (47). Long-term cardiovascular outcomes in pediatric patients with Hashimoto thyroiditis are largely unknown due to lack of longitudinal prospective studies. In addition, clinical studies aimed to address benefit of L-T4 administration on future cardiovascular health are required. Available data indicate that statins also have certain immunomodulatory properties (125). Hence, the impact on conventional dyslipidemia treatment on thyroid autoimmunity should be explored in the future.

CONCLUSIONS

Available evidence suggests that AIT is associated with profound changes of lipid profile, which are driven not only by a decrease in thyroid hormones, but also by chronic inflammation and disturbed redox balance. In light of the current scientific data, novel biomarkers of altered lipoprotein metabolism might provide more complete information regarding lipid profile of Hashimoto AIT patients and subsequent cardiovascular risk. However, lack of reliable results in pediatric AIT patients urges the need for more comprehensive studies aimed to explore characteristics of dyslipidemia in children with Hashimoto AIT and implications for their future cardiovascular health.

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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Gender Differences at the Onset of Autoimmune Thyroid Diseases in Children and Adolescents

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Background: The incidence of autoimmune thyroid diseases (ATD) may vary with the beginning of reproductive function, although few reports differentiate the incidence before and during the onset of puberty, examining gender bias. We analyzed onset of ATD in a pediatric population to assess gender differences in onset age, disease subtype, pubertal status, autoimmune co-morbidity, family history and treatment, focusing on the interaction between gender and pubertal stage.

Patients and methods: We retrospectively recorded 382 children and adolescents with ATD. In each patient physical examination was considered. The presence of other associated autoimmune diseases (AAD) and familial predisposition was also recorded.

Results: Predominant prevalence was noted in females compared to males ($p < 0.001$), both in Hashimoto's diseases (HD or HT) and Graves' disease (GD) ($p < 0.001$). Mean age at diagnosis showed no significant difference between sexes ($p > 0.05$). A higher prevalence in pubertal subjects was noted compared to prepubertal ($p < 0.001$, particularly HT in early and GD in late pubertal stage), without sexes difference intra- (prepubertal vs. pubertal) and inter-puberty groups (prepubertal vs. early pubertal vs. late pubertal). Both in HT and in GD, the prevalence of autoimmune associated diseases (AAD) was higher in males compared to females ($p = 0.04$), with similar distribution according to the pubertal maturation. The familial predisposition was similarly distributed in both genders ($p > 0.05$) and into pubertal stages ($p > 0.05$).

Conclusions: Females are more prone to develop ATD during puberty, earlier in HT than in GD. The effect of puberty is not different between genders, suggesting the role of additional factors other than hormones. The screening for detection of ATD is recommended in all patients with positive family history and other autoimmune diseases, mostly in males. Considerations of gender in pediatrics could be important to define pathogenic mechanisms of ATD and to help in early diagnosis and clinical management.

Keywords: gender difference, autoimmune thyroid disease, children, adolescents, thyroiditis

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INTRODUCTION

Autoimmune thyroid diseases (ATD), which includes both Hashimoto's thyroiditis (HT) and Graves' disease (GD), are the most common etiology of acquired thyroid dysfunction in pediatrics (1–3) ATD are characterized by the production of anti-thyroid antibodies, by an infiltration of autoreactive B and T lymphocytes into the thyroid parenchyma and by alterations in thyroid function (hyperthyroidism in GD, normal function or subclinical/overt hypothyroidism in HT) (4).

Both polyautoimmunity and familial autoimmunity are typical findings of ATD (5, 6), supporting that the risk of developing disease is related to genetic susceptibility, with environmental factors playing a role in triggering disease in susceptible individuals (7). Different genetic factors are associated not only with susceptibility to a certain disease, but also with specific autoantibodies and disease phenotypes (7).

In pediatrics, ATD usually occur in puberty (1, 2) but they may occur at any time, rarely even in children under 1 year of age (2). The incidence of autoimmune disease may vary with the onset of reproductive function, although the incidence before and during the onset of puberty. In particular, the consideration of gender exist in examining the data in pediatric endocrinology, whereas a higher incidence rate of ATD in the adult female population is well-described (7). Reasons underlying a higher rate of ATD diagnosis in women are unclear. Moreover, we lack a real knowledge on gender-specific phenotypes.

That being so, we analyzed a pediatric population with onset of ATD to assess the gender differences as regard onset age, disease subtype, pubertal status, autoimmune co-morbidity, family history and treatment, focusing on the interaction between gender and pubertal stage. The gender specific characteristics of disease across puberty may help early diagnosis and clinical management.

METHODS

Patients

We retrospectively recorded 382 children and adolescents with ATD (HT and GD) who have been referred to the Pediatric Endocrinology and Diabetology Unit of the Fondazione IRCCS Policlinico San Matteo for diagnosis and treatment over a decade (2009–2019).

ATD diagnosis was based on the finding of one or more positive thyroid autoantibodies and a characteristic thyroid ultrasound, lacking homogeneity, with a hypogenic or mixed echopattern.

In each patient, physical examination was performed. The presence of eventual associated autoimmune diseases (AAD) at the ATD diagnosis was also recorded.

Positive family history of autoimmune thyroid disorders was collected (only first-degree relatives were considered significant) in order to analyze the effect of familial predisposition on the development of ATD.

The protocol was performed with the approval of the Ethical Committee of Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and according to the Declaration of Helsinki. All participants or their responsible guardians were asked their written consent after being informed about the nature of the study.

Physical Examination

Physical examination of patients at first diagnosis included evaluation of height, weight, BMI (calculated as body weight in kilograms divided by height in meters squared), stages of puberty according to Marshall and Tanner (8, 9).

Anthropometric and blood pressure measurements were performed as previously described (10).

Pubertal development was classified as: prepubertal = Tanner 1; early puberty = Tanner 2–3; late puberty = Tanner 4–5.

Biochemical Data

TSH, FT4 and FT3 and anti-thyroid peroxidase antibodies (TPOAb), anti-thyroglobulin antibodies (TGAb) anti-thyrotropin receptor antibodies (TRAb), were measured as previously reported (11).

Thyroid ultrasound studies were performed by the same operator (VC) with patients in a supine position with the neck hyperextended, by using an Aloka machine (Aloka Prosound α5, Aloka, Tokyo, Japan) with a 7–13 MHz linear transducer.

Statistical Analysis

Qualitative variables were described as counts and percentage and the chi square test was used to compare the differences between groups. The Shapiro-Wilk test was used to test the normal distribution of quantitative variables. As quantitative variables were normally distributed, the results were expressed as the mean value and standard deviation (SD) and compared between groups with *t*-test.

RESULTS

Overall Population With ATD

Out of the 382 patients with ATD (361 HT and 21 GD) included in the study, 325 (85%) were females and 57 (15%) were males ($p < 0.001$). Mean age at the diagnosis was 11.58±0.21 years without significant sexes difference (females 11.55 ± 0.23 vs. males 11.73 ± 0.47, $p = 0.7$).

Pre-pubertal status was present in 79 (20.9%) of our patients, and pubertal condition in 298 (78%), $p < 0.001$; in particular early puberty was recorded in 188 (49.74%) and late puberty in 111 (29.37%), $p < 0.001$, without sexes difference intra- (prepubertal vs. pubertal $p = 0.6$) and inter-puberty groups (prepubertal vs. early pubertal vs. late pubertal, $p = 0.86$), **Figure 1**.

AAD was noted in 100 (26.18%) of our overall population, with higher prevalence in males compared to females (37.0% vs. 24.31%, $p = 0.04$). Mean age of the subjects with poliautoimmunity was similar compared to patients with a single disease (11.45 ± 0.24 vs. 11.93 ± 0.81, $p = 0.8$) and there was no difference between gender or according to pubertal stage distribution ($p > 0.05$). Celiac disease was detected in 58 of subjects (58%; 47F/11M, $p < 0.01$ and in 2 cases type 1 diabetes was also present), type 1 diabetes in 19 (19%; 13F/6M, $p < 0.01$), autoimmune gastritis 6 (6%; 3M/3F, $p < 0.01$), vitiligo 11

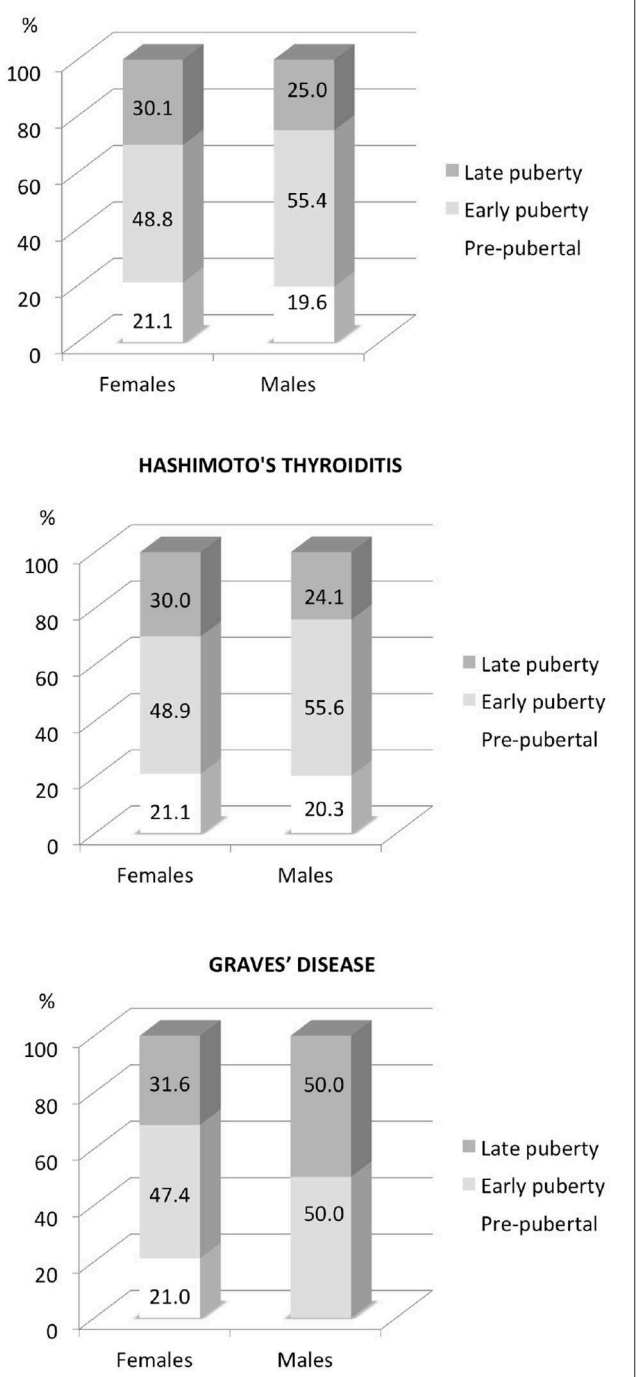

FIGURE 1 | Sex distribution of autoimmune thyroid diseases according to pubertal stages.

(11%; 9F/2M, $p < 0.01$), and alopecia in 9 (9%; 8F/1M, $p < 0.01$) children.

Positive family history of autoimmune diseases was reported in 204 of our patients (53.83%), without difference in prevalence between males and females ($p = 0.7$). Mean age at onset was not different in patients with or without a positive family history (11.77±0.26 vs. 11.29 ± 0.35, respectively, $p = 0.3$), as well as the gender lineage did not make any difference ($p = 0.25$). Distribution of the pubertal maturation in the groups with or without positive family history was similar taking into account the gender bias ($p = 0.21$ and $p = 0.84$, respectively).

At the onset of disease, hormonal treatment was started in 204 (53.4%; 183 with L-thyroxine and 21 with metimazole) of children and the rate was similar in males and females ($p = 0.8$) with no gender difference also according to pre- and pubertal condition ($p = 0.6$ and $p = 0.8$, respectively).

Hashimoto's Thyroiditis (HT)

Out of the 382 patients with ATD, 361 subjects were diagnosed with HT, with predominance of female population ($F = 306$, 84.7% vs. M = 55, 15.2%, $p < 0.001$). Mean age at diagnosis was 11.58 ± 0.21 years without significant difference between gender (females 11.55 ± 0.23 vs. males 11.73±0.47, $p = 0.7$).

Pre-pubertal status was detected in 21.3% of our patients, and pubertal condition in 78.7%, $p < 0.001$; in particular, early puberty was recorded in 50.57% and late puberty in 28.14%, $p < 0.001$, without difference between gender intra- (prepubertal vs. pubertal, $p = 0.61$) and inter-puberty groups (prepubertal vs. early pubertal vs. late pubertal, $p = 0.9$), **Figure 1**.

AAD was diagnosed in 27.14% of the study sample with ATD, with higher prevalence in males compared to females (36.84% vs. 19.38%, $p < 0.01$). The mean age of the patients with poliautoimmunity was similar in comparison with those carrying only one disease (11.45 ± 0.24 vs. 11.93 ± 0.81, $p = 0.8$); no difference between gender was found ($p = 0.9$). Celiac disease was detected in 56 of subjects (56%; 47F/9M, $p < 0.01$), type 1 diabetes mellitus in 17 (17%; 13F/4M, $p < 0.01$), autoimmune gastritis in 6 (3F/3M; 6%; $p < 0.01$), vitiligo in 11 (11.2%; 8F/2M, $p < 0.01$), and alopecia in 9 (9.1%; 8F/1M, $p < 0.01$) children. Distribution of the pubertal stages in the patients with or without associated autoimmune diseases was not different according to the gender ($p = 0.9$ and $p = 0.4$, respectively).

Positive familiarity for autoimmune diseases was reported in 54.47% of our patients, without a different prevalence between males and females ($p = 0.7$). Mean age at onset in subjects with positive familiarity was not different compared to patients without (11.77 ± 0.26 vs. 11.29 ± 0.35, respectively, $p = 0.3$) and no difference was evident according to gender lineage ($p = 0.25$). Even the distribution of the pubertal stages in the groups with or without a positive family history was not different taking into account the gender bias ($p = 0.2$ and $p = 0.8$, respectively).

At the onset of disease, hormonal treatment with L-thyroxine was started in 183 of children (47.9%) and the rate was similar in males and females ($p = 0.8$). No significant difference between gender according to the presence of both polyautoimmunity and puberty ($p = 0.3$ and $p = 0.5$, respectively). In our series, all patients were adequately treated, with a good control of signs and symptoms.

Graves' Disease (GD)

Out of the 382 patients with ATD, 21 subjects were diagnosed with GD, with a higher prevalence in females ($F = 19$, 90.4% vs. M = 2, 9.5%, $p < 0.001$). Mean age at diagnosis was not

different in females compared to males (12.14 ± 0.74 vs. 12.76 ± 2.76 years, $p = 0.6$).

Pre-pubertal status was recorded in 19.0% of patients and pubertal condition in 81%, $p < 0.001$, with predominance of late puberty (early puberty 33.0%vs. late puberty in 47.62%, $p = 0.03$); no gender difference between intra- (prepubertal vs. pubertal $p = 0.8$) and inter-puberty groups (prepubertal vs. early pubertal vs. late pubertal, $p = 0.9$) was documented, **Figure 1**.

Associated autoimmune diseases was detected in 2 males patients (9.52%, $p < 0.01$), that presented both CD and type 1 diabetes, and were at late puberty.

A positive familiarity for autoimmune diseases was reported in 42.86% of patients, without difference in prevalence between males and females ($p = 0.8$). Mean age at onset was not different in subjects with or without a positive family history (12.84±0.70 vs. 11.0±0.50, respectively, $p = 0.4$) and according to the gender lineage ($p = 0.25$). Distribution of the pubertal stages in the groups with or without familiarity was not different according to the gender ($p = 0.3$).

At the onset of disease, metimazole was started in all patients, with a good response in each of them.

DISCUSSION

Predominance of ATD, both HT and GD, was confirmed in females pediatric and adolescent population. Puberty represents a crucial period for ATD development, without significant gender difference according to pubertal stages. In HT early puberty is a period at greater risk for diagnosis, whereas in GD late puberty is more represented. Poliautoimmunity is more frequent in males compared to females. By contrast, familial predisposition was similarly between genders and into pubertal stages.

The gender differences existing in autoimmune thyroid disease follow a female bias (7) even in a pediatric population, which has displayed a female preponderance of 2 : 1 (3). Different factors may underlie this striking gender difference, including genetic effects, gender differences in sex hormones, immune response and organ vulnerability (7).

Evidence in favor of a genetic basis for the ATD is abundant and the factors that might influence the development of autoimmune disease may be related to genetic susceptibility, chromosomal differences, or epigenetics (12–16).

Genetic studies of patients with autoimmune disorders, including ATD, have shown the role of the major histocompatibility complex (MHC), as compared with other genome areas (17). The association between HLA genes and autoimmune disorders shows a gender bias toward females (17), justifying the predominance of autoimmune diseases, including ATD, in the female population (3, 5, 17, 18).

Findings reported in our series are in line with the evidence that ATD is often accompanied by other organ-specific autoimmune disorders, confirming that common genetic factors may prejudice an individual to developing autoimmunity (7). Although the exact pathogenic mechanism of the coexistence of autoimmune diseases has not been clearly elucidated, the role of HLA haplotypes, such as HLA-B8 and -DR3, in the overlapping of autoimmune disorders was well-supported by higher frequency of these haplotypes in several diseases (19, 20).

We confirmed the higher prevalence of ATD in females. Moreover, we showed that the prevalence of AAD was higher in males compared to females. This result supports that non-HLA genes, such as Polymorphisms in cytotoxic T-lymphocyte anti- gen-4, acid phosphatase locus 1, Discs large homolog 5, interleukin-10, and apolipoprotein E, may be also associated with susceptibility to multiple autoimmune disease (7, 21). Additionally, the stronger susceptibility in women, may support a possible pathogenic role for mechanisms related to genes on the X-chromosome, as reported in animal models (22–25), even though the role of Y chromosome in autoimmune disease and in poliautoimmunity could be not excluded.

The observed familial predisposition to GD and HT confirms the crucial role of the genetic component in the development of this disease. Indeed, we documented that more than 50% of subjects with ATD presented a positive family history, supporting the notion that maternal and paternal inheritance may also contribute to the pathogenesis of autoimmune disease. The lack of the gender specific familial risk suggests that the interaction of genes and environmental factors may underlie parental inheritance of autoimmunity (26).

In adults, a difference between males and females are reported in age at onset of ATD (27). In this study in pediatrics, the gender difference is not evident, but we noted the importance of pubertal stages given the higher prevalence in pubertal compared to prepubertal subjects. These results may confirm that exposure to hormonal changes during puberty plays a fundamental role in immune function (5, 7, 17, 28–30) and consequently in the development of ATD. In our population this is not gender specific, supporting that factors other than hormones, such as environmental effects, could influence the effect of puberty on the disease state (7).

We are aware that there are some limitations in our study starting from recognizing that the retrospective design has numerous disadvantages with an inferior level of evidence. Of note, we did not observe a gender difference in the severity among ATD, as it has been previously reported (7). However, we recorded only the start and the response to treatments, as an index of severity of the disease, and our data do not exclude the effect of the young age on a good prognosis (3, 5, 7). On the other hand, other factors, such as type of symptoms, goiter, ophthalmopathy, hormonal levels would have been necessary to confirm the severity of the disease. Additionally, in this report no environmental factors related to susceptibility to GD and HT, as well as to disease manifestations, have been taken into account (7, 26). Further prospective studies are mandatory to gain a better insight into this research topic.

In conclusion, females are more prone to develop ATD during puberty, in particularly HT in early stage and GD in late stage. The impact of puberty is not different in females and males, supporting the role of additional factors other than sex hormones. We recommend screening for detection of ATD in all children and adolescents with positive family history and other autoimmune diseases, especially in males.

Considerations of gender in pediatrics could be important to define pathogenic mechanisms of ATD and to help in early diagnosis and clinical management.

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 Ethical Committee of Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kids.

AUTHOR CONTRIBUTIONS

VC, RN, and DL designed experiments and wrote and supervised the manuscript. CR recorded data of the patients and wrote the manuscript. AI, RAm, FB, AC, and FV recorded data of the patients. AD performed statistical analysis. RAl performed biochemical evaluation. All authors have read and approved the paper.

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Long-Term Follow-Up and Outcomes of Autoimmune Thyroiditis in Childhood

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Background: Autoimmune thyroiditis (AIT) is the most common cause of acquired hypothyroidism in children. The natural outcome of AIT in childhood has been reported previously however follow-up duration is generally short and results variable.

Objectives: To characterize clinical and biochemical findings at presentation of AIT, evaluate long-term outcomes and assess which factors at presentation predict evolution over time.

Study cohort: 201 children under 18 years of age at presentation (82% female) were enrolled. Subjects were divided into five subgroups according to thyroid stimulating hormone (TSH) level at referral.

Results: Mean follow-up was 8.1 years (range 0–29 years). At presentation, 34% of patients had overt hypothyroidism, 32% subclinical hypothyroidism (SCH), 16% compensated hypothyroidism, 14% were euthyroid, and 3.7% had Hashitoxicosis. Children with overt hypothyroidism were younger (10.6 vs. 13.2 years) and had higher thyroid peroxidase antibody titers. At the time of the study, levothyroxine (LT_4) therapy was required in 26% of children who were euthyroid at presentation, 56% of SCH patients, 83–84% of those with TSH above 10 mIU/L, and 57% of those with Hashitoxicosis. Over the years, 16% of children presenting with overt hypothyroidism stopped therapy. Free T_4 at presentation was the only predictor of outcome over time.

Conclusions: Our findings suggest that only 26% children who were euthyroid at presentation developed hypothyroidism, whereas over 50% of those with SCH went on to require treatment. Of those presenting with overt hypothyroidism, 16% recovered with time. The only predictive parameter for LT_4 therapy at the end of the study was free T_4 levels at presentation. Long-term follow-up is required to determine ongoing therapy needs and screen for additional autoimmune diseases.

Keywords: autoimmune thyroiditis (AIT), Hashimoto's thyroiditis, thyroid autoantibodies, goiter, Hashitoxicosis

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INTRODUCTION

Autoimmune thyroiditis (AIT), also known as Hashimoto's thyroiditis, is the most common cause of acquired hypothyroidism in childhood, with a prevalence of 1 to 3%, peaking during adolescence (1–3). There is a female predominance, with a female-to-male ratio of 4–8:1 (2). AIT is characterized by thyroid destruction due to an autoimmune-mediated process, resulting in gradual thyroid failure with or without goiter. The diagnosis of AIT is suggested by the presence of anti-thyroid antibodies against peroxidase (TPOAb) and/or thyroglobulin (TGAb) and by a typical hypo-echogenic and heterogeneous ultrasound pattern. Children are commonly referred for endocrine evaluation due to thyroid enlargement or on the basis of abnormal thyroid function results discovered as part of a medical workup for variable complaints (often unrelated to thyroid dysfunction) or for positive family history of AIT. At the time of diagnosis, thyroid function in children may be variable ranging from euthyroidism (52.1%), to overt (22.2%) or subclinical hypothyroidism (SCH) (19.2%) or, more rarely to either subclinical or overt hyperthyroidism (6.5%) (4).

Predictive factors for progression from euthyroidism or SCH to overt hypothyroidism, or for recovery from hypothyroidism over time, have been investigated in some studies, with variable results (3–19). Some of the studies included a small number of patients (5, 7, 8, 12, 19) and were of short duration (9, 10, 13, 16). Moreover, the definition of SCH, as well as indications for levothyroxine (LT_4) supplemental therapy, differ among authors (15) and include goiter shrinkage in some instances (20). de Vries et al. (18) reported that almost all of their patients required LT_4 treatment either at referral or during follow-up. Conversely, other studies suggested favorable outcomes for patients who were euthyroid or had SCH at referral (6–9, 11, 16), whereas only 25–50% of those with initial thyroid dysfunction experienced normalization of thyroid function over time (1, 8, 9, 12–14, 17).

Predictive factors for the development of overt hypothyroidism included: elevated TGAb and the presence of goiter (6), as well as elevated TPOAb, elevated thyroid stimulating hormone (TSH) and the presence of celiac disease (16).

The objectives of this retrospective study were to evaluate the long-outcomes of AIT in children with variable thyroid function at presentation, to evaluate the prevalence of euthyroid and/or SCH patients developing overt hypothyroidism, and the prevalence of patients with overt hypothyroidism becoming euthyroid after long-term treatment, and to identify predictive factors of long-term outcomes.

SUBJECTS AND METHODS

Study Cohort

We enrolled 201 subjects in the study, all under 18 years of age at diagnosis. They were followed up on a 6-monthly basis at Ha'Emek Medical Center or in outpatient clinics affiliated with the hospital. All patients had positive TGAb and/or TPOAb. Most subjects also had at least one of the following: abnormal thyroid function, enlarged thyroid gland, morphological changes on thyroid ultrasound. Patients were referred for evaluation by a pediatric endocrinologist due to one or more of the following: abnormal thyroid functions, elevated thyroid antibodies, presence of goiter and various complaints that were related to abnormal thyroid function. Patients with diabetes mellitus type 1 (DMT1) diagnosed prior to the diagnosis of AIT, and those with Down or Turner syndromes, were excluded from the study. LT_4 supplemental therapy was initiated in overt hypothyroidism, when TSH levels were above 10 mIU/L, and in a few individuals with TSH $<$ 10 mIU/L for the purpose of goiter shrinkage. LT_4 dose was adjusted to maintain TSH concentrations within a normal range. Data were collected retrospectively from computerized medical files and included: clinical findings and thyroid function at presentation, during follow-up and at the last visit; LT_4 therapy initiation; additional autoimmune diseases and family history of autoimmune and thyroid diseases.

Hormone Analysis

Serum TSH (normal range 0.4–4.2 mIU/L) and free thyroxine (FT_4; normal range 10–20 pmol/L) concentrations were measured by direct automated chemiluminescent immunoradiometric assays using ADVIA Centaur (Bayer Corporation, Tarrytown, NY). TGAb and TPOAb were measured by direct automated chemiluminescent immunoradiometric assay using Immulite 2000 (Siemens, Llanberis, Gwynedd, UK). TGAb and TPOAb were considered positive above 35 U/mL.

Subgroups

Patients were classified into five subgroups according to TSH level at presentation: (i) hyperthyroid (TSH $<$ 0.03 mIU/L and $FT_4 \geq$ 20 pmol/L); (ii) euthyroid (TSH 0.4–4.2 mIU/L and FT_4 10–20 pmol/L); (iii) SCH (TSH 4.3–10 mIU/L); (iv) compensated hypothyroid (TSH 10.1–20 mIU/L with normal FT_4); (v) hypothyroid (TSH $>$ 20 mIU/L). Since our practice is to initiate LT_4 therapy in patients with TSH above 10 mIU/L even if FT_4 is within the normal range, we divided patients into two subgroups according to their TSH levels: those with TSH 4.3–10 mIU/L were defined as SCH while those with TSH above 10 mIU/L and FT_4 above 10 pmol/L were defined as compensated SCH.

Statistical Analysis

Statistical analyses were performed with SAS v9.4 statistical software package (Cary, NC). Study groups were compared by Kruskal–Wallis test (continuous variables) and Chi-square or Fisher's Exact Test (categorical variables). Pairwise comparisons were made using Wilcoxon two-sample tests and Chi-square or Fisher's exact test for continuous and categorical variables, respectively, using Bonferroni correction. Univariate analyses were performed for the study outcome: LT_4 therapy at the end of the study follow-up. Stepwise logistic regression was used for multivariable analysis to establish independent predictors for LT_4 therapy at the end of the follow-up period. Significance was set at $p < 0.05$.

This study was approved by the Ethics Committee of Ha'Emek Medical Center.

RESULTS

The study population consisted of 201 subjects (female-to-male ratio of 4.5:1) with mean age of 11.7 ± 3.4 years (range 2.25–17.75 years). Reasons for referral were goiter or thyroid dysfunction and/or increased thyroid autoantibodies identified during a work-up performed for various other complaints (hair loss, obesity, weight gain, short stature, pubertal delay, fatigue and headaches).

Mean TSH at presentation was 55 ± 145 mIU/L (range 0.02–1,225) and FT_4 12.7 ± 6.7 pmol/L (range 1.2–67). TSH at presentation, was undetectable in 3.7% of subjects, within the normal range in 14%, slightly increased in 32%, and elevated (>10 mIU/L) in 50%. FT_4 was below the normal range in 29% of patients. TGAb was positive in 83% and TPOAb in 95% of cases. Thyroid gland enlargement was identified in 66% of patients by neck examination.

Fifty-five patients (28%) had an additional autoimmune disease diagnosed either before or after AIT diagnosis. Autoimmune comorbidities included celiac disease (15 patients), pernicious anemia (nine patients), rheumatic diseases (six patients), vitiligo (five patients), DMT1 (three patients), alopecia areata (three patients) and others (14 patients). Positive family history of thyroid diseases was reported in 80 patients (40%), including AIT, Graves' disease, papillary thyroid carcinoma, and multinodular goiter. Follow-up duration was 8.1 years (range 0–29).

The cohort was divided into five subgroups according to TSH level at diagnosis: hyperthyroid (seven patients), euthyroid (27 patients), SCH (60 patients), compensated SCH (30 patients), and hypothyroid (64 patients). Clinical and biochemical findings in the different subgroups are presented in **Table 1**. Subjects with overt hypothyroidism were younger than euthyroid subjects ($p = 0.0011$). As expected FT_4 levels were significantly lower ($p < 0.0001$) in overtly hypothyroid children compared to those with SCH and euthyroid patients. TGAb levels at presentation did not differ between groups, but TPOAb values were higher in the overtly hypothyroid group than in the euthyroid group ($p = 0.0004$). Gender did not differ between groups.

At LT_4 initiation, patients with overt hypothyroidism were significantly younger than those in the euthyroid, SCH, and hyperthyroid groups ($p = 0.0016$) and had higher TSH and lower FT_4 levels.

During the follow-up period, LT_4 therapy was initiated in 100% of subjects presenting with overt or compensated hypothyroidism; however, at the time of the study, 5 (16%) and 11 (17%) of those patients, respectively, no longer required treatment.

No differences in variables at presentation were found between patients who were off therapy compared to those who required therapy at the end of the follow-up period. As for the other groups, three patients (43%) presenting with hyperthyroidism, 27 patients (44%) with SCH and 20 (74%) of the euthyroid group did not receive treatment. Follow-up duration, age at last visit and thyroid autoantibodies at the time of the study did not differ among groups (**Table 1**).

A comparison of thyroid antibody levels in the whole cohort revealed lower levels at the study endpoint compared to levels at the time of referral. Univariate analysis revealed that patients who underwent therapy at presentation were younger (11.4 vs. 12.7 years, $p = 0.036$), had higher TSH (73 vs. 5.0 mIU/L, $p < 0.0001$), lower FT_4 (11.7 vs. 15.8 pmol/L, $p < 0.0001$) and higher TPOAb (920 vs. 521 U/mL, $p = 0.002$). Gender and TGAb did not differ.

At the end of the study, 122 patients were receiving LT_4 therapy, while 64 were not. Looking for factors at presentation predictive of LT_4 therapy at the end of the follow-up period, multivariable logistic regression performed for age, gender, TSH, FT_4, TPOAb, TGAb, goiter, familial history of thyroid disease and additional autoimmune diseases revealed that only FT_4 level at presentation was predictive for a future need for LT_4 therapy.

DISCUSSION

In this study, we examined clinical and laboratory characteristics at presentation of AIT in children and adolescents and its natural progression. We found that about 75% of children euthyroid at diagnosis remained in this stable condition over time, whereas only 16% of those presenting with overt hypothyroidism attained remission.

The primary cause for referral to our service was thyroid function abnormality or elevated autoantibodies (68%) and the most prevalent complaint was thyroid enlargement (32%). Interestingly, goiter was identified in 66% of the patients on physical examination at presentation, in keeping with the high prevalence of goiter reported in previous studies (10, 11).

The prevalence of AIT is about 1.2 to 3%, with 87% of cases asymptomatic at presentation and spontaneous resolution occurring in 50% (1, 2). The benign evolution of euthyroid AIT in children has been reported in several studies (7–9, 12–14). Our finding, that only 26% of children who were euthyroid at presentation developed hypothyroidism during the follow-up period is consistent with these data, suggesting that most euthyroid children remain disease free over time. On the other hand, in the study of de Vries et al. (18) almost all patients were treated with LT_4 at referral or during follow-up, however, their data suggested that appropriately monitored LT_4 therapy, even in euthyroid children, does not appear to be harmful.

SCH is characterized by serum TSH levels between 4.2 and 10 mIU/L with normal FT_4 levels. In the presence of thyroid autoantibodies, the diagnosis of AIT is most likely (20). Lazar et al. (21) demonstrated spontaneous normalization of TSH values in most patients with SCH without supplemental therapy. Female gender and initial TSH above 7 mIU/L were predictors of sustained highly elevated TSH. In this study however, the cohort was not limited to subjects with AIT. The risk of deterioration of thyroid status over time is higher in AIT compared to idiopathic SCH (53 vs. 11%) (5, 14, 15), as observed in our study, where 56% of those with SCH required treatment over time.

The presence of additional autoimmune diseases (6, 15, 16) and of genetic syndrome such as Turner and Down syndromes

TABLE 1 | Comparison of clinical and biochemical parameters in the five subgroups.

At diagnosis	Hyperthyroidism	Euthyroidism	SCH	Compensated SCH	Hypothyroidism	*P*-value
Hormonal status	TSH < 0.03 mIU/L FT_4 > 20 pmol/L	TSH 0.4–4.2 mIU/L FT_4 10–20 pmol/L	TSH 4.3–10 mIU/L	TSH 10.1–20 mIU/L	TSH > 20 mIU/L	
No. of patients	7 (3.7%)	27 (14.3%)	60 (32%)	30 (16%)	64 (34%)	
Gender (F:M) % male	6:1 (14%)	25:2 (8%)	43:17 (28%)	22:8 (27%)	57:7 (11%)	
At presentation						
Age (years)	13.8 ± 2.6 (8.2–16.2)	13.2 ± 3.0 (7.7–17.8)	11.5 ± 2.9 (5.2–17.5)	12.0 ± 3.8 (4.5–17.6)	10.6 ± 3.4 (2.3–17.6)	0.005
TSH (mIU/L)	0.03 ± 0.01 (0.02–0.04)	2.4 ± 1.0 (0.9–4.1)	7.1 ± 1.7 (4.4–9.8)	13.5 ± 3.0 (10.1–19.6)	152.1 ± 226 (21.7–1224.7)	<0.0001
FT_4 (pmol/L)	34.5 ± 16.1 (19.6–67.0)	14.8 ± 2.2 (11.3–19.2)	14.1 ± 2.3 (9.4–19.8)	13.3 ± 1.7 (10.3–17.1)	7.8 ± 3.1 (1.2–14.2)	<0.0001
TPOAb (U/mL)	681 ± 448 (65–1,000)	397 ± 321 (26–1,000)	721 ± 1005 (10–5,730)	645 ± 397 (35–1,000)	1,195 ± 2,010 (11–13,372)	0.01
TGAb (U/mL)	357 ± 408 (30–1,136)	451 ± 723 (28–3,000)	485 ± 868 (20–3,000)	529 ± 1,092 (25–4,284)	876 ± 1,463 (30–6,500)	0.5
At LT_4 therapy initiation						
Age (years)	17.4 ± 4.5 (12.5–23.3)	14.8 ± 3.9 (9.2–22.4)	13.5 ± 3.7 (6.0–21.8)	12.8 ± 4.5 (5.0–23.3)	10.6 ± 3.4 (2.3–17.8)	0.0001
Years between diagnosis and LT_4 therapy	4.4 ± 3.6 (1.07–9.5)	2.7 ± 2.1 (0–8.1)	1.4 ± 2.2 (0–11.3)	0.27 ± 1.3 (0–12.4)	0.05 ± 0.271 (0–2.17)	<0.0001
TSH at LT_4 initiation (mIU/L)	47.6 ± 69 (6.4–150.6)	36.0±49.2 (0.9–132)	17.2 ± 28.0 (5.0–150.4)	21.9 ± 29.1 (5.5–150)	153.1 ± 2,283 (5.3–1,223)	<0.0001
FT_4 at LT_4 initiation (pmol/L)	10.4 ± 6.9 (6.9–14.4)	11.4 ± 4.3 (3.4–16.4)	13.2 ± 2.4 (8.8–19.8)	13.0 ± 2.7 (4.4–17.8)	7.7 ± 3.1 (1.2–13.7)	<0.0001
Time of study						
Age (years)	21.6 ± 6.7 (15–33)	19.5 ± 6.3 (8.1–31.1)	18.7 ± 5.5 (9.4–31.0)	20.0 ± 5.9 (9.3–35.0)	18.9 ± 5.8 (5.1–33.0)	0.76
Follow-up duration (years)	8.0 ± 6.5 (0.4–19)	6.4 ± 6.1 (0–20.8)	7.6 ± 6.1 (0–20.8)	8.7 ± 6.7 (0.1–29.4)	9.3 ± 5.5 (0.8–22.0)	0.11
TSH (mIU/L)	5.2 ± 4.3 (0.02–11.6)	3.4 ± 3.4 (0.36–18.6)	6.4 ± 3.4 (0.4–18.6)	15.1 ± 53.3 (0.3–296)	13.1 ± 31.8 (0.04–196)	0.15
FT_4 (pmol/L)	14.9 ± 8.4 (12.3–28.4)	14.9 ± 9.9 (9.9–19.9)	14.9 ± 7.2 (9.9–19.9)	15.7 ± 6.4 (11.4–21.1)	14.9 ± 5.6 (2.2–28.4)	0.9
TGAb (U/mL)	460 ±599 (34–1,255)	411 ± 833 (30–3,000)	540 ± 980 (20–3,000)	1,655 ± 4,363 (39–15,907)	443 ± 565 (21–3,000)	0.58
TPOAb (U/mL)	515 ± 456 (76–1,000)	390 ± 291 (24–1,000)	692 ± 866 (11–1,000)	632 ± 380 (29–1,300)	552 ± 535 (10–3,000)	0.59
No of patients undergoing LT_4 therapy at time of study (%)	4 (57%)	7 (26%)	33 (56%)	25 (83%)	53 (84%)	<0.0001

TSH normal range 0.4–4.2 mIU/L.
FT_4 normal range 10–20 pmol/L.
SCH, sub clinical hypothyroidism, TPOAb, TPO abs; TGAb, Thyroglobulin antibodies.

(14, 15) has been shown to increase the risk of deterioration in thyroid function. In our study, therefore, patients with these syndromes were excluded, as were children with DMT1 in whom thyroid seropositive antibodies were detected after the diagnosis of diabetes mellitus, as the natural history of AIT in these children differs (22).

Amongst our cohort, a small number of patients were treated with LT_4 therapy for goiter shrinkage despite TSH levels below 10 mIU/L, an approach that is suggested in a few studies (23, 24). Even though LT_4 therapy has been shown to reduce thyroid autoantibodies in patients with SCH and overt hypothyroidism, its beneficial effect on the evolution of thyroid function (11, 25), and on metabolic (26) and neurocognitive outcome (27–29) remains uncertain. In the United States, the prevalence of positive thyroid antibodies, in the disease-free population above 12 years of age, is about 10%, it is more prevalent in females and increases with age (30). TPOAb are significantly associated with thyroid function but TGAb are not (30). In our study, elevated TPOAb were associated with initiation of LT_4 at presentation, but were not predictive for long-term LT_4 treatment, whereas, in our multivariate analysis, only FT_4 values at presentation predicted future treatment requirements in children with AIT.

Normalization of thyroid function over time has been reported to occur in 30–50% of children presenting with overt hypothyroidism (8, 9). In our study, supplemental therapy was initiated in all such patients with remission occurring in only 16% by the end of the follow-up period. Although this remission rate is lower than that reported in other studies, it suggests that reassessment of thyroid status is important given that 16% of those with overt autoimmune hypothyroidism may not require lifelong LT_4 therapy.

Hyperthyroidism was the presenting symptom in seven patients (3.7%), with four developing hypothyroidism over time. Although hyperthyroidism is an uncommon presentation of AIT, awareness of this condition is important and should be differentiated from Graves' disease in order to avoid unnecessary suppressive therapy (3, 31).

A family history of thyroid disease was present in 40% of children with AIT, including Hashimoto's thyroiditis, Graves' disease, multinodular goiter and papillary thyroid carcinoma, suggesting a genetic susceptibility to thyroid disease. In addition, other autoimmune diseases were present in 28% of the cohort. These findings highlight the need for screening patients with AIT for additional autoimmune diseases.

This large, long-term retrospective study adds further information to the controversial issue of the natural history of AIT in childhood. The major limit of this study is its retrospective nature, with variable duration of follow-up among individuals.

In conclusion, this study indicates that 26% of children with normal thyroid function at presentation develop overt hypothyroidism, over 50% of those with initial SCH may eventually require LT_4 treatment and 16% of those presenting with overt hypothyroidism may recover over time. FT_4 at presentation appears to be the only factor predicting evolution of thyroid status. Further long-term studies on large cohorts of patients are needed to better clarify the natural history of AIT and identify prognostic factors for therapeutic intervention.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study involving human participants was reviewed and approved by Ha'Emek Medical Center Helsinki Comittee. Written informed consent for participation was not provided by the participants' legal guardians/next of kin, nor was written consent required from the local ethics committee since the study was retrospective collecting data from medical files.

AUTHOR CONTRIBUTIONS

OA collected the data and reviewed and revised the manuscript. TA, SR, and GE-A reviewed and revised the manuscript. YT-R drafted the initial manuscript, designed the study, coordinated and collected the data, and revised the manuscript.

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Clinical and Biochemical Characteristics of Severe Hypothyroidism Due to Autoimmune Thyroiditis in Children

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Introduction: In the majority of countries, autoimmune thyroiditis is the main cause of acquired hypothyroidism in children. Typically, the natural course of the disease is initially insidious and the diagnosis is incidental. There are some children who develop severe hypothyroidism without a proper diagnosis. The aim of the study was to analyze the clinical and biochemical profiles of children with severe primary hypothyroidism due to autoimmune thyroiditis.

Materials and Methods: We analyzed the records of 354 patients diagnosed between 2009 and 2019 with autoimmune thyroiditis. Only patients with TSH above 100 μIU/mL, associated with decreased free thyroxine and the presence of antithyroid antibodies, were enrolled in the study. The analysis encompassed clinical symptoms, thyroid and biochemical status, bone age, and imaging.

Results: Twenty-six children were enrolled in the study. The mean age at diagnosis was 10.26 ± 3.3 years, with a female preponderance of 1.8:1. The most frequent symptom was growth impairment (77%) and weight gain (58%). Goiters were present in 42% of patients. Less common findings were pituitary hypertrophy (four patients) and hypertrichosis (three patients). Median values at the time of diagnosis were TSH 454.3 uIU/ml (295.0–879.4), anti-TPO antibodies 1,090 IU/ml, and anti-Tg antibodies 195 IU/ml. Anti-TSHR ab were evaluated only in six out of the 26 patients. The characteristic biochemical profile was correlated with the grade of hypothyroidism, and the strongest correlations were found with CBC parameters, lipid profile, aminotransferases, and creatine.

Conclusion: In children with severe hypothyroidism, the most sensitive symptoms are growth arrest and weight gain despite the fact that, in some children, the auxological parameters at presentation could be within normal values for the population. The specific biochemical profile closely correlates to the severity of thyroid hormone deficiency and involves mostly erythropoiesis, liver function, and kidney function. Pituitary enlargement

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should be considered in each child with severe hypothyroidism. It is necessary to conduct prospective studies evaluating the actual frequency of anti-TSHR antibodies and pituitary enlargement in children with extremely high TSH, especially those presenting without goiters.

Keywords: severe autoimmune hypothyroidism, symptoms, hypertrichosis, pituitary hypertrophy, children

INTRODUCTION

Autoimmune thyroiditis (AIT) is the main cause of acquired hypothyroidism in children (1, 2). Typically, the natural course of disease is initially insidious and many patients are diagnosed incidentally before they present overt hypothyroidism (3). There are also some children who develop severe hypothyroidism for several months without a proper diagnosis.

The most common form of AIT is classic Hashimoto's disease with goiter—a high level of antithyroid antibodies and infiltration of the thyroid gland by macrophages and lymphocytes that form the specific thyroid lymphatic tissue (4). Some patients develop atrophic autoimmune thyroiditis, which is characterized by thyroid gland fibrosis, reduction of blood perfusion, and severe damage of thyroid tissue resulting in rapid progression of severe hypothyroidism. This atrophic thyroiditis is considered the form of autoimmune thyroid disease associated with lower levels of anti-thyroid antibodies in comparison to the goitrous form of AIT. It is typical that the majority of patients with atrophic AIT are diagnosed at the phase of advanced overt hypothyroidism (5). The diagnosis of AIT in those patients is usually delayed, probably because of the lack of goiter. In such children, some characteristic changes in the clinical and biochemical picture can be observed. Although the symptoms of hypothyroidism seem to be well-known, they are still often overlooked. Stereotypically, a child with hypothyroidism is an obese, short, slow, and sleepy patient with a goiter. Nonetheless, particular symptoms usually have a specific pattern: in children with hypothyroidism, there is an observed redistribution of subcutaneous tissue due to myxedema rather than simple obesity; growth arrest is more characteristic than absolute short stature; and weight gain is observed with normal or even reduced food intake. Some patients also present non-specific symptoms like fainting and headaches. In laboratory tests, the most characteristic abnormalities are lipid disorders (6) and anemia (7, 8), but in some cases liver (9) and kidney (10) function could also be impaired.

The aim of our study was to analyze the clinical and biochemical profiles of children with severe primary hypothyroidism due to autoimmune thyroiditis.

MATERIALS AND METHODS

The study was retrospective. The medical records of 354 patients with AIT diagnosed between 2009 and 2019 in our institution were reviewed. Only patients with severe hypothyroidism (SH) were enrolled in the study. The criteria of inclusion were thyroid-stimulating hormone (TSH) value above 100 μIU/mL associated with free thyroxine (fT4) concentration below reference values and the presence of antithyroid antibodies: anti-thyroid peroxidase antibodies (anti-TPO ab) and/or anti-thyroglobulin antibodies (anti-Tg ab). Twenty-six patients (17 girls, nine boys) aged 10.26 ± 3.3 years met these criteria.

Serum concentrations of TSH, fT4, free triiodothyronine (fT3), anti-Tg ab, anti-TPO ab, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, fasting glucose, total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and complete blood cell count (CBC) were analyzed. TSH receptor antibodies (TSHR ab) were measured in six out of 26 patients. Bone age (BA) using the Greulich and Pyle method (11) was evaluated in 14 out of 26 patients. An ultrasound of the thyroid gland was performed in each patient using 7.5–11 MHz linear transducer. In patients with specific indications, magnetic resonance imaging of pituitary gland was also performed.

The study was approved by the Bioethics Committee at the Medical University of Warsaw.

Biochemical Analysis

The serum concentrations of TSH, fT4, fT3, anti-Tg ab, and anti-TPO ab were measured by the immunofluorescence method using the Architect i1000SR Analyzer (Abbott Diagnostics, Abbott Park, Illinois, USA). The anti-TSHR ab levels were measured by electrochemiluminescence immunoassay (ECLIA) with the Cobas e801 Analyzer (Diagnostics Roche, Basel, Switzerland). ALT and AST activity, creatinine, and urea concentrations were measured by the dry chemistry method using Vitros 5600 Analyzer (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). The glomerular filtration rate (GFR) was calculated using creatinine level, age, sex, and height by Bedside Schwartz method (12).

Fasting glucose was determined in blood serum using Vitros 5600 Analyzer (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). The lipid profile parameters were determined using Vitros 5600 Analyzer (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). CBC was measured in blood collected in EDTA samples using Sysmex-XN-1000i hematological analyzer (Sysmex Europe, Norderstedt, Germany): red blood cell (RBC) count, hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC) count, and platelet (PLT) count. Blood samples were obtained from patients after overnight fasting.

Reference values of analyzed laboratory parameters are shown in **Table 1**.

TABLE 1 | Average values of all evaluated biochemical parameters in SH children.

Parameter	Results	Reference limits
Thyroid		
TSH (μIU/ml)	454.3 (310.4 – 899.0)	0.58 – 3.59
fT4 (ng/dl)	0.39 (0.39 – 0.43)	0.84 – 1.47
fT3 (pg/ml)	1.48 ± 0.66	2.33 – 4.35
Tg ab (IU/l)	934.0 (197.4 – 1902.7)	<4.1
TPO ab (IU/l)	195.4 (38.3 – 647.1)	<5.6
Liver		
ALT (U/l)	49.0 (25.0 – 108.0)	10 – 30
AST (U/l)	73.0 (41.0 – 98.0)	10 – 40
Fasting glucose (mg/dl)	81.7 ± 8.0	70 – 99
Kidneys		
Creatinine (mg/dl)	0.7 (0.6 – 0.8)	0.2 – 0.7
Urea (mg/dl)	28.1 ± 7.0	15 – 36.4
Lipids		
total-C (mg/dl)	255.0 (214.0 – 343.0)	<170
LDL-C (mg/dl)	170.4 (145.0 – 244.0)	<110
HDL-C (mg/dl)	57.6 ± 22.9	>45
TG (mg/dl)	109.5 (67.0 – 173.0)	<90
CBC		
RBC ($\times 10^6$/μl)	4.1 ± 0.6	4.7 – 6.1
Hgb (g/dl)	12.0 ± 1.5	14 – 18
MCV (fl)	87.8 ± 5.3	78 – 95
MCH (pg)	29.2 ± 1.7	26 – 32
MCHC (g/dl)	33.4 ± 1.0	31 – 35
WBC ($\times 10^3$/μl)	6.7 ± 2.0	4 – 10
PLT ($\times 10^3$/μl)	256.8 ± 64.5	150 – 400

Data are presented as mean ± standard deviation or median with interquartile range as appropriate.

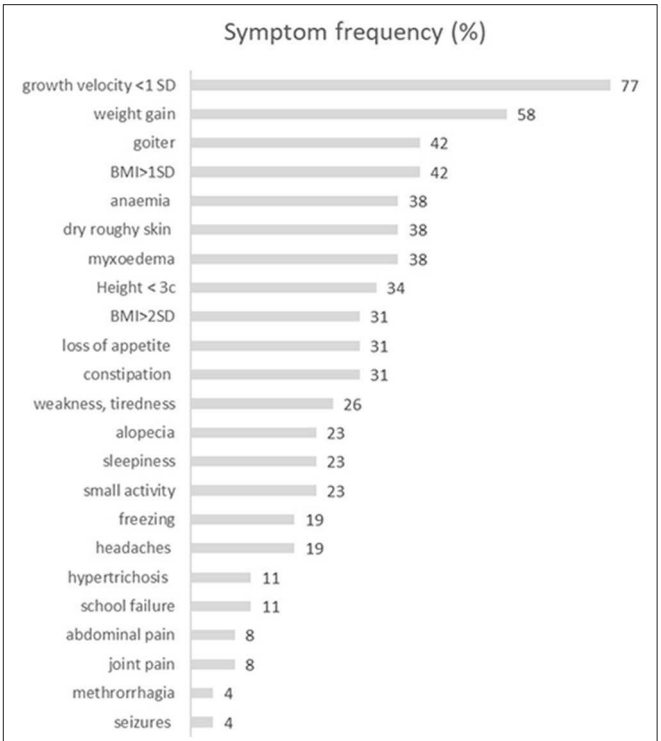

FIGURE 1 | The characteristics of the frequency of symptoms reported by patients with severe hypothyroidism. BMI, body mass index [kg/m^2].

Statistical Analysis

Statistical analysis was performed using Statistica 13.1. Data was checked by the Shapiro-Wilk normality test. Results were reported as means ± standard deviation (SD), median and interquartile ranges (IR), or as percentages, as appropriate. Correlations between variables were evaluated using the Spearman's correlation analysis for non-normally distributed data and the Pearson's correlation test for normally distributed data. A $p < 0.05$ was considered significant.

RESULTS

Clinical Symptoms in SH Children

The mean age at diagnosis was 10.26 (range 3.0–14.85), with girls' preponderance 1.8:1 (17:9). Growth arrest was the most frequent symptom (77%), whereas absolute short stature (height <3rd percentile for Polish population) was present only in 38%. The second most frequent symptom was weight gain, reported in 58% of patients; simultaneously, absolute obesity was found in 38% ($n = 10$) of patients (**Table 2**). Only 11 out of 26 patients (42%) had a goiter confirmed by ultrasound volume evaluation (**Figure 1**). The rarest symptoms were headaches, reported by four (15%) patients, and among them, seizures in one patient (4%). In those four patients, the CNS imaging was performed and anterior pituitary hypertrophy of variable grade was found without focal changes. Three patients had substantial hypertrichosis on the whole skin area, which disappeared when euthyroidism was achieved again. The characteristics of all clinical symptoms and findings in the SH children are presented in **Figure 1**. We also analyzed the diagnostic delay in SH children. The mean putative time from the occurrence of the first symptoms to the moment of diagnosis ranged from 6 months to 3 years.

Laboratory Findings

The evaluation of CBC revealed that 38% ($n = 10$) of patients had reduced RBC, 42% ($n = 11$) had decreased Hb value, and MCV in the majority of our patients was normal or slightly increased, with only one child having low MCV value.

Average values of laboratory results in all SH children are presented in **Table 1**. Parameters of lipid profile and kidney and liver function are shown in **Table 3**.

Relations of TSH, fT4, and fT3 Levels With Other Biochemical Parameters and CBC

In SH children, TSH levels were significantly positively correlated with AST ($R = 0.46$, $p = 0.026$) and urea

TABLE 2 | The auxological characteristics and hormonal and antibodies profile in patients with severe hypothyroidism.

No	Age (years)	Sex	TSH (mIU/l)	fT4 (ng/dl)	fT3 (pg/ml)	Anti-TPO (nl< 5.6)	Anti-Tg (nl< 4.1)	Anti-TSHR (positive >1.75IU/L)	Goiter	Bone age (years)	Height SDS	BMI (kg/m^2)	BMI (SDS)	Puberty Tanner scale
1	3	F	>1,000	0.41	1.4	49.0	17.0		Yes	2	−3.99	19.1	2.42	B1P1
2	4.59	F	279.64	0.43	2.37	9340.9	61.2		No		0.16	28.9	8.50	B1P1
3	5.34	F	859.74	<0.4	0.99	140.0	2630.6		No	2.5	−1.8	19.6	2.39	B1P1
4	5.67	M	534.45	<0.4	0.99	4899.6	143.6		No	4	−2.13	14.9	−0.54	G1P1
5	6.75	F	332.4	0.4	1.6	2001.0	1400.0		Yes	6	−1.29	18.3	1.21	B1P1
6	8.5^{twin}	F	>1,000	0.15	0.92	187.0	36.8	0.8	No	3.5	−3.5	24.5	3.18	B1P1
7	8.5^{twin}	F	359.0	0.48	2.74	383.6	416.9	0.8	No	5	−2.4	21.7	2.04	B1P1
8	8.59	F	171.22	0.5	2.52	7.7	250.9		Yes		1.06	30.4	4.59	B1P1
9	9.08	F	>1,000	<0.4	0.99	65.5	784.8	1.8	No	8	−1.53	22.2	1.75	B1P1
10	9.67	M	278.37	0.45	2.5	2756.7	283.5		Yes		−0.34	20.3	1.04	G1P1
11	10	F	561.43	<0.4	0.99	124.5	26.6	0.33	No		0.18	22.2	1.75	B1P1
12	10	F	316.97	<0.4	1.49	2668.9	440.8		No	8	−1.8	24.3	2.47	B1P1
13	10.17	F	355.78	<0.4	1.31	1804.4	15.7		No		−0.36	23.4	1.96	B1P1
14	10.5	M	134.59	0.48	1.86	541.4	225.2		No	9	1.66	24.8	2.45	G1P1
15	10.58	M	362.13	0.51	1.49	476.1	29.8		No		−1.3	23.5	1.65	G1P2
16	11.68	M	962.85	<0.4	1.11	1185.8	554.8	0.8	No	8.5	2.17	18.6	0.15	G1P1
17	11.75	F	899.0	0.04	0.25	1589.7	1761.0	0.77	Yes		−1.06	24.3	1.92	B2P2
18	12	F	>1,000	<0.4	1.49	653.0	69.7		Yes		−0.21	18.0	−0.36	B2P2
19	12.09	M	613.39	<0.4	0.99	1723.3	21.9		No	11	−2.06	25.9	2.08	G2P2
20	12.17	M	447.21	<0.4	1.08	2806.2	739.4		Yes		0.06	22.5	1.19	G2P2
21	13.42	F	133.94	0.41	2.18	233.2	158.0		Yes		−1.46	17.1	0.88	B3P3
22	13.84	F	461.48	<0.4	1.1	1584.7	3712.0		Yes	12.5	−3.05	25.7	1.86	B3P3
23	14.67	M	310.4	<0.4	0.99	207.7	39.8		No		−0.22	25.8	2.17	G3P3
24	14.67	F	561.98	0.36	0.98	873.3	45.8		Yes	12	−3.6	19.1	−0.37	B3P4
25	14.67	F	100.28	0.49	1.93	994.6	165.5		Yes		0.61	19.1	−0.38	B4P4
26	14.85	M	>1,000	0.22	0.74	88.8	15.5		no	12.5	−2.1	21.5	1.04	G2P3

TSH, thyroid stimulating hormone at presentation; fT4, free thyroxine value at presentation; fT3, free triiodothyronine value at presentation; anti-TPO, anti-thyroxine peroxidase antibodies and anti-Tg, antithyroglobulin antibodies (normal values in brackets); anti-TSHR, anti-TSH receptor antibodies; Height SDS, height values in SD standardized for age and sex; BMI, body mass index; BMI SDS, BMI values in SD standardized for age and sex; B, breast development; P, pubic hair development. Patients No. 6 and 7. were monozygotic twins.

levels ($R = 0.50$, $p = 0.043$). Negative correlations were found between TSH and RBC ($R = -0.75$, $p < 0.0001$) and between TSH and Hgb ($R = -0.74$, $p < 0.0001$). TSH concentrations and MCV were positively correlated ($R = 0.46$, $p = 0.019$). Surprisingly, lipid profile turned out to not be significantly correlated with TSH value in SH children, although lipid parameters were highly increased (**Tables 1**, **3**).

Free T4 levels were strongly negatively correlated with total-C ($R = -0.66$, $p = 0.001$) and LDL-C ($R = -0.61$, $p = 0.009$). Positive correlations were found between fT4 and certain CBC parameters such as RBC ($R = 0.45$, $p = 0.023$), Hgb ($R = 0.45$, $p = 0.024$), MCHC ($R = 0.49$, $p = 0.015$), and PLT ($R = 0.46$, $p = 0.021$). Free T4 concentration was negatively correlated with MCV ($R = -0.57$, $p = 0.003$). Unfortunately, the evaluation of fT4 associations was limited, because in most patients at diagnosis we received the laboratory result of fT4 concentration as a value of "< 0.4 ng/dl" (**Table 2**), so for statistical calculations we determined the value arbitrarily as 0.39 ng/dl.

We found a statistically significant negative correlation between fT3 levels and ALT ($R = 0.46$, $p = 0.046$) and AST ($R = -0.68$, $p = 0.001$). A strong negative correlation was found between fT3 and total-C ($R = -0.67$, $p = 0.002$). Free T3 also correlated with CBC parameters: positively with RBC ($R = 0.53$, $p < 0.05$) and Hgb ($R = 0.47$, $p = 0.031$), and negatively with MCV ($R = -0.58$, $p < 0.05$) and MCH ($R = -0.45$, $p < 0.05$).

Bone age was delayed more than 1 year in nine out of 14 children in whom it was determined. The median difference between chronological age and BA was 0.4 years, with the range from 0 to 5 years. We found a significant negative correlation between TSH value and absolute BA ($R = -0.55$, $p = 0.040$), but no correlation between TSH and degree of BA delay (delta in years) ($p = 0.08$).

No relationships between anti-Tg ab or anti-TPO ab and any evaluated parameter were found in our study. Antibodies against TSHR were evaluated only in six out of 26 SH patients at the presentation and in one of them the value was positive: 1.8 IU/l (by the cut-off 1.75 IU/l). None of our patients had thyroid ophthalmopathy.

TABLE 3 | Liver and kidney function tests in children with severe hypothyroidism.

No	Age (years)	TSH (mIU/L)	ALT (U/L)	AST (U/L)	Creatynine (mg/dl)	Urea (mg/dl)	GFR (ml/min/1.73 m^2)	Lipid profile			
								Total-C (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)
1	3	>1,000	62	90	0.6		56.4	183	27	134.8	106
2	4.6	279.64	25	39				221			
3	5.3	859.74	114	133	0.7	33	60.8	613	88	494.4	153
4	5.7	534.45	49	85	0.7	27	61.7	235	58	146	153
5	6.7	332.4	52	58	0.6	30	77.8	286	85	189.6	57
6	8.5	>1,000	25	49	0.5	28	93.3	230	36	145	119
7	8.5	359.0	19	43	0.5	39	98.3	208	50	148	48
8	8.6	171.22	21	29	0.6	23	94.2	214			
9	9.1	>1,000	139	98				289	86	192.8	51
10	9.7	278.37	151	86	0.6	21	94.2	214	30		477
11	10	561.43	44	92	0.8	27	72.8	411	51	348	81
12	10	316.97	35	46	0.5	27	61.4				
13	10.2	355.78	48	55	0.9	27	63.8	400	27	195.4	296
14	10.5	134.59						189	62	114.6	62
15	10.6	362.13			0.8	28	69.2	281			224
16	11.7	962.85	108	144	1.1	38	50.7	253	71	156	58
17	11.75	899.0	59	101.7	1.1	41	53.7	343	23	244	383
18	12	>1,000	26	41				283			162
19	12.1	613.39	113	128	1.1	41	52.4	375	84	254	113
20	12.2	447.21	97	98	0.8	19	79.5	366	57	272	184
21	13.4	133.96	24	40	0.6	14	106.0	208	78	114.8	76
22	13.8	461.48	16	25	0.4		146.6	255	80	160.6	72
23	14.7	310.4	82	159	1.5	25	45.9				
24	14.7	561.98	108	73	0.8		85.9				
25	14.7	100.28	14	16	0.8	31	86.2	164	44	99.4	103
26	14.9	>1,000									

DISCUSSION

Clinical Symptoms

Growth arrest in children is the most sensitive symptom of thyroid hormone deficiency because of the thyroid's strong impact on growth hormone synthesis and action. Nevertheless, in our group of patients, only 77% reported decreased growth velocity as the main problem. We can easily explain this observation taking into consideration the age of our patients and their pubertal status. All patients in whom the growth retardation was not reported were at the age of 13–14 years and had almost reached their final height and completed their pubertal development. Additionally, comparing the number of children with growth inhibition and absolute short stature, we can conclude that growth arrest, rather than height below the third percentile, is the most sensitive symptom of hypothyroidism. Similarly, comparing the frequency of weight gain (58%) and BMI >2SD (38%) in our group of SH patients, it can be concluded that it is not obesity, but fast weight gain that is typical of hypothyroidism.

The next observation concerns goiter. It seems to be a strange finding that goiter occurence is so rare and not of very high volume in SH children under such a strong stimulation of extremely increased TSH (**Table 2**). In the literature, there is evidence that some patients with atrophic AIT could be positive for anti-TSHR ab (13, 14) that act as TSH-blocking factors. They are usually polyclonal and bind to the leucine-rich repeat region of the extracellular domain of the TSHR, similar to stimulating antibodies present in patients with Graves' disease (15, 16). Blocking antibodies cut off the signal pathway of TSHR, and this can cause atrophic changes in the thyroid gland. Nevertheless, in our group, the number of patients in whom anti-TSHR ab were determined was too small to make reliable conclusions. In our opinion, in hypothyroid patient without goiter, anti-TSHR antibodies should be the obvious element of laboratory workshop.

For the last 10 years, reports evaluating large groups of children with severe hypothyroidism have been scanty and most of them concern cases with some unusual manifestations. Cabrera et al. (17) reported a relatively large group of 62 children with severe primary hypothyroidism. In this group, eight (24%) patients at prepubertal age experienced pseudoprecocious puberty with such symptoms as thelarche and/or menarche in girls and isolated testicular enlargement in boys, which regressed

during the thyroid hormone replacement. In de Vries et al., the group of 114 children and adolescents with autoimmune thyroiditis reported normal onset and duration of puberty; however, in their study, only 40 children were hypothyroid with TSH value between 11.8 and 236 mIU/l (18). In our study also, none of the patients presented symptoms of precocious puberty. The only abnormality related to puberty in our group was excessive menstrual bleeding in one girl at the age of 14.7 years. It is a typical symptom of hypothyroidism in menstruating girls, which is connected with impaired production of coagulation factors in the liver (19). Additionally, primary hypothyroidism is often associated with increased prolactin level, which can cause puberty arrest and oligomenorrhea or secondary amenorrhea. Khawaja et al. reported 16 patients younger than 20 with severe hypothyroidism (TSH >50 mIU/l) where two girls presented precocious puberty with precocious menarche and breast development with prepubertal response to gonadotropin-releasing hormone stimulation test (20).

Skin symptoms present in our patients, such as myxedema, dry, rough skin as well as loss of head hair are widely known as symptoms of hypothyroidism and are easily identified by patients and doctors. As an unusual skin symptom presented by SH children, we consider substantial hypertrichosis of individual intensity, which disappeared in the course of thyroid hormone treatment. Hypertrichosis accompanied by hair loss has been reported in hypothyroid patients (21). The question is why the same patient can suffer loss of head hair and, simultaneously, significant hypertrichosis on other skin areas. To explain this phenomenon, one has to take into account the fact that skin symptoms are dependent not only on thyroid hormone deficiency, but also on excessive TSH level. Thyroid hormone receptor Beta 1 has been proved to be expressed in the human hair follicle (22), but also TSHR messenger RNA (mRNA) and protein have been detected in human scalp hair follicles (23). Western blot and immunohistochemical analyses of skin specimens have confirmed the presence of TSHR protein in keratinocytes and fibroblasts (24). Moreover, it has been found that TSH treatment can induce the proliferation of cultured keratinocytes and fibroblasts (24). We can presume that the hypertrichosis observed in SH children could be dependent on prolonged TSH excess, whereas the head hair loss could rather result from thyroid hormones deficiency.

It is worthy to notice that in our group of SH children, at the moment of diagnosis none have the associated autoimmune comorbidities such as celiac disease, adrenal insufficiency, or diabetes mellitus, which is often observed in adult patients with Hashimoto's thyroiditis. Our data are concordant with observation of Ruggeri et al. (25), who reported that association between HT and other autoimmune diseases increases with age and occurs most frequently in adults.

Laboratory Characteristics

It is widely known that anemia is associated with hypothyroidism (26). Its prevalence in adults has been reported at 14–43% in overt hypothyroidism (26, 27). The evaluation of vitamin B12, iron, and folic acid levels in hypothyroid patients with anemia has not revealed any significant differences (26, 27). In our group of SH children, anemia was present with similar frequency (38%), although one might have expected a higher prevalence in severe hypothyroidism. The positive correlation of TSH level and MCV in our study supports the hypothesis of Das et al. (28), which suggests that the basic background of anemia in hypothyroidism is a deficiency of erythropoietin dependent on thyroid hormone deficiency. Nevertheless, the differences in the values of Hgb, MCV, and RBC among our patients also suggest that the pathophysiology of anemia in hypothyroidism is much more complicated and must have various reasons including iron deficiency in some cases.

Hypothyroidism is closely associated with abnormal liver function resulting in atherogenic lipid profile and elevated aminotransferases. Liver function tests return to normal during thyroid hormone replacement (29). Nevertheless, increased prevalence of non-alcoholic fatty liver disease in hypothyroid adults with abnormal alanine aminotransferase according to the grade of hypothyroidism has been reported (30).

In our study, we found an increase of cholesterol and its fractions in SH patients, but we did not observe any evident correlations of lipid parameters with the TSH level. On the contrary, very clear associations were found between fT4 or fT3 concentrations and lipid profile. We suppose that perhaps extremely increased TSH values should be evaluated for statistical use in logarithmic scale, because the deviations of TSH values in our study were very high in comparison to deviations in lipids concentrations. In our group of SH children, aminotransferases were elevated in the majority of patients in whom they were measured ($n = 23$). We observed a preponderance of AST elevation: it was detected in 82% ($n = 19$) of children and ALT increased in 65% ($n = 15$) (**Tables 1**, **3**). The value of TSH correlated positively only with AST concentration. Most authors suggest that more frequent AST than ALT elevation in hypothyroidism results from associated myopathy, not only the liver injury (30). On the other hand, other factors could also be involved in pathomechanism of the injury, including oxidative stress and decreased ceruloplasmin level, which is reported in hypothyroid patients (31, 32).

Myopathy and rhabdomyolysis have been reported as the factors partially responsible for the increase of creatinine, which is released from muscles (33). Serum creatinine elevation has been observed in adults as well as in children with hypothyroidism (34). This change can normalize during the treatment with thyroxine; however, in a long-standing study by Elgadi et al. (35), the authors suggest that renal impairment induced by hypothyroidism in children is not as benign as has been previously considered. In their study, GFR did not normalize completely even after 5 years of thyroxine therapy in some patients (35).

In our group of SH children, creatinine concentration was elevated in four patients, but calculated GFR was decreased in 11 children and was determined only in 15 out of 26 patients. It shows that this is not considered a routinely examined parameter in hypothyroid patients. The pathomechanism of renal impairment in hypothyroidism is not fully understood. It is suggested that thyroid hormones have an influence on muscle

function, circulating volume, and cardiac function, and also have a direct effect on the kidney (36).

Imaging

Bone Age

In general experience, BA in hypothyroidism is delayed proportionally to the grade and duration of thyroid hormones deficiency. In our group, the BA was determined in patients with significant height deficiency (below third percentile) and we had a unique opportunity to observe monozygotic twin girls at the age of 8.5 years with severe hypothyroidism with a different delay of BA (3 and 5 years, respectively) (**Table 2**). They had similar thyroid hormone deficiency, but the duration of hypothyroidism was longer in the girl with greater BA delay.

Thyroid Ultrasonography

In ultrasound scans, thyroid glands in our patients were hypoechogenic, 11 out of 26 patients (42%) had goiter, and a small thyroid was found in three children (11.5%). Thyroid blood perfusion was increased in 53.8%, and reduced in 11.5%. Nodules or focal changes were present only in two out of 26 SH children. They were of benign character.

Pituitary MRI

An interesting finding was pituitary hyperplasia found in four SH children suffering from headaches. One of those four children also had seizures. The incidence of pituitary hyperplasia secondary to hypothyroidism is unknown and probably underestimated. Pituitary MRI in patients with hypothyroidism is recommended only when some suggestive symptoms are present, i.e., vision disturbances, seizures, or severe headaches. A recent review of literature by Cao et al. (37) reported only 17 pediatric cases published from 1980 to 2017 (retrieved from Pubmed). On the other hand, Shukla et al. (38) published a review of pituitary hyperplasia in adult patients with hypothyroidism, which suggests that it is an underestimated problem and the frequency of pituitary hyperplasia has not been clearly identified. Khawaja et al. (20) reported that pituitary enlargement is observed in 70% of patients with TSH value >50 mIU/L and in 84% of patients with TSH higher than 100 mIU/L. Additionally the authors suggested that in younger patients, the frequency of pituitary enlargement can be even higher, but in their study there were only 16 patients below 20 years of life (20).

In our group of SH children, the MRI was performed only in four out of 26 children and the indications for this examination were headaches or neurological symptoms, not hypothyroidism. In these four children, everyone had an enlarged anterior pituitary and the function of tropic hormones secreted in pituitary was not impaired (**Supplementary Table 1**).

In SH patients, a deep deficiency of thyroid hormones causes excessive over-secretion of thyrotropin-releasing hormone (TRH) resulting in hypersecretion of TSH and prolactin. Increased prolactin and enlarged pituitary can suggest false pituitary adenoma, moreover the overlapping clinical symptoms such as growth arrest and weight gain associated with pituitary-suprasellar tumors could be differentiated with other childhood suprasellar tumors, i.e., craniopharyngioma. The differential diagnostics can be easy considering thyroid tests results because patients with craniopharyngioma typically present secondary and not primary hypothyroidism. More difficult could be the differentiation from prolactinoma. Another problem is not to overlook TSH-secreting adenomas, which were sporadically reported in patients with severe longstanding hypothyroidism due to the possible autonomisation of thyrotrophs (39, 40). Histology studies in humans revealed some characteristic changes of pituitary cells known as "thyroidectomy cells" (41) and additionally, it is suggested that thyrotroph hyperplasia is attributed to a loss of inhibitory feedback of the hypothalamo-pituitary axis (42). In concert to these findings, it could be presumed that patients without goiter positive for blocking anti-TSHR ab might be particularly predisposed to pituitary enlargement because of the interference of these antibodies with the ultrashort loop (TSH-pituitary) (43), which could additionally enhance the pituitary enlargement.

FINAL CONCLUSIONS

In children with severe hypothyroidism, the most sensitive symptoms are growth arrest and weight gain, despite the fact that, in some children, the auxological parameters at presentation could be within normal values for the population. The specific biochemical profile is closely correlated with severity of thyroid hormone deficiency and involves mostly erythropoiesis, liver function, and kidney function. Pituitary enlargement should be considered in each child with severe hypothyroidism. It is necessary to conduct prospective studies evaluating the actual frequency of anti-TSHR antibodies and pituitary enlargement in children with extremely high TSH, especially without goiters.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee at the Medical University of Warsaw. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AK designed the study, wrote, and supervised the manuscript. EW-S performed statistical analysis and wrote the manuscript. DL and MR recorded data of the patients, wrote the manuscript, and collected the literature data. All authors have read and approved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00364/full#supplementary-material

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Does Hashimoto's Thyroiditis Increase the Risk of Cardiovascular Disease in Young Type 1 Diabetic Patients?

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Background: Immunological and hormonal disorders have undoubted influence on the development of atherosclerotic process. Autoimmune diseases accompanying type 1 diabetes (T1D) may additionally accelerate atherosclerosis progression and increase the risk of cardiovascular events in the future. The influence of subclinical hypothyroidism on the cardiovascular system, in particular, has recently aroused great interest. The aim of our study was to assess intima-media thickness (cIMT) of common carotid arteries and the occurrence of classical atherosclerosis risk factors together with selected new biomarkers of cardiovascular diseases in young patients with type 1 diabetes mellitus coexisting with Hashimoto's disease (HD).

Patients and Methods: The study included 50 adolescents and young adults with T1D with mean age 17.1 ± 3 years, with mean diabetes duration of 10.5 ± 3.3 years, including 20 patients with diagnosed HD: T1D and HD(+), and 30 patients with no additional diseases: T1D and HD(–). Twenty-two healthy, age-matched volunteers formed control group (C). We analyzed mean HbA_1c value from all years of disease, BMI, blood pressure, lipids, new biomarkers of atherosclerosis (hsCRP, adiponectin, myeloperoxidase, NT-proBNP peptide, vitamin D), and cIMT of common carotid arteries.

Results: In the group of patients with T1D and HD(+), significantly higher BMI was found: 23.3 ± 4.4 vs. 21.28 ± 2.9 in group HD(–) and 19.65 ± 2.4 kg/m^2 in group C ($p = 0.003$), and higher waist circumference: 79 ± 10.9 vs. 75.10 ± 7.6 in group HD(–) vs. 69.0 ± 7.4 cm in group C ($p < 0.001$). The mean value of HbA_1c was higher in group T1D and HD(+): 8.8% than in group HD(–): 8.1% ($p = 0.04$). Significantly higher concentration of hsCRP and lower vitamin D were observed in T1D and HD(+) in comparison to T1D and HD(–) and the control group. The IMT index in the HD(+) group was 0.46 ± 0.05 mm and was comparable to the HD(–) group but significantly higher than in healthy controls: 0.41 ± 0.03 mm ($P < 0.05$).

Conclusions: Young patients with type 1 diabetes mellitus and with coexisting Hashimoto's thyroiditis have a higher BMI, a higher waist circumference, and a higher HbA_1c value, which altogether may cause faster development of macroangiopathy in the near future. Additional risk for cardiovascular disease may result from low vitamin D and increased hsCRP concentration in this group of patients. Coexistence of Hashimoto's thyroiditis did not significantly affect the cIMT value in the studied population.

Keywords: diabetes type 1, Hashimoto's thyroiditis, cardiovascular risk, obesity, children, young adults

INTRODUCTION

Cardiovascular diseases (CVD) are the major chronic complications of type 1 diabetes mellitus (T1DM) and cause increased mortality (1). The estimated life expectancy is 14 years less for women and 17 years for men with childhood onset T1D (2). The risk of atherosclerosis development and early ischemic heart disease in T1DM patients is several times higher than in the general population (3). T1DM in children has been identified as a high-risk factor for premature development of CVD (4). Type 1 diabetes mellitus is also associated with a significantly higher prevalence of additional autoimmune diseases, including the incidence of Hashimoto's disease (HD) estimated at 3% to even 50% (5). The influence of subclinical hypothyroidism, including HD, on increased cardiovascular risk remains a current topic of research (6–9).

Over the last decade, atherosclerosis has been identified as an inflammatory disease involving pro-inflammatory cytokines that activate the expression of endothelial adhesion molecules, together with proteases and also other mediators (10). Inflammation concerns the formation of all stages of atherosclerotic lesions, including fatty acids, most commonly prevalent in children (11). The causes of inflammation in the vessel wall are not fully explained. According to one of the current hypotheses, atherosclerosis is an autoimmune disease. There is an increasing evidence provided by observing patients with diagnosed autoimmune diseases, especially lupus erythematosus, rheumatoid arthritis, or antiphospholipid syndrome. In the course of these diseases, atherosclerotic lesions develop rapidly and extensively, much faster and more often than in the general population. It seems that immunological dysregulation in the course of these diseases is crucial in accelerating the process of the autoimmune vascular damage. This suggests that the onset of atherosclerosis may be related to genetic predisposition to autoimmune diseases (12–14).

In the preclinical phase of the atherosclerosis process, great attention is paid to numerous "new biomarkers," their usefulness in estimating the risk of cardiovascular disease, and explaining the complicated and still not fully understood pathogenesis of this disease (15). The last years confirmed the importance of high-sensitivity c-reactive protein (hsCRP) determination (16, 17). Clinical usefulness of many other biomarkers is discussed, of which oxidative stress markers, adiponectin, vitamin D, and atrial natriuretic peptide—NT-proBNP are documented both in basic and clinical studies (18, 19).

Non-invasive, ultrasonography-based studies performed among young people have shown the relationship between all known, traditional risk factors and abnormalities of blood vessel structure and function (20, 21). Recent studies have shown that T1DM already in children, adolescents, and young adults is associated with the greater carotid intima-media thickness (cIMT), the recognized marker of early structural atherosclerotic lesions (22). Nowadays, it is well-known that thickness of cIMT increases in patients with DMT1 as the disease progresses, and arterial parameters depend on the metabolic control but also on coexisting obesity, hypertension, and dyslipidemia (23–25).

Noteworthy, studies in patients who suffer from type 1 diabetes mellitus and additionally with coexisting autoimmune diseases regarding CVD risk factors, new biomarkers, and vascular status have not been conducted so far. Thus, the issue whether autoimmune diseases accompanying diabetes may further accelerate the progression of atherosclerosis and increase the risk of future cardiovascular events stays to be clarified. The current problem faced by young patients with all chronic diseases and especially DMT1 among them is not only life expectancy but also quality of life, which largely depends on the condition of the cardiovascular system.

Therefore, the purpose of the study was to evaluate cIMT (carotid intima-media thickness), classical cardiovascular risk factors, and selected new biomarkers of atherosclerosis in young patients with DMT1 with coexisting HD. We wanted to explain whether and how additional autoimmune disease in the course of type 1 diabetes mellitus in young people leads to accelerated development of atherosclerosis. We assumed that the new knowledge may help to create the appropriate therapeutic goals for these patients to minimize their cardiovascular risk and to understand better the mechanisms of atherosclerosis connected with thyroid autoimmunity.

PATIENTS

We recruited consecutive adolescents and also young adults diagnosed with type 1 diabetes remaining under the routine care of Children's Hospital and outpatient clinic in Olsztyn. The inclusion criteria for the study group were ages over 10 and under 26 and duration of illness at least 5 years. Criteria for exclusion from the study group were other types of diabetes, coexistence of other autoimmune disease (e.g., celiac disease), multiple autoimmune diseases in one patient, the occurrence of microvascular complications, previous recognition

of hypertension or hyperlipidemia, and/or taking any additional drugs apart from insulin treatment and levothyroxine (from 0.5 to 2.0 mcg/kg body mass/day orally) if classified into the HD group.

Due to the diagnosis confirming the presence or absence of additional disease, patients were qualified to particular study groups: (1) group with diabetes mellitus type 1 and Hashimoto's thyroiditis ($n = 20$), and (2) group with DMT1 without additional accompanying disease ($n = 30$). Patients were qualified to particular groups on the basis of the results of periodic screening tests according to the guidelines presented by the Polish Diabetological Society and ESPE. The diagnosis of Hashimoto's thyroiditis was stated on the basis of standard criteria: elevated serum TSH level, decreased thyroid hormone (fT4) concentration always accompanied by elevated thyroid antibodies (aTPO and/or aTG) titer, and typical ultrasound. Among our patients, three persons were recognized with clinical hypothyroidism with decreased fT4. Two patients at recognition had elevated antibodies and had typical US picture indicating thyroid autoimmunity but with normal TSH and fT4, and the other 15 studied patients were diagnosed with subclinical hypothyroidism (elevated thyroid antibodies and TSH, fT4 within the norm with different degrees of thyroid gland involvement in ultrasonography). All included into the study were treated chronically with levothyroxine to keep TSH and fT4 within the normal range. To the Hashimoto group, we included patients with at least 1-year history of the additional diagnosis, with confirmed current euthyreosis (actual TSH and fT4 within normal range) status in laboratory tests.

The reference group consisted of 22 healthy, age-matched volunteers. They were healthy, slim, normotensive students, young doctors, children of staff, and their friends. All of them had tests that excluded autoimmune and other diseases. People with mental disorders, including eating disorders (e.g., anorexia and bulimia), were not qualified for the study. The control group included individuals after exclusion of atherosclerosis risk factors (diabetes, hyperlipidemia, hypertension, and obesity) and without family history of cardiovascular diseases. Individuals in the reference group did not take any drugs. The recruitment process is presented in the flow chart (**Figure 1**).

The study protocol was approved by the Bioethics Committee of the Warmia and Mazury Chamber of Physicians and Dentists in Olsztyn, Poland. In each case of a juvenile patient (below 16 years), his or her parents'/guardians', and in the case of persons aged 16 years and over, their personal, informed written consent forms were obtained—in order to participate in the study.

METHODS

All patients had to undergo physical examination. Their height and weight were measured in a standard way by using a Harpenden stadiometer and a digital scale. Then their body mass index (BMI) was counted on the base of a standard formula. In order to adjust for age and sex, the BMI standard deviation score (BMI-SDS) was calculated and assessed using age- and sex-specific BMI growth charts according to a local Polish OLAF study (26). Patients were divided as normal weight, overweight, or obese depending on the BMI-SDS. Waist circumference was measured with clinic centimeter and converted to waist-SDS. There were two measurements of the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) at the right arm, each one after a 10-min rest with the use of calibrated sphygmomanometer of the proper cuff size, and the readings were averaged.

Laboratory Analyses

For laboratory tests, venous blood was collected after 8–12 h of fasting. Eight milliliters of blood was collected and then centrifuged for 10 min at 2,000 turns per minute. Several of the variables (HbA_1c, lipids, vitamin D, hormones, and thyroid antibodies) were performed on an ongoing basis in the hospital laboratory using standard methods. HbA_1c was evaluated in two ways: the last value in time when the blood sample was taken, and the mean value from the total time of disease duration. The remaining material (serum) was stored at a temperature of −80°C until the determination. Adiponectin (Adp), natriuretic peptide (NT-proBNP), and myeloperoxidase (MPO) markers were analyzed immunoenzymatically using ELISA kits that are commercially accessible (Parameter Human Immunoassays, R&D Systems, Inc., Minneapolis, USA). hsCRP was determined owing to the immunoturbidimetric method [Tina-quant hsCRP (Latex) HS, Roche; Hitachi 912, La Roche, Japan]. Serum levels of free thyroxine (fT4) and TSH were calculated on electrochemiluminescence, ECLIA, with Cobas E411 analyzer (Roche Diagnostics). The range of normal values for fT4 was between 1.1 and 1.7 ng/dL, and that for TSH was between 0.28 and 4.3 (μIU/l). Anti-TPO and anti-TG antibodies were measured in all samples using ECLIA with Modular Analytics E170 analyzer (Roche Diagnostics). The positive values for antibodies were >34 IU/mL for anti-TPO-Abs and >115 IU/mL for anti-TG-Abs.

Ultrasound Measurements

The procedure of ultrasound measurements was conducted in the timeslot between 8:00 and 10:00 a.m., and after a fasting period from 8 to 12 h. Measuring of intima-media thickness (IMT) in the right and left common carotid arteries was conducted as described in previous methodology, with our own modification (27, 28). Measuring covered end-diastolic (minimum diameter) IMT of the far walls (the distance between the leading edge of the first echogenic line and the leading edge of the second echogenic line) within a distance larger than 1 cm from the bifurcation. The mean value of six measurements (three from the left and three from the right carotid artery) was included in the analyses. The representative images for two groups (DMT1 and controls) with graphic scheme for IMT are presented in the **Supplementary Figures 1–3**.

Statistical Analysis

The statistical analysis was performed using Statistica 12.0 (Stat Soft, USA). All the continuous variables were tested for normal distribution by the Kolmogorov-Smirnov, with Lilliefors correction and Shapiro-Wilk tests. For variables meeting the

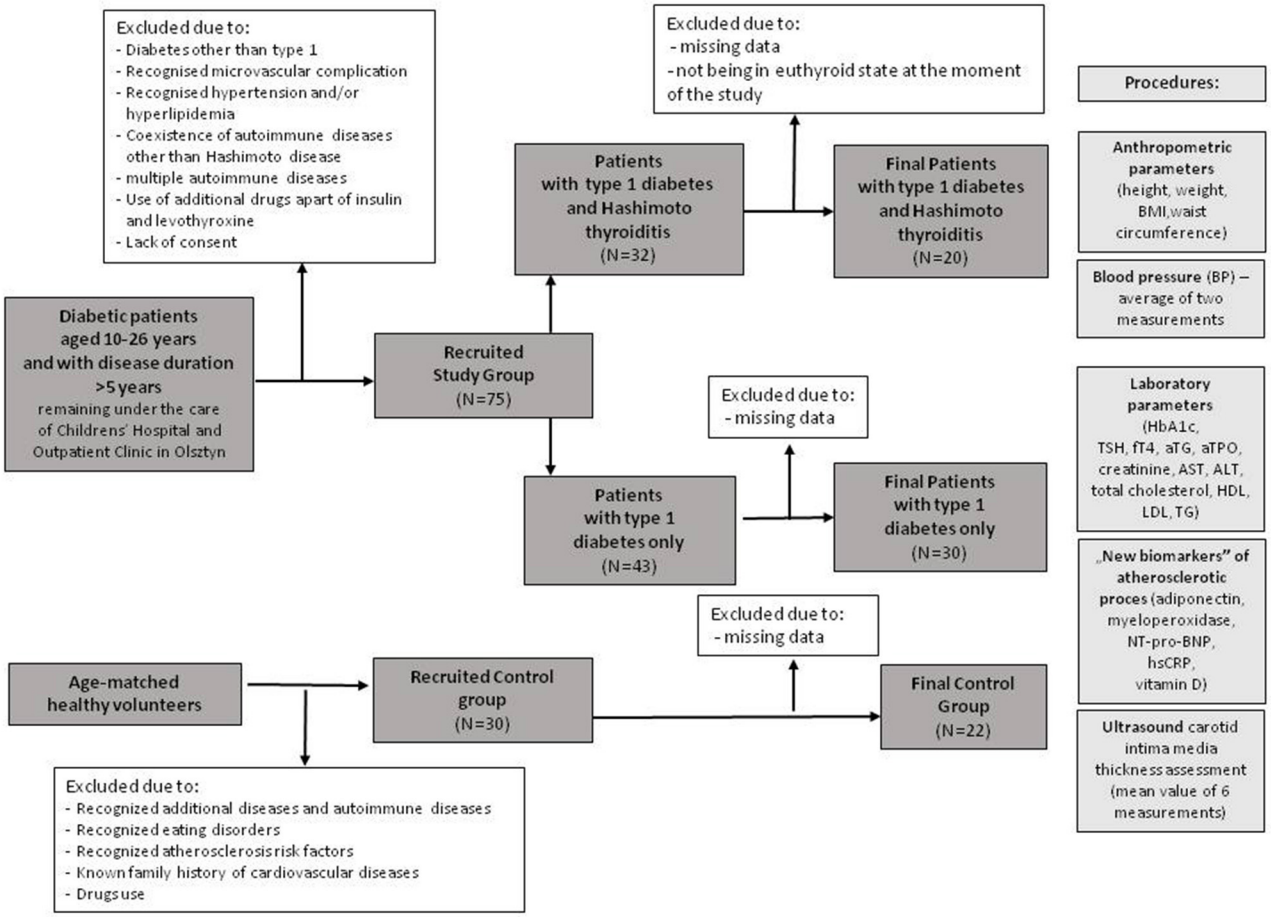

FIGURE 1 | The flow chart of the selection for the study groups.

criteria of normal distribution, the Student's *t*-test was used when comparing two variables. In the analysis of more than two groups, the analysis of variance was used with Tukey's *post-hoc* RIR test for unequal numbers. The results are presented as mean ± standard deviation *(SD)*. Non-parametric tests were used for variables not meeting the criteria of normal distribution. Mann-Whitney U non-parametric test was applied to compare quantitative variables. In the case of comparisons for more than two groups, the ANOVA rang Kruskal-Wallis test and the median test with *post-hoc* tests of multiple comparisons were used for all samples. Results are shown as median (Me) and interquartile range or mean and SD. We performed a *post-hoc* sample size calculation basing on our outcome to achieve a power of 1-β = 0.70–0.80 for the ANOVA Kruskal-Wallis test at level α = 0.05. Under these assumptions, an amount of a minimum 20 participants per group is required.

The analysis of correlations was performed using the Spearman test with the determination of the rank-order (rho) correlation coefficient. In order to detect independent determinants of IMT, multivariate regression analysis was performed. Only variables for which the *p*-value in a univariate analysis was <0.05 were included in this model. All comparisons were adjusted to age, gender, BMI, and blood pressure values. Statistically significant results were found at the level of $P < 0.05$.

RESULTS

We recruited a total of 50 patients with diabetes type 1 (20, 40% males), aged mean 17.1 + 3 years, with mean diabetes duration of 10.3 + 3.1 years, mean HbA_1c from the whole disease at 8.4 + 1.3%, and HbA_1c at the time of the analysis at 8.7 + 1.2%. Ninety-two percent of the patients were treated with continuous subcutaneous insulin infusion (CSII). Twenty patients (7, 35% males) were diagnosed with HD [T1D HD(+)]. Thirty patients had T1D without any other additional diseases [T1D HD(–)]. Studied groups were similar in mean age, diabetes duration, metabolic control, and daily insulin requirement. Body mass was higher in the HD(+) group ($P = 0.006$). TSH level was significantly ($P = 0.002$), and fT4 insignificantly higher in the T1D HD(+) group, although all values stayed within the normal range. The control group consisted of

TABLE 1 | General characteristics of the study groups.

	DMT1 total $N = 50$	DMT1 group with Hashimoto's thyroiditis $N = 20$	DMT1 group without Hashimoto's thyroiditis $N = 30$	Control group $N = 22$	*P*-values
Age (years)	17.1 ± 3	17.6 ± 3.1	16.8 ± 3.0	16.5 ± 5.0	0.62**
Gender (M/F) [*n* (%)]	20 (40%)/30 (60%)	7 (35%)/13 (65%)	13 (45%)/17 (57%)	9 (41%)/13 (59%)	
Diabetes duration (years)	10.3 ± 3.1	10.8 ± 3.5	10.0 ± 2.8		0.33*
Age of onset (years)	6.8 ± 3.6	6.8 ± 3.4	6.8 ± 3.9		0.90*
Body mass (kg)	63.4 ± 14.5	68.3 ± 15.3[a]	60.3 ± 13.3	54.0 ± 13.8	0.006**
Height (cm)	170.6 ± 11	170.8 ± 11.6	170.5 ± 11.2	164.4 ± 13.0	0.13**
HbA_1c mean (total disease duration time) (%)	8.4 ± 1.3	8.8 ± 1.4	8.1 ± 1.1		0.06*
HbA_1c last (%)	8.7 ± 1.2	9.0 ± 1.2	8.6 ± 0.4	5.4 ± 0.3	0.17*
Daily insulin requirement (UI/kg/24 h)	0.8 ± 0.18	0.8 ± 0.17	0.8 ± 0.16		0.65*
Remission period (months)	7.9 ± 7.6	6.6 ± 7.6	8.8 ± 7.6		0.35*
TSH uIU/L	2.9 ± 0.9	3.5 ± 0.6[a,b]	2.4 ± 0.8	2.33 ± 1.24	0.002**
fT4	1.33 ± 0.28	1.37 ± 0.19	1.31 ± 0.33	1.32 ± 0.24	0.61**
aTPO IU/ml	94 ± 185	209 ± 255[a,b]	17 ± 16	18 ± 10	<0.005**
aTG IU/ml	432 ± 987	1030 ± 1370[a,b]	34 ± 68	53 ± 36	<0.005**
Creatinine mg/dl	0.75 ± 0.16	0.76 ± 0.16	0.75 ± 0.16	0.76 ± 0.2	0.92**
AST U/L	25.1 ± 18	31.6 ± 26.4	21.6 ± 7.4	23.5 ± 6.5	0.07**
ALT U/L	28 ± 10	29.4 ± 11.3	27.1 ± 10.0	24.6 ± 7.1	0.27**

**p-values in t-student test [difference between Hashimoto (+) and Hashimoto (–) patients].*

***p-values in ANOVA variance test (differences between both diabetic and control groups).*

[a]$p < 0.05$—compared to the control group.

[b]$p < 0.05$—compared to the diabetes and Hashimoto (–) group in post-hoc tests. The data are presented as mean ± SD.

22 (9, 41% males), age/gender-matched healthy volunteers. The general characteristic of the study groups is shown in **Table 1**.

First, we analyzed classical risk factors of cardiovascular diseases. We found significantly higher BMI and SDS–BMI in patients with T1D and HD(+) compared to T1D and HD(–) and to control groups ($p = 0.003$, $p = 0.010$, respectively). Nine patients (45%) from the HD(+) group were found to be overweight or obese. Waist circumference was higher in the HD(+) group compared to both the remaining groups ($p < 0.001$), and waist-SDS was significantly higher in comparison with the control group ($p = 0.002$). SBP was higher among both HD(+) and HD(–) compared to controls ($p < 0.001$), and DBP was the highest in T1D and HD(+) ($p = 0.008$). Within lipid parameters, we found significant differences in the triglycerides level, with the highest values in the T1D and HD(+) groups ($p = 0.005$ in comparison to controls). The HbA_1c value, averaged from the whole disease period, was higher in HD(+) ($p = 0.04$) and comparable with the HD(–) group when the last value from the time of the current analysis was considered (**Table 2**, **Figure 2**).

Next, we analyzed the differences in new biomarkers of the atherosclerotic process. We showed significant differences in the myeloperoxidase level that was higher in both diabetic groups in comparison with controls ($p = 0.012$), hsCRP, higher in T1D and HD(+) compared to T1D and HD(–) (**Figure 3A**), and to the control groups ($p < 0.001$), as well as in the vitamin D level, which we found lower in both diabetic groups compared to healthy ones ($p < 0.001$) (**Figure 3B**, **Table 2**).

Finally, we analyzed the IMT value. The thickness of the intima-media of the common carotid arteries was significantly higher in both diabetic groups: 0.46 ± 0.05 mm in T1D with HD(+), 0.45 ± 0.04 mm in T1D with HD(–) compared to the control group: 0.41 ± 0.03 mm (both $p < 0.05$) (**Figure 4**). IMT correlated significantly positively with BMI-SDS, SBP, and mean HbA_1c, and negatively with vitamin D (**Figure 5**). In **Table 3**, we present other results of the correlation analysis between IMT and studied classical risk factors and new biomarkers of cardiovascular disease in the group of HD(+) patients. In the multivariate regression model regarding this group, IMT was associated significantly with SDS-BMI and vitamin D level ($R^2 = 0.48$, B = 0.18, $p < 0.04$).

DISCUSSION

The crucial finding of our study is that young patients (teenagers and young adults) with T1D and coexisting additional Hashimoto's disease HD(+) have much more unfavorable profile of classical cardiovascular risks factors compared to T1D peers without any additional disease. We found higher body mass, waist circumference, blood pressure values, and triglycerides concentration, as well as poorer metabolic control, evaluated as mean glycated hemoglobin from the whole disease period and

TABLE 2 | Comparison of clinical parameters, lipid levels, metabolic control, and analysis of the concentration of "new biomarkers" of the atherosclerotic process between study groups.

	DMT1 group with Hashimoto's thyroiditis *N* = 20	DMT1 group *N* = 30	Control group *N* = 22	*P*-values*
BMI (kg/m^2)	23.3 ± 4.4[a,b]	21.28 ± 2.9	19.65 ± 2.4	0.003
BMI-SDS	1.015 (0.14–1.36)[a,b]	0.24 (−0.2–0.93)	−0.1 (−0.3–0.28)	0.010
Waist (cm)	79 ± 10.9[a,b]	75.1 ± 7.6	69.0 ± 7.4	<0.001
Waist-SDS	0.98 (0.52–1.9)[a]	0.47 (−0.13–1.18)	−0.14 (−0.27–0.23)	0.002
SBP (mmHg)	125 ± 15[a]	121.5 ± 11[a]	109 ± 9	<0.001
DBP (mmHg)	74 ± 8[a]	71.1 ± 6	69 ± 5	0.008
Total cholesterol (mg/dl)	180 ± 30	176 ± 25	164 ± 29	0.160
LDL (mg/dl)	96 ± 31	101 ± 28	89 ± 28	0.100
HDL(mg/dl)	57 ± 10	57 ± 11	59 ± 11	0.150
TG (mg/dl)	108 ± 354[a]	82 ± 27	75 ± 39	0.005
HbA_1c mean %	8.8 ± 1.4	8.1 ± 1.1	–	0.040**
HbA_1c last %	9.0 ± 1.2[a]	8.6 ± 1.1[a]	5.4 ± 0.2	<0.001
Adiponectin (ng/ml)	8764.3 (6659–14616)	7704.6 (4816–10231)	9746.6 (4933–11333)	0.650
Myeloperoxidase (ng/ml)	184.6 (120–325)[a]	200.8 (95–281)[a]	96.8 (72–139)	0.012
NTproBNP (pg/ml)	29.5 (17.8–40.0)	23.4 (15.2–43.8)	28.9 (17–37)	0.080
hsCRP (mg/L)	0.98 (0.4–2.49)[a,b]	0.36 (0.23–0.69)	0.2 (0.1–0.31)	<0.001
Vitamin D (ng/ml)	17.9 ± 7.9[a]	18.5 ± 8.1[a]	25.4 ± 5.7	<0.001

The data are presented as mean ± SD or median (interquartile range).
**ANOVA Kruskal-Wallis test.*
***t-student test.*
[a]*p < 0.05—compared to the control group.*
[b]*p < 0.05—compared to the diabetes group in post-hoc tests.*

just the last value. In the present study, we also confirmed that patients with recognized HD had higher concentration of hsCRP compared not only to the healthy group but also to the T1D HD(–) group. Moreover, the myeloperoxidase level was higher, whereas the vitamin D concentration was lower in both groups of diabetic patients. However, the cIMT value was comparable between HD(+) and HD(–) T1D patients, and in both groups, considerably higher than in the control group.

In the current study, we chose to evaluate the population of teenagers and young adults with T1D. To the best of our knowledge, they represent the rarely studied population of diabetic patients. CV risk factors are quite commonly studied in diabetic children groups or in adult ones when clinical complications and apparent macroangiopathy have already appeared. Our study provides the unique possibility to get the knowledge on CV risk factors status in T1D patients being almost or already young adults, with quite a long time of diabetes duration (at least 5 years), yet without confirmed cardiovascular complications. As the main target, however, we decided to investigate the group with coexistence of autoimmune hypothyroidism. Additional autoimmune diseases, among them mainly thyroid autoimmunopathies, are frequent comorbidities of T1D. Their prevalence increases with diabetes duration (29), and generally, the frequency of additional autoimmune diseases is increasing in the last decades (5). Just a few studies published so far have presented the data of single cardiovascular risk factors, specifically dyslipidemia, in pediatric diabetic patients with coexisting thyroid autoimmunity (30, 31).

Multiple studies in patients with subclinical hypothyroidism have shown the association with cardiovascular abnormalities, like impaired endothelial function, increased IMT, left ventricular dysfunction, heart failure, coronary artery disease, and cardiovascular death. Many of these studies proved the substantial contribution of dyslipidemia, hypertension, obesity, insulin resistance, and metabolic syndrome in these complications (7, 32). The recently published meta-analyses on early atherosclerosis in SH patients showed that severity of thyroid hormones disturbance is closely associated with the degree of arteries' function and structure, but other factors, like additional diseases, could not be ruled out (32, 33). The scientific research on the relevance of low-normal thyroid function on components of the metabolic syndrome (MS) shows that the state is significantly associated with all components of MS (34). Several studies in obese and also in non-obese individuals, with thyroid function at normal range, presented the results of association between elevated thyroid antibodies with insulin resistance and hsCRP (16, 35). Low-normal thyroid function may be implicated into atherosclerosis development via connections with insulin resistance and metabolic syndrome (36). All our patients with HD(+) were in laboratory euthyroid state, and to make this group more homogeneous, all included subjects were treated with supplemental dose of levothyroxine.

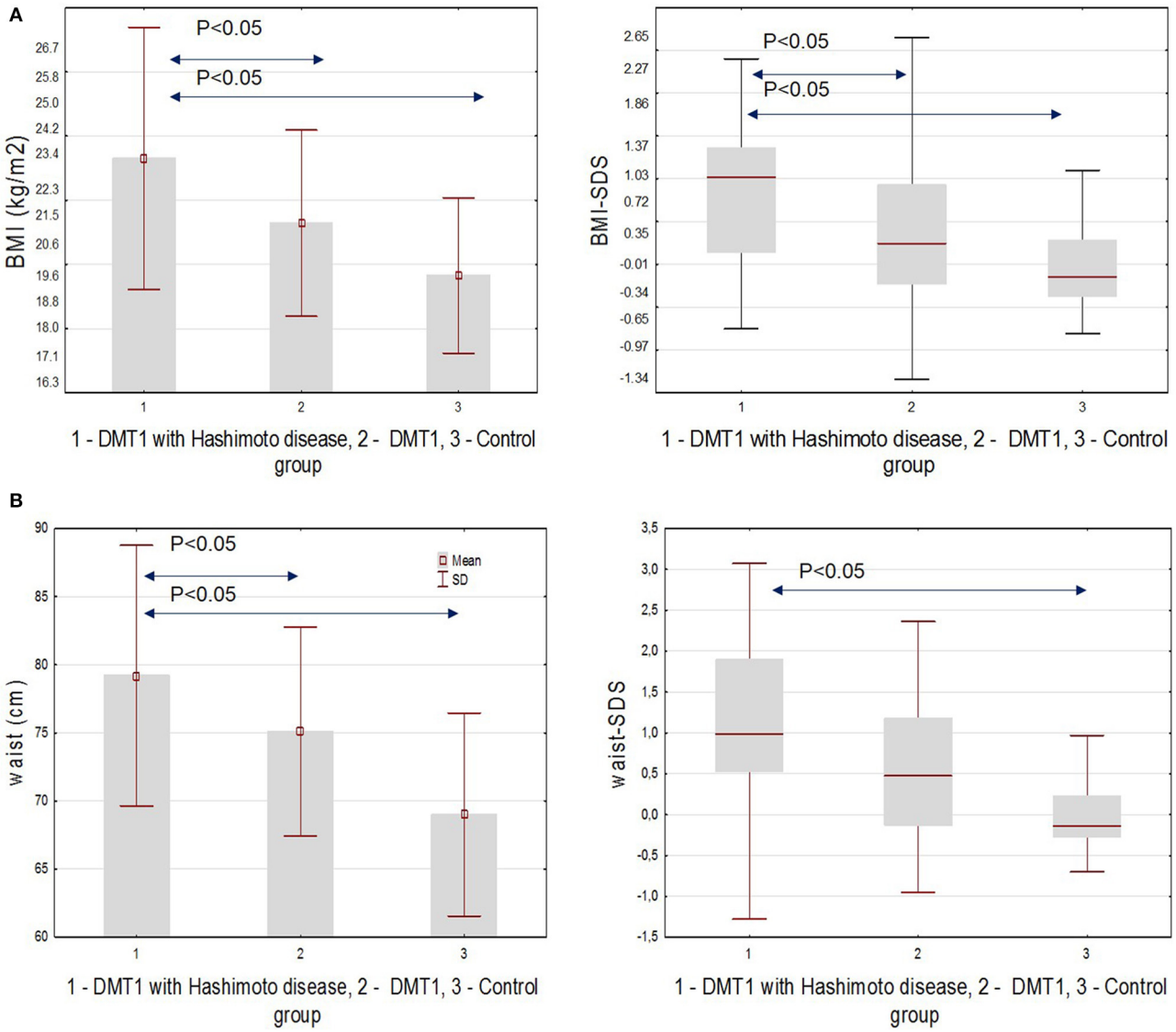

FIGURE 2 | (A) BMI and SDS-BMI in the studied groups. **(B)** Waist circumference and waist-SDS in the studied groups.

Notwithstanding, we observed significant differences in the TSH level between the HD(+) and HD(−) groups, which may be one of the explanations of observed differences in CV risk factors intensity.

Studies considering early atherosclerosis risk in childhood thyroid autoimmunity are scarce, and the results are inconsistent. In euthyroid girls newly diagnosed with HD, increased total cholesterol and hsCRP levels were found, like in our study, but also increased cIMT and no differences in BMI contrary to our results (37, 38). In another study conducted on a large group of Spanish children, higher levels of thyrotropin were found in obese young patients. The difference between obese and normal weight may be related to higher incidence of thyroid autoimmunity in the overweight patients (39). Isolated increased TSH was found to be common in other obese pediatric population, without significant relationship to autoimmune status (40). Recently published meta-analysis clearly indicated that obesity was independently significantly associated with hypothyroidism, recognition of HD, and thyroid antibodies (41).

Our current analyses proved that more altered parameters associated with CV risk were found in the group of T1D with HD. This group had the highest BMI, expressed also as SDS-BMI, and waist circumference, which is the key index in insulin resistance recognition in clinical settings. Almost half of the group fulfilled criteria for overweight or obesity. Ciccone et al. presented the results of the study in women with Hashimoto's thyroiditis, where they found that IMT is increased only in obese and overweight patients. This correlation between Hashimoto's thyroiditis and IMT seemed to be independent of TSH and thyroid hormone values. They conclude that HD represents

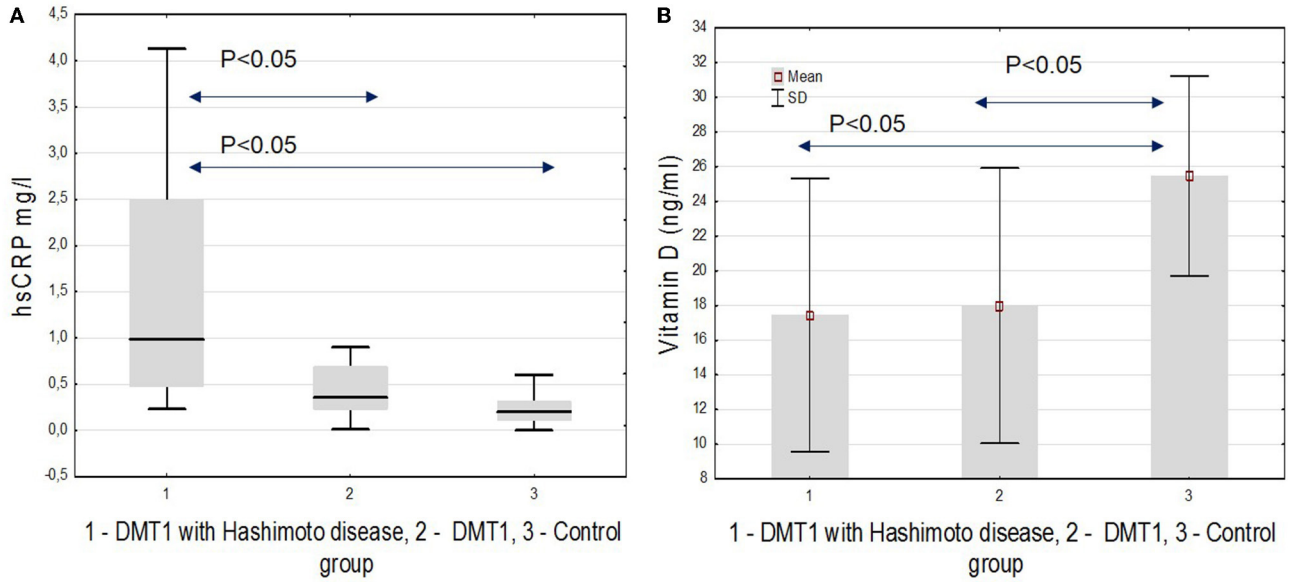

FIGURE 3 | (A) hsCRP. **(B)** Vitamin D in the studied groups.

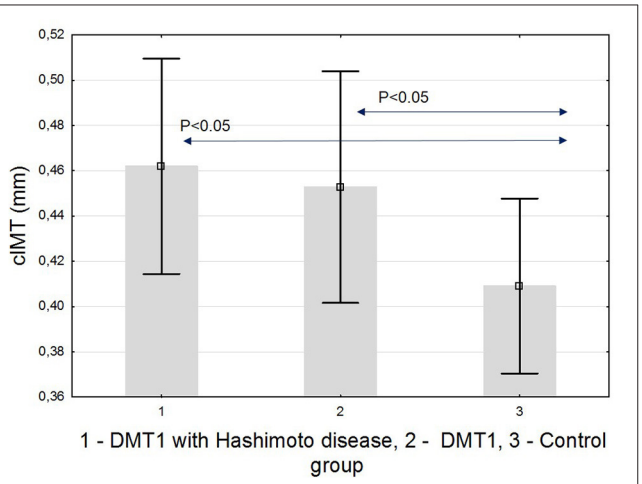

FIGURE 4 | cIMT in the studied groups.

a marker of atherosclerosis development when combined to adiposity (42).

The increasing prevalence of obesity worldwide is parallel to increasing numbers of not only cardiovascular diseases or cancers but autoimmune conditions as well. Obesity is regarded as a chronic low-grade inflammation process, where many inflammatory markers and cytokines are overproduced and over-activated. This process may result in increased pathogenic processes leading up to increased outbreak of type 1 diabetes, higher numbers of autoimmune thyroiditis, and cardiovascular disease in the future. Obesity seems to be a core environmental contributing factor to the onset and development of autoimmune diseases (43).

The excess in body weight has become an urgent problem among patients with T1D. It is reported that T1D is being recognized with higher SDS-BMI nowadays, and there is a trend for increasing BMI with diabetes duration (44). As many as 30% of young diabetic patients are overweight or obese (45, 46). Very alarming data come from recent DCCT/EDIC published studies. This is a crucial, longitudinal observation for T1D intensive insulin treatment implementation. The results of the study proved, firstly, that intensive insulin treatment regimens result in improvement in metabolic control and significant reduction in IMT and all vascular complications rates, CVD, and myocardial infarctions among them (47). Recent observation, however, found that these subjects from the intensive group, who experienced excessive weight gain, had increased IMT and total CVD event after 15 years observation, comparing to the group treated conventionally, thus with poorer metabolic control. Weight gain in long-term observation seems to nullify the success of intensive insulin therapy and improved metabolic control (48). The issue whether evolving, obesity-connected autoimmunity in T1D patients additionally exacerbates the risk of early CVD remains unexplained.

In our group of T1D and HD(+) patients, we also confirmed elevated triglycerides level. The amount of already published data proved that thyroid dysfunction and autoimmune process, even in young population, is connected with impaired lipid metabolism. Severe atherogenic dyslipidemia may occur in overt hypothyroidism, while in euthyroid AIT patients, the alterations are discrete. The lack of thyroid hormones is related to reduced clearance of TG-rich particles. Hypertriglyceridemia has been associated with the increased production of small, dense LDL (49). Long-term consequences of childhood AITD-associated dyslipidemia remain unknown, but the short–term data reveal improvement in lipid profile with L-thyroxine

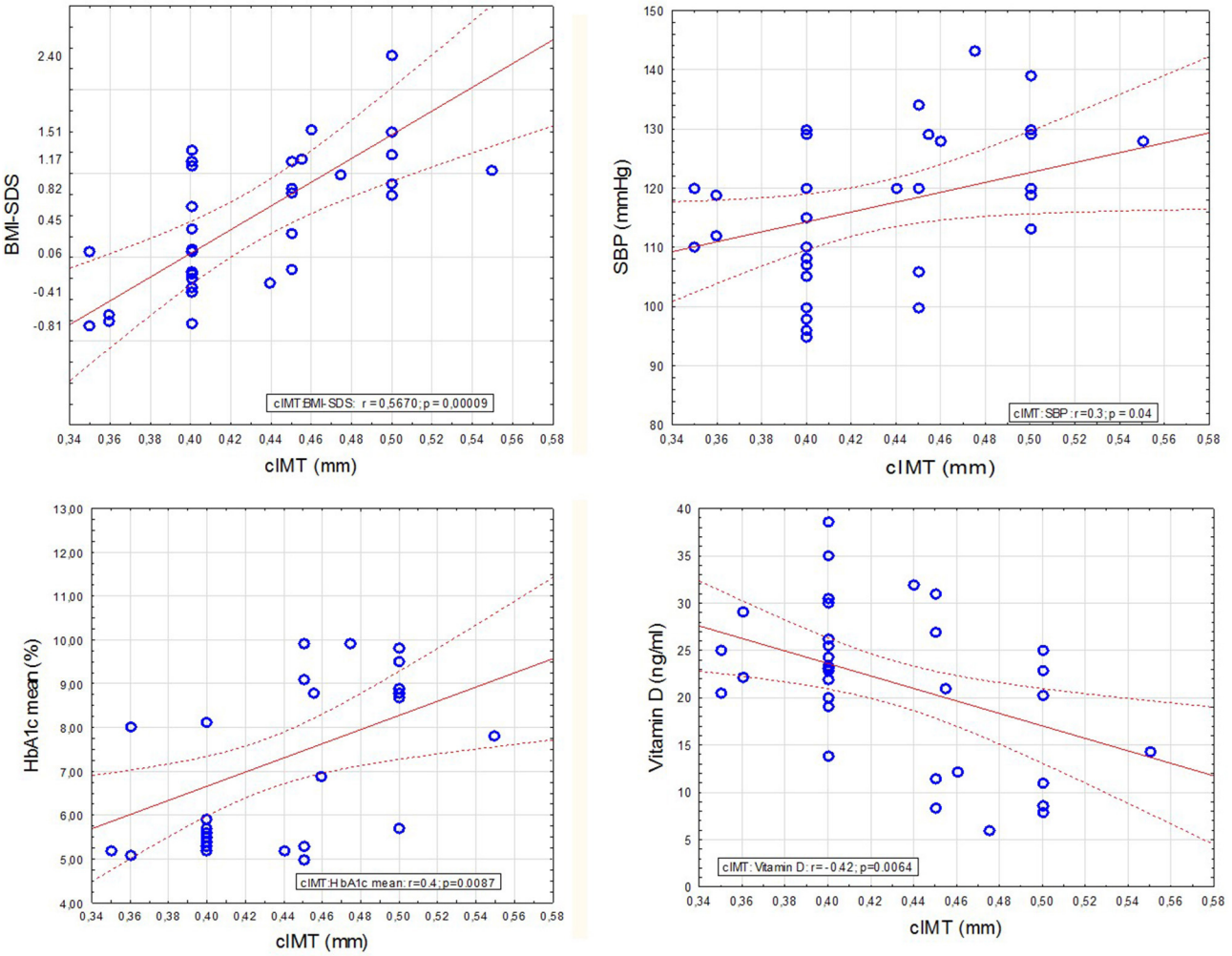

FIGURE 5 | Analysis of IMT correlation with selected variables in patients with diabetes type 1 and Hashimoto's thyroiditis.

treatment [reviewed in (50)]. Atherogenic dyslipidemia is a huge, recognized but undertreated and pending problem among young people with T1D (46, 51, 52).

In our study, we decided not to limit analyses only to traditional risk factors but to investigate selected new biomarkers of atherosclerosis as well. The relevance of hsCRP as a new and independent atherosclerosis biomarker, associated mainly with obesity and low-grade inflammatory state, is established (10, 17). A correlation between cIMT and adiponectin, leptin, and high C-reactive protein (hsCRP) has been demonstrated in obese children (53, 54). Here in our study, we proved higher hsCRP level in patients with HD(+). Our data showed decreased concentration of vitamin D in both groups of diabetic patients. Some studies reported correlation between the deficiency of vitamin D and the risk of autoimmune disease (55, 56). Several observations found decreased vitamin D levels in obese and T1D patients (57, 58). What is more interesting, supplementation with vitamin D was associated with an improvement in peripheral vascular function in diabetic children (57, 58). The discussion whether supplementation with vitamin D may be preventive in general or selected population in CVD prevention is open (59).

Both our presented diabetic groups had significantly higher cIMT compared to the healthy group. The difference between the HD(+) and HD(−) groups was not apparent, although many differences in cardiovascular risk intensity were noticed and discussed above that indicated that HD(+) should be at higher risk. In our patients, IMT was correlated with BMI, SBP, and HbA_1c. These results are in line with already published data considering young diabetic patients (24, 60). Both our diabetic groups had poor metabolic control. The mean disease value was far from recommendations. Unfortunately, this is a well-recognized clinical problem that pediatric and young population is very problematic in keeping proper metabolic control even with modern technologies (61). We think that it is possible that chronic hyperglycemia at this teenager age remains the main contributor to IMT, like some other authors found (62). However, contrary to ours and the studies discussed above, the

TABLE 3 | Correlation analysis between cIMT and other studied variables in DMT1 with Hashimoto's thyroiditis group.

	cIMT
Age	Rho = 0.280, $P = 0.075$
Diabetes duration	Rho = 0.030, $P = 0.860$
BMI	Rho = 0.580, $P < 0.001$
BMI-SDS	Rho = 0.560, $P < 0.001$
Waist SDS	Rho = 0.490, $P < 0.001$
SBP	Rho = 0.300, $P = 0.04$
DBP	Rho = 0.370, $P = 0.013$
Total cholesterol	Rho = 0.019, $P = 0.900$
LDL-cholesterol	Rho = 0.001, $P = 0.100$
HDL-cholesterol	Rho = 0.080, $P = 0.570$
Triglicerides	Rho = 0.140, $P = 0.340$
HbA_1c mean	Rho = 0.400, $P = 0.009$
Adiponectin	Rho = −0.040, $P = 0.810$
Myeloperoxidase	Rho = 0.170, $P = 0.350$
NT proBNP	Rho = 0.230, $P = 0.170$
hsCRP	Rho = 0.280, $P = 0.075$
Vitamin D	Rho = −0.420, $P = 0.006$

results of SEARCH CVD Study clearly stated that CV risk factors burden increased gradually in young people with T1D, BMI was a major risk modifiable factor that was predicting carotid IMT, and HbA_1c alone could not explain the value of IMT (24). So far, additional autoimmune processes were not included into such analyses among T1D patients.

cIMT was proved to be increased in clinically overt hypothyroidism, and the decrease was noticed after thyroxin treatment (63). However, there are also some reports that prove the increased cIMT in euthyroid, non-diabetic state but connected with autoimmune thyroid condition (64). The issue of significance of pharmacological treatment among patients with SH remains open. In some studies, thyroxin replacement was related to significant reduction in carotid IMT, and improving lipid profile (65, 66).

The issue whether HD as an autoimmune condition may be responsible for autoimmune, inflammation-based endothelial dysfunction itself remains to be elucidated. However, some studies demonstrated these early vessels impairment in HD patients to be independent from other risk factors for CVD (67). An increased ongoing inflammatory status might contribute to increased insulin resistance in both obese and non-obese AIT patients even with euthyroidism (35). It should be established whether HD is an independent cardiovascular risk factor. The possible pathogenic mechanism of the connection between HD, type 1 diabetes, obesity, and early atherosclerosis remains unclear. However, several hypotheses can be discussed. In patients without T1D, it was proved that IMT is related to hormone levels, even when their values remain within the normal range. In our group, we confirmed that the T1D HD(+) patients had higher TSH level despite the pharmacological treatment, and the level of thyroid antibodies remained high, indicating ongoing autoimmune process. The lack of difference in IMT values between HD(+) and HD(−) patients can be explained by probably the strongest influence of poor metabolic control in all diabetic patients. Metabolic control, expressed as the HbA_1c level, is known to be the strongest cardiovascular risk factor in children with T1D. Recently published DCCT/EDIC study population data clearly revealed that HbA_1c is associated with numerous traditional CVD risk factors, and that this association cannot alone be an explanation of its effect on the CVD risk. It is concluded that aggressive management of traditional non-glycemic CVD risk factors is indicated in all T1D patients and, together with excellent metabolic control, remains the primary objective (68). However, it cannot be entirely excluded that autoimmune thyroiditis may itself be causing inflammation of autoimmune origin that keeps atherosclerosis process accelerated in the long run. Randomized, controlled, and longitudinal studies on larger patient groups with T1D and HD(+) are needed to prove the benefits of additional early levothyroxine replacement on reducing the CVD risk in young patients with diabetes type 1, additionally to continuous efforts for improving metabolic control. Long-term cardiovascular consequences of T1D in today's young patients, affected additionally by autoimmune hypothyroidism, remain unknown due to lack of longitudinal prospective studies.

LIMITATIONS OF THE STUDY

We are aware that there are certain limitations of our study implicating a careful interpretation of the study results. The main limitation of our study is the small sample size of the population and the small number of patients included into every studied group. Moreover, all patients came from the same one center. However, the sample size calculation allowed us to carry out the designed study. Another limitation is that we did not perform screening tests for other autoimmunities, except for celiac disease. There are no screening recommendations because of the rare occurrence among diabetic type 1 patients. Additional diseases, other than thyroid and celiac, are diagnosed on the basis of clinical presentation firstly. We did not recruit into the study group these patients with recognized autoimmune disease other than Hashimoto's thyroiditis, so we cannot exclude that among our patients, there might have been any additional subclinical autoimmune processes.

CONCLUSIONS

Young patients suffering from type 1 diabetes mellitus and with coexisting Hashimoto's thyroiditis have a higher BMI, a higher waist circumference, and a higher HbA_1c value, which altogether may cause faster development of macroangiopathy in the near future. Additional risk for cardiovascular disease may result from low vitamin D and increased hsCRP concentration in this group of patients. Coexistence of Hashimoto's thyroiditis did not significantly affect the cIMT value in the studied population. Explaining whether and how additional autoimmune diseases in the course of type 1 diabetes mellitus in young people lead to accelerated development of atherosclerosis can help not only to

create the right therapeutic goals for these patients to minimize their cardiovascular risk but also may be the next step in understanding the autoimmune mechanisms of atherosclerosis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee of the Warmia and Mazury Chamber of Physicians and Dentists in Olsztyn, Poland. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BG-O designed the study, performed the statistical analysis, and drafted and wrote the manuscript. HB-S and BS analyzed the data, participated in the study conception, and designed and contributed to a great extent to the discussion. BK and DC participated in patients' recruitment, collecting the data, and analyses. BŻ-R performed the laboratory analyses of new biomarkers and analyzed them. AB was involved in the design, conception, analysis, and revision of the manuscript. All authors contributed in discussions and read and approved 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/fendo.2020.00431/full#supplementary-material

Supplementary Figure 1 | The graphic scheme of IMT measurement.

Supplementary Figure 2 | IMT ultrasonography representative image for diabetic patient.

Supplementary Figure 3 | IMT ultrasonography representative image for healthy control.

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Increasing Co-occurrence of Additional Autoimmune Disorders at Diabetes Type 1 Onset Among Children and Adolescents Diagnosed in Years 2010–2018—Single-Center Study

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Objectives: The prevalence of type 1 diabetes mellitus (T1D) in children is growing, but its relation to other autoimmune disorders that coexist since the onset of diabetes is not recognized. The objective of this study was to assess the incidence of T1D and the prevalence of autoimmune illnesses additionally coexisting since the diabetes mellitus onset in children during a period of 9 years' observation.

Methods: In this retrospective study, the incidence rate (IR) of the T1D was calculated as the total number of all cases that were newly diagnosed per 100,000 population people between 0 and 18 years of age. The selected age groups (0–4, 5–9, 10–14, and 15–18 years) were examined, respectively. The studied group included 493 children (264 [53.55%] boys) between 0 and 18 years old newly diagnosed with T1D in one of the Polish centers in the years 2010–2018. Other autoimmune illnesses diagnoses were obtained from medical records taken from the first hospital treatment, when T1D was recognized.

Results: The annual standardized IR of T1D increased from 19.2/100,000 in year 2010 to 31.7/100,000 in 2018 (1.7-fold over 9 years' observation), with an increase in the incidence rate ratio (IRR) by 4% per year. The highest growth in IR was recorded in 5- to 9-year-olds (from 19.61 in 2010 to 43.45 in 2018). In 61 (12.4%) of the studied group, at least one additional autoimmune disease was diagnosed. The prevalence doubled from 10.4% in the year 2010 to 20.8% in the year 2018. Autoimmune thyroid illnesses were found in 37 children (7.5%); their incidence increased from 6.3% to almost 2-fold, 12.5%, in 2018. In 26 children (5.3%), celiac disease was recognized; the prevalence increased from 4.2 to 9.8% in the study period. The prevalence of additional autoimmune thyroid disease was higher in glutamic acid decarboxylase–positive antibodies ($\chi^2 = 3.4$, $p = 0.04$) patients, the oldest age group (15–18 years) ($\chi^2 = 7.1$, $p = 0.06$), and in girls ($\chi^2 = 7.1$, $p = 0.007$).

Conclusions: The standardized IR of T1D in children increased 1.7-fold over the 9-year observation period, and IRR increased 4% per year. Additional autoimmunity represents a significant comorbidity in patients with new-onset T1D. The number of children diagnosed with additional autoimmune diseases that accompany T1D is rapidly growing in all age groups throughout recent years.

Keywords: diabetes type 1, autoimmune thyroid diseases, celiac disease, children, epidemiology

INTRODUCTION

The prevalence of type 1 diabetes mellitus (T1D) has received much attention lately, and the rapid increase in the number of patients should not be disregarded. According to the ninth edition of *IDF Diabetes Atlas* 2019 (1), it is estimated that 1,110,100 young people younger than 20 years have T1D worldwide. Type 1 diabetes incidence in children increases by 2–5% annually worldwide, according to large epidemiologic studies (2), and it depends on the geographic region; for example, in Asian countries, the incidence rates (IRs) are usually very low (3), whereas the rates in some European countries, for example, Finland, are indisputably high (4). In European cases, the incidence among girls is currently the highest in the age group between 5 and 9 years, whereas that of boys is highest in the 10- to 14-years age group (5). In years 2010–2014 in Poland, incidence rate ratio (IRR) increased 1.5-fold (by 12.73% annually) in children and adolescents aged 0–17 years (6).

It has been reported recently, among them in meta-analysis, that frequency of autoimmune diseases (AD) in general, has increased significantly over the last 30 years, with thorough research of risk factors and environmental impacts on susceptibility to AD (7, 8). The global prevalence of AD in pediatric age is ∼5%, and the most frequent autoimmunities are represented by autoimmune thyroid diseases (AITDs) (9). The incidence of autoimmune hypothyroidism in children is rated at 1–2% with a dominance of female 4:1 (10). Even subclinical course of the disease is known to result in many adverse effects such as increased risk of congestive cardiac failure and coronary heart disease events. What is more, subclinical hypothyroidism is likely to cause cognitive impairment and non-specific symptoms, for example, fatigue and mood changes (11). Hyperthyroidism constitutes 15% of children's thyroid disorders, and most of the cases can be attributed to hyperthyroidism of autoimmune origin, Graves disease (GD) (10).

It is now recognized that the prevalence of additional autoimmunity is increasingly encountered in clinical practice of pediatric diabetologists. Additional ADs (AADs) frequently occur in the same individual over the course of T1D, suggesting strong shared genetic susceptibility and pathological mechanisms. Patients with T1D demonstrate an increased exposure to other autoimmune disorders, for example, AITD (Hashimoto thyroiditis and GD, 15–30%), Addison disease (0.5%), autoimmune gastritis (5–10%), celiac disease (CD; 4–9%), and vitiligo (2–10%) (12, 13). Revealed high prevalence of associated autoimmune conditions generated the need for early screening of these diseases (14).

There is increasing knowledge about the prevalence of AAD in the course of long-lasting T1D in children with T1D (15, 16). Notably, the association of T1D with other autoimmune illnesses that coexist from the onset of diabetes and the actual trend of this connection over the years are not fully recognized. We aimed to estimate the prevalence of patients diagnosed with the most common autoimmune disorders: AITD and CD at the diagnosis of T1D and the changes in prevalence of these diseases during 9 years (2010–2018) observation. Second, we intended to search for possible clinical factors connected with multiple autoimmunities since T1D onset. We hypothesized that together with increasing prevalence of diabetes type 1 in children over the years, the number of patients diagnosed with AAD will also increase.

PATIENTS AND METHODS

The study was planned as a retrospective, hospital records–based study including 493 children and adolescents (264 boys and 229 girls, aged 0–18 years), who were diagnosed for T1D between 2010 and 2018 at the Department of Pediatrics, Endocrinology, Diabetology with Cardiology Division, Medical University of Białystok, Poland. This is the only center for diabetic children in the Podlasie Voivodeship Region (northeast part of Poland), where all young patients with newly diagnosed T1D from the region are treated. The IR of the T1D was calculated as the total number of all cases that were newly diagnosed per 100,000 people between 0 and 18 years of age. General population figure was obtained from the Central Statistical Office of Poland (Polish: Glowny Urzad Statystyczny) (17). The selected age groups (0–4, 5–9, 10–14, and 15–18 years) were examined separately. Laboratory and anthropometric data were obtained from electronic medical records, body mass index (BMI), and standard-deviation-score (SDS) for BMI was calculated using age- and sex-specific BMI growth charts according to local Polish OLAF study (18).

The diagnosis of T1D was made on the basis of criteria for diabetes type 1 recognition according to the International Society for Pediatric and Adolescent Diabetes guidelines: history of polyuria, polydipsia, and weight loss with an elevated random plasma glucose of ≥11.1 mmol/L or fasting plasma glucose of ≥7 mmol/L (19). Diabetic autoimmunity was confirmed on the basis of at least one positive titer of autoantibodies to islet cells (ICAs), glutamic acid decarboxylase (GADA), and also the protein tyrosine phosphatase (IA2). Analysis was performed in the same laboratory for all the study period. The diagnosis date referred to the date of the very first injection of insulin, according to EURODIAB criteria (20), and our previous publications. The

age of the patients was calculated in completed years on the day of T1DM diagnosis (21, 22).

Serologically screening tests for thyroid autoimmunity included anti-thyroid peroxidase antibodies (aTPO) and antithyroglobulin (aTg), and, in cases of hyperthyroidism suspicion, anti-thyroid stimulating hormone (TSH) antibodies (TR-Ab). Confirmation of thyroid autoimmunity was based on the elevated titer of aTPO, aTg, or TR-Ab. Both hypothyroid and hyperthyroid autoimmunity was taken into consideration. Children were screened for the thyroid diseases using TSH and free thyroxine (fT4) levels at the moment of diagnosing. Furthermore, in each case of newly diagnosed T1D, according to our local recommendations, thyroid ultrasound has been performed since 2016. The clinical entities found in AITDs are diverse and vary depending on whether it is in a state of autoimmune hypothyroidism (HT, Hashimoto disease) or hyperthyroidism (GD). Patients suffering from HT represented clinical and biochemical characteristics of hypothyroidism and demonstrated an elevated TSH with presence of elevated anti-TPO and/or aTg autoantibodies. Graves disease was diagnosed in children with large goiter, hyperthyroidism in laboratory tests and positive thyrotropin receptor (TR-Ab) antibodies, anti-TPO antibodies, and aTg antibodies. Subclinical hypothyroidism, without confirmed autoimmunity, was not taken into consideration into further analyses. The diagnosis of AITDs was set on the basis of clinical, laboratory, and ultrasound investigations and was always set with experienced pediatric endocrinologist, and pharmacological treatment was introduced if needed.

To diagnose celiac autoimmunity, anti-tissue transglutaminase (anti-tTGA) antibodies were performed. With titer of $\geq$10 IU/mL or with questionable results, anti-endomysial (EMA) and anti-reticulin (ARA) antibodies were also implemented. In this case, small intestine–associated autoimmunity was recognized. The CD was diagnosed on the basis of the revised criteria of CD diagnosis according to the European Society Pediatric Gastroenterology, Hepatology, and Nutrition criteria (21, 23) and always consulted with experienced gastroenterologist. Patients positively serologically tested for tTGA, EMA, and ARA had a gastroscopy performed if needed for diagnosis. Children recognized with the disease started gluten-free diet since diabetes onset. We did not test our patients for Addison disease because it is out of obligatory screening in Poland. Yet, none of the children represented clinical symptoms of this or any other ADs that may accompany T1D (gastritis, vitiligo, etc.).

Laboratory Analyses

The analyses were performed with routine laboratory methods in hospital laboratory on an ongoing basis. Fasting blood sample for analysis was collected in the morning. Serum levels of TSH, fT4, and triiodothyronine (fT3) were assessed with the use of electrochemiluminescence "ECLIA" with Cobas E411 analyzer (Roche Diagnostics, Warsaw, Poland). Values within the norm presented the range between 0.28 and 4.3 μIU/L for TSH, between 1.1 and 1.7 ng/dL for fT4, and between 2.6 and 5.4 ng/dL for fT3. Anti-TPO, aTg, and TR-Ab antibodies were analyzed in all samples with the use of ECLIA with Modular Analytics E170

TABLE 1 | General characteristics of the study group.

Parameters at diagnosis	No. of valid results	% of valid results	Median [interquartile range]	Minimal value	Maximal value
Age at diagnosis (years)	493	100.00	9.50 [5.50–12.50]	0.50	18.00
0–4			*n* = 109		
5–9			*n* = 151		
10–14			*n* = 172		
15–18			*n* = 61		
HbA_{1c} (%)	369	75	10.70 [9.46–12.42]	6.00	21.0
pH	487	99	7.37 [7.28–7.41]	6.93	7.64
HCO_3	220	45	16.60 [8.50–20.95]	2.60	43.4
C-peptide (ng/mL)	295	60	0.47 [0.28–0.79]	0.00	1.1
GADA (U/mL) [86.82%—positive]	433	88	63.20 [8.67–280.03]	0.27	5,775
IA2A (U/mL) [86%—positive]	434	88	188.05 [8.16–777.51]	0.00	3,626
ICA (JDF U) [64.91%—positive]	431	87	20.00 [10.00–40.00]	0.00	640
TPO (IU/mL)	474	96	5.00 [5.00–6.90]	5.00	600.00
ATG (IU/mL)	474	96	10.00 [10.00–10.00]	3.15	4,000.00
TTGA (UI/mL)	461	93	10.00 [10.00–10.00]	0.4	1,700
BMI	470	95	15.7 [14.2–18.1]	8.9	35.01
SDS-BMI	429	87	−0.43 [−1.08 to 0.29]	−3.40	5.07
Gender (%), *n*	Boys: 53.55% *n* = 264			Girls: 46.45% *n* = 229	

analyzer (Roche Diagnostics). The positive values for antithyroid antibodies were >1.75 U/L for TR-Ab, >34 IU/mL for anti-TPO-Abs, and >115 IU/mL for aTg-Abs. Tests for beta cells and thyroid and celiac antibodies were performed after recovery from ketoacidosis and the initiation of subcutaneous insulin therapy, usually between the fifth and the seventh day of the child's stay in the ward (in our hospital, hospitalization usually lasts 8–10 and is related to patient's education).

The study was performed in accordance with the Guidelines of Good Clinical Practice. The protocol was approved by the Medical University in Białystok, Poland Bioethical Committee. Parents/legal guardians and their children both provided their written informed consent. The study adhered to ethical standards including ethics committee approval and consent procedure. Also, standard biosecurity and institutional safety procedures were adhered to.

Statistical Analysis

The IR of the T1D was calculated as the total number of all cases that were newly diagnosed per 100,000 population people at 0- to

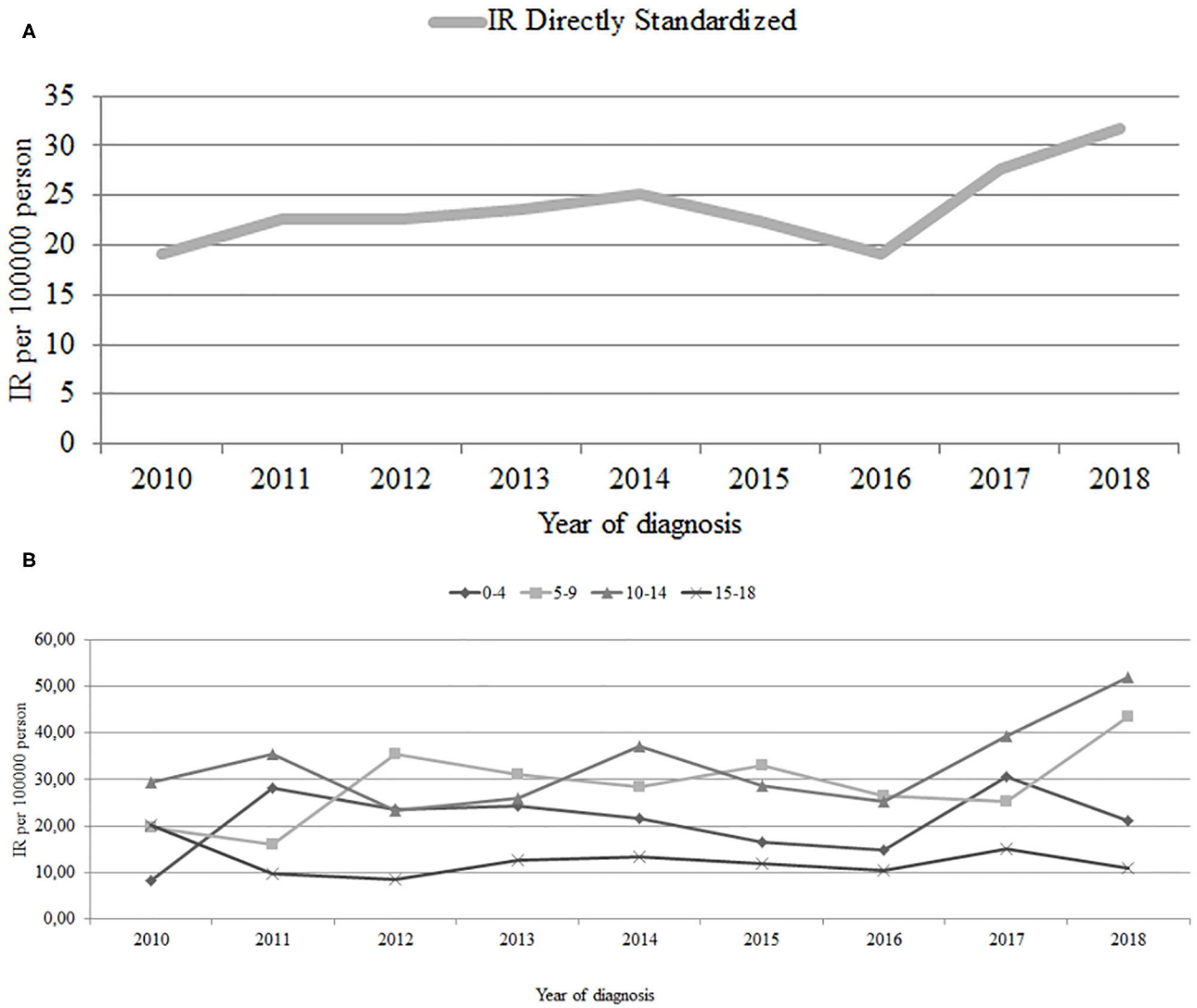

FIGURE 1 | (A) T1D IR Directly Standardized in years 2010–2018 in all age groups (0–18 y.o.) A *incidence rate directly standardized* is the number of new cases occurring in a specified population during a year, expressed as the number of cases per 100,000 population of Podlaskie Voivedeship aged 0–18 yrs, standardized to the age-matched population of Poland from 2010. Slope = 0.948, $P = 0.057$. **(B)** T1D incidence rate (IR) in years 2010–2018 in certain age groups. A *crude incidence rate* is the number of new cases occuring, in a specified population during a year, expressed as the number of cases per 100,000 population of Podlaskie. Voivodeship in these age groups.
0–4 y.o. slope = 0.561, $p = 0.566$.
5–9 y.o slope = 1.78, $p = 0.095$.
10–14 y.o slope = 1.82, $p = 0.127$.
15–18 y.o slope = 1.78, $p = 0.521$.

18-year age per year. A multifactorial Poisson regression model was made to assess the IRR, depending on age and year. For the needs of the model for age, dummy variable was created, in which the 0- to 4-year age group is the base (reference) variable. Model fit was assessed using the Pearson goodness-of-fit test. One-way linear regression models were used in the time trend analysis. For IRR by age group and year, exact Poisson 95% confidence intervals (CIs) were calculated. The IRs were directly standardized by age according to the population of Poland 2010. The prevalence of T1D and additional AID was evaluated using χ^2 or Fisher exact test when appropriate. A multiple logistic regression analysis was used to recognize independent factors and their influence on additional autoimmunity prevalence. The odds ratios with 95% CIs were calculated. p-value lower than 0.05 was determined as statistically significant. The results are presented as mean ± SD, or numbers (n), and percentages (%). Statistically significant results were found at $p < 0.05$. The data were calculated using Stata/IC 12.1 package from StataCorp LP, College Station, TX, USA and STATISTICA 13.0 software, StatSoft Polska Sp. z o.o. Kraków, Poland.

RESULTS

We analyzed the data from total 493 pediatric patients (264 male and 229 female) aged 0 to 18 years [median (interquartile range) of age at diagnosis 9.50 (5.50–12.50) years], diagnosed successively between 2010 and 2018. During the study period, 23 children were diagnosed with other types of diabetes (11 with maturity-onset diabetes of the young 2, six with type 2, and six were insulin dependent, but autoantibody negative—not included into the present analysis as T1D). Two of our patients were diagnosed with AITD, and one, CD before the diagnosis of T1D. The general characteristics of the studied group are presented in **Table 1**. Mean IR of T1D among children and adolescent patients during the study period was 23.78/100,000 age-matched population. We found that incidence of diabetes mellitus type 1 in Podlaskie Voivodeship keeps rising. During the time of the study, the age-standardized annual incidence increased from 19.2/100,000 in 2010 up to 31.7/100,000 in 2018 over 9 years, in total 1.7-fold in the observed period. Annual fluctuations, with the lowest IR in 2016, were noticed. Time trend analysis revealed increase in incidence ratio (slope 1.06, $p = 0.039$), although statistically insignificant when standardized for age ($p = 0.057$) (**Figure 1A**). The increase in the IRR was 4% per year.

We divided the studied population into certain age groups and assessed the IR one by one. We discovered the growth in three of the age groups (from 8.22 to 21.08 in group 0–4 years old; from 19.61 to 43.45 in group 5–9 years old; from 29.26 to 52.01 in group 10–14 years old) and decrease in the oldest (15–18 years) age group of patients, from 20.16 to 10.95. The overall upward trend in incidence of T1DM seems to come from the increase in groups 5–9 years old and 10–14 years old, in which the rise in IR was the greatest. The lowest mean IR was found in group 15–18 years old (12.52/100,000), and the highest IR was noticed in group 10–14 years old (32.89/100,000). In other age groups, it was, respectively, 20.95 in group 0–4 years old and 28.78 in group 5–9 years old. In the time trend analysis, we did not prove significant differences when selected age groups were studied (**Figure 1B**). The Poisson regression model adjusted for the year of diagnosis and age group demonstrated the incidence risk ratio increasing 1.37 in children aged 5–9 years, 1.57 for children aged 10–14 years, and 0.61 for children aged 15–18 years. The year of diagnosis increased the IRR 1.04 times (4%) (**Table 2**). The differences in the standardized incidence ratios of diagnosed T1D cases between male and female were not statistically different and are presented in **Table 3**. In **Supplementary Table 1**, we included the data of incidence ratios with 95% CI in separated age groups in every studied year.

Next, we assessed the prevalence of additional autoimmunity and AD in all new-onset cases of T1D in subsequent years of analysis. In total, thyroid autoimmunity was recognized in 66 patients (13.39%), and AITD was recognized in 37 patients (7.51%) during the study period. Celiac autoimmunity was recognized in 38 patients (7.7%), whereas CD was found in 26 patients (5.3%). We found that general specific autoimmunity (other than diabetic antibodies) at T1D diagnosis remained stable during the study period (from 25% of patients in 2010 up to 23.6% in 2018; $\chi^2 = 1.2$ $p = 0.26$). As for AADs (celiac and AITD), their prevalence fluctuated during the study: it started from 10.4% in 2010, next increased in years 2012–2014, and then fell in years 2015–2017 to eventually finish at 20.83% in 2018. In

TABLE 2 | Model of Poisson regression for IRR based on a group of 0–4 years old.

Age group (years)	IRR (95% CI)	*P*
0–4	Base	
5–9	1.37 (1.06–1.75)	0.013
10–14	1.57 (1.23–1.99)	0.000
15–18	0.61 (0.44–0.83)	0.002
Year	1.04 (1.00–1.07)	0.021

Pearson goodness of fit = 33.0039.

TABLE 3 | Age-standardized incidence rates (per 100,000 person-years) of type 1 diabetes according to gender.

	IR (95% CI) age standardized		
Year	Girls	Boys	Total
2010	17.5 (9.8–24.4)	21.2 (13.2–29.2)	19.2 (13.7–24.6)
2011	24.2 (15.2–22.2)	21.3 (13.1–29.4)	22.7 (16.6–28.8)
2012	20.9 (12.5–29.3)	24.2 (15.4–33)	22.6 (16.5–28.7)
2013	21.3 (12.6–29.6)	25.5 (16.4–24.6)	23.5 (17.2–29.7)
2014	23.3 (14.2–32.2)	26.9 (17.4–36.4)	25.1 (15.6–31.7)
2015	19.9 (11.5–28.3)	24.7 (15.7–33.8)	22.4 (16.2–28.3)
2016	21.6 (12.9–30.2)	16.7 (9.2–24.3)	19.1 (13.20–24.8)
2017	29.6 (19.2–39.9)	25.9 (16.5–35.4)	27.7 (20.7–34.7)
2018	25.2 (15.9–34.6)	37.9 (26.7–49.2)	31.7 (24.4–39.1)

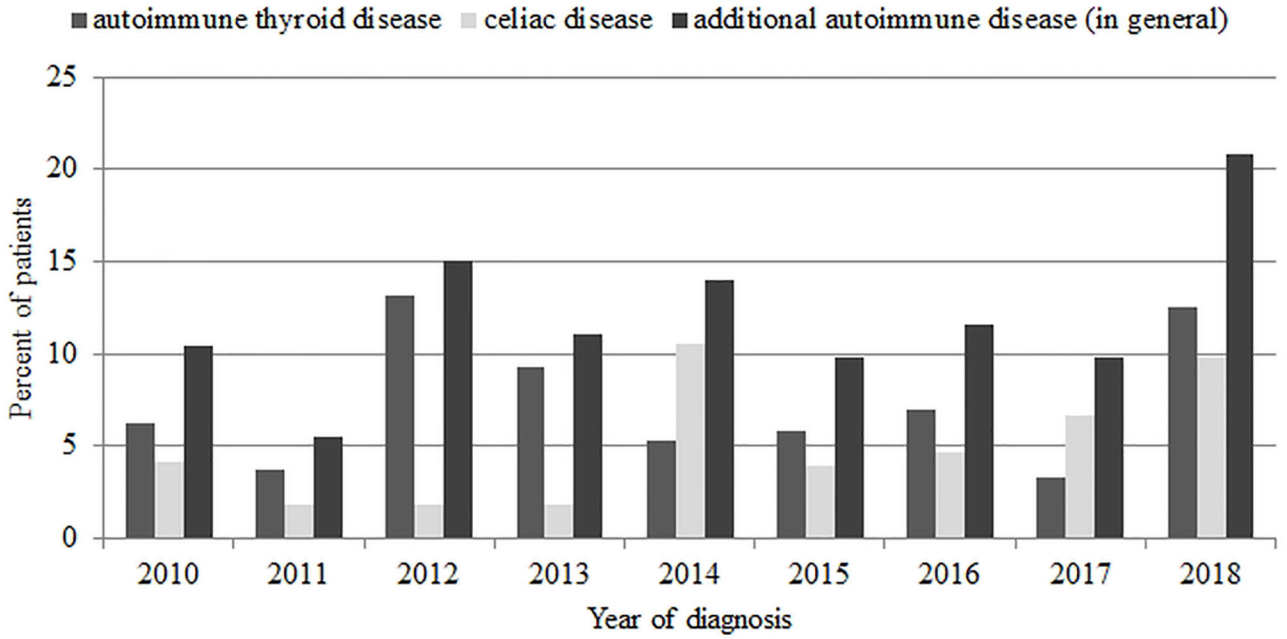

FIGURE 2 | Prevalence of additional autoimmune diseases (autoimmune thyroid disease and celiac disease) in new-onset type 1 diabetes mellitus patients.

TABLE 4 | Other organ autoimmunity and autoimmune diseases among DMT1 patients aged 0–18 years diagnosed in years 2010–2018.

Annual distribution												
Year	**Total**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**χ^2 and *p*-value total**	**χ^2 and *p*-value 2010 vs. 2018**
T1D, *n*	493	48	54	50	54	57	51	43	61	72		
TAI, *n*	66	8	10	9	8	6	6	4	5	10	$\chi^2 = 5.2$	$\chi^2 = 2.3\ p = 0.12$
(%)	(13.5)	(16.6)	(18.5)	(18.0)	(14.8)	(10.5)	(11.8)	(9.3)	(8.2)	(13.8)	$p = 0.7$	$Y = 1.6\ p = 0.19$
												Fi $p = 0.09$
TTGA, *n*	38	5	7	1	3	6	3	2	4	7	$\chi^2 = 7.8$	$\chi^2 = 0.4\ p = 0.50$
(%)	(7.8)	(10.4)	(13.7)	(1.9)	(5.6)	(10.5)	(5.9)	(4.6)	(6.5)	(9.8)	$p = 0.45$	$Y = 0.09\ p = 0.70$
												Fi $p = 0.30$
AI, *n*	99	12	15	10	10	11	9	6	9	17	$\chi^2 = 5.7$	$\chi^2 = 1.2\ p = 0.26$
(%)	(20.1)	(25)	(27.7)	(18.8)	(18.5)	(19.3)	(17.6)	(13.9)	(14.7)	(23.6)	$p = 0.68$	$Y = 0.7\ p = 0.37$
												Fi $p = 0.18$
AITD, *n*	37	3	2	7	5	3	3	3	2	9	$\chi^2 = 8.7$	$\chi^2 = 1.2\ p = 0.20$
(%)	(7.5)	(6.25)	(3.7)	(13.2)	(9.3)	(5.3)	(5.9)	(7)	(3.3)	(12.5)	$p = 0.36$	$Y = 0.6\ p = 0.40$
												Fi $p = 0.20$
CD, *n*	26	2	1	1	1	6	2	2	4	7	$\chi^2 = 10.4$	$\chi^2 = 1.3\ p = 0.20$
(%)	(5.3)	(4.2)	(1.8)	(1.9)	(1.8)	(10.5)	(3.9)	(4.6)	(6.7)	(9.8)	$p = 0.2$	$Y = 0.6\ p = 0.42$
												Fi $p = 0.21$
AAD, *n*	61	5	3	8	6	8	5	5	6	15	$\chi^2 = 8.5$	$\chi^2 = 2.7\ p = 0.09$
(%)	(12.4)	(10.4)	(5.6)	(15.1)	(11.1)	(14.0)	(9.8)	(11.6)	(9.8)	(20.8)	$p = 0.38$	$Y = 1.9\ p = 1.50$
												Fi $p = 0.07$

n, no. of patients; X^2 - Chi-squared; Y, Yates correction; Fi, Fisher exact test; T1D, type 1 diabetes mellitus; TAI, thyroid-positive autoimmunity; TTGA, anti-tissue transglutaminase antibodies positive; AI, autoimmunity (thyroid + celiac); AITD, autoimmune thyroid disease; CD, celiac disease; AAD, additional autoimmune disease (total).

the end, this gives a 2-fold increase in the percentage of patients with AADs during the 9-year study period (**Figure 2**, **Table 4**).

In further analysis, considering the frequency of autoimmunity in T1D age groups, we did not show statistically significant differences ($\chi^2 = 1.7$, $p = 0.63$). As for AITD, it was similar; we found higher prevalence of these diseases in the oldest age group, although it was not statistically significant ($\chi^2 = 7.1$, $p = 0.06$) (**Figure 3**). The detailed comparison of the differences in mean age between additional autoimmunity "positive" and "negative" patients is presented in **Table 5**. We

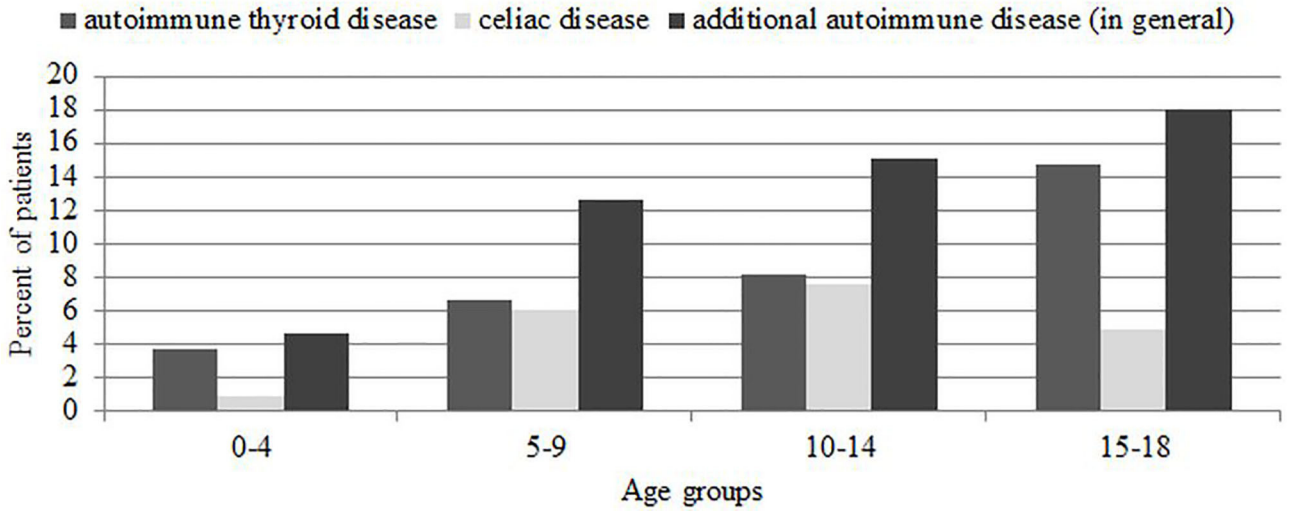

FIGURE 3 | Prevalence of autoimmune thyroid disease and celiac disease in new-onset T1D. Comparison between age groups.

TABLE 5 | Mean age at diagnosis of T1D in the groups of "positive" and "negative" patients in terms of total autoimmunity, AITD, thyroid autoimmunity, celiac disease, TTGA autoimmunity, and additional autoimmune disease.

	Additional autoimmunity "positive" patients	Additional autoimmunity "negative" patients	*p*-value
	Mean age		
Autoimmunity	10.13 ± 4.42	8.80 ± 4.58	0.009
AITD	11.14 ± 4.33	8.91 ± 4.56	0.004
Thyroid autoimmunity	10.14 ± 4.68	8.92 ± 4.55	0.040
Celiac disease	10.69 ± 3.52	8.98 ± 4.62	0.064
TTGA autoimmunity	10.03 ± 4.29	9.02 ± 4.59	0.203
Additional autoimmune disease	10.70 ± 3.96	8.85 ± 4.62	0.004

found that patients with additional autoimmmune problem, especially thyroid disease, were significantly older. Thyroid autoimmunity varied significantly between male (9.13%) and female (18.5%) ($\chi^2 = 9.2$, $p = 0.002$), as well as thyroid AD (male 4.55%, female 10.92%, $\chi^2 = 7.2$, $p = 0.007$) (**Figure 4**). Such differences were not found for CD.

Finally, we analyzed the prevalence of AAD according to the presence of GADA antibodies. We discovered that children with positive anti-GAD antibodies were much more likely to develop thyroid autoimmunity at diagnosis of T1D than patients with negative anti-GAD, and this difference was observed as statistically significant ($p = 0.024$). We also obtained a significant difference in frequency of AITD between both groups ($p = 0.045$). As presented in **Figure 5**, in GADA(+) patients, 8.54% had AITD, and 14.33% had thyroid autoimmunity at diagnosis of T1D, whereas in GADA(–) patients, it was 3.42 and 6.9%, respectively, $p < 0.05$ (**Figure 5**). We have also assessed the possible relationship between the age of patients and the titers of GADA, ICAs, IA2, and antithyroid antibodies without showing a statistically significant difference (Student *t*-test; data not shown).

Furthermore, we performed a multiple logistic regression analysis, which showed that female gender ($p = 0.01$), year of onset ($p = 0.01$), and GADA positive ($p = 0.04$) influenced the presence of additional thyroid autoimmunity at diabetes type 1 onset. In this analysis, we did not confirm the independent influence for the age at onset. As for AITD, the independent influencing factors were female gender ($p = 0.03$), GADA-positive antibodies ($p = 0.01$), and age at onset ($p = 0.01$) (**Table 6**).

Autoimmune hyperthyroidism was found in three of our patients. Their cases were included into the AITD group. Four of the children had developed both CD and AITD; they were treated separately in the analyses. None of our patients represented clinical symptoms of any other autoimmune disorder.

DISCUSSION

In our study, we presented high, in our opinion, overall prevalence of AADs (thyroid and celiac) in children and adolescents who newly received the diagnosis of T1D. Our results showed increasing T1D IRs over the observed years, accompanied with growing prevalence of other coexisting ADs, with the 2-fold increase in AITD. To the best of our knowledge, this work represents the first report considering the epidemiological trends and distribution by demographic data (gender and age) of the most common AAD—AITD and CD—among newly diagnosed T1D pediatric patients. Therefore, we believe that our study may provide new insight into the relation of T1D and other autoimmunities in pediatric population. Moreover, we reported significant association between specific diabetic autoimmunity (GADA) and female preponderance with increased prevalence of thyroid autoimmunity and AITD. Last

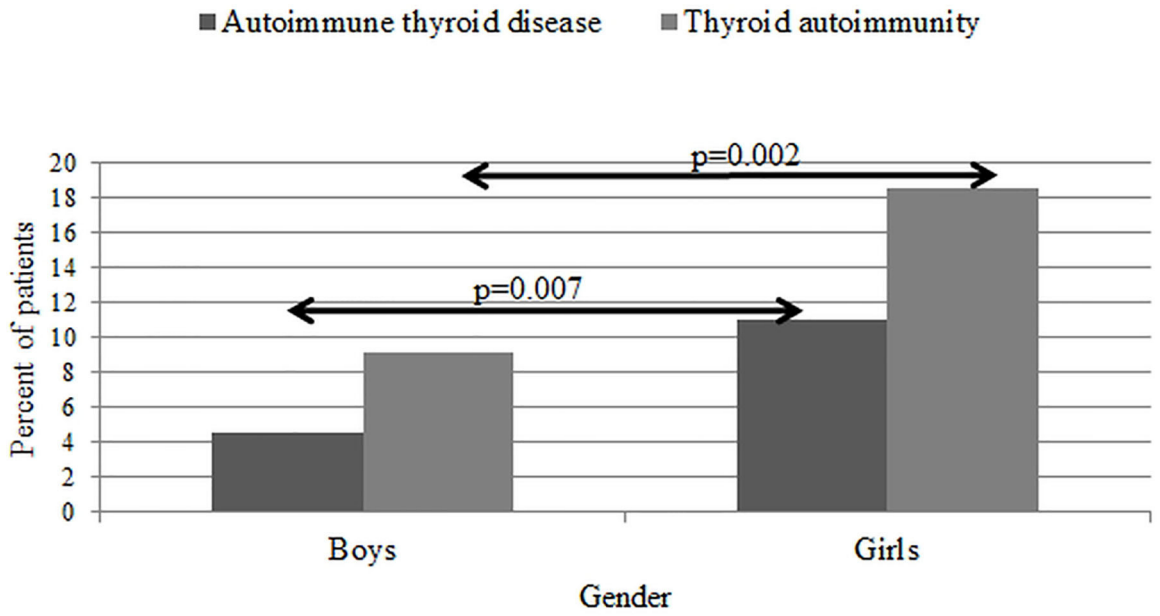

FIGURE 4 | Prevalence of patients with thyroid autoimmunity and autoimmune thyroid disease at diabetes type 1 diagnosis.

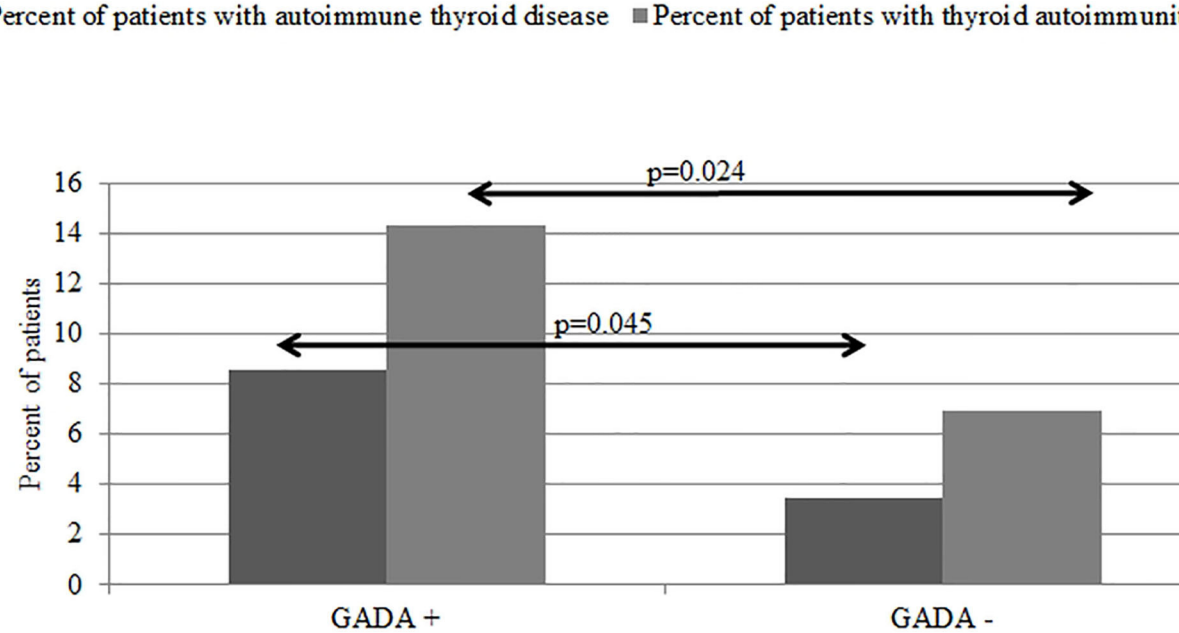

FIGURE 5 | Prevalence of autoimmune thyroid disease and thyroid autoimmunity in patient with positive or negative GADA autoantibodies.

but not least, we observed a trend for increase of AAD at T1D onset with the older age at diagnosis.

In our present results, we observed increasing T1D incidence over the observed 2010–2018 years; the overall IRR amounted to 4% a year (1.7-fold), and we noticed the highest IR in children aged 10–14 years. According to the latest data, incidence of T1D is increasing irrespectively of the genetics characteristic for the region and geographical location. Numerous research tried to find demographic trends concerning age and gender (6, 24). The recent literature emphasizes the trend of increased prevalence of T1D among all age groups of people (25–27). Observed worldwide IR of T1D incidence in children is increasing by 2–5% annually. Nevertheless, IRs vary among regions of the world. In Asia, the incidence of T1D is very low: China (Shanghai), 3.1 per

TABLE 6 | Independent factors influencing additional thyroid autoimmunity and thyroid disease at diabetes type 1 onset in a multiple logistic regression analysis.

	OR	95% CI	*p*-value
Additional thyroid autoimmunity			
Gender	1.9	(1.1–3.4)	0.01
Year of onset	0.9	(0.7–1.0)	0.01
GADA antibodies	1.0	(1.0–1.0)	0.04
Age at onset	1.2	(0.9–1.6)	0.09
Autoimmune thyroid disease			
Gender	1.9	(0.9–4.0)	0.03
Year of onset	1.0	(0.9–1.1)	0.4
GADA antibodies	2.1	(0.7–6.1)	0.01
Age at onset	1.6	(1.1–2.3)	0.01

CI, confidence interval; OR, odds ratio.

100,000 (28). Some research was made, looking for a possible link between these differences in IR and HLA specific to Caucasians and Asian populations (29, 30). In Europe, newly diagnosed cases of T1D are increasing by 3.4% per year (25). According to the large nationwide studies, in Poland, incidence of T1D is also increasing year after year (6). In the previous study considering our region, the lowest IR was observed in the youngest group of children aged (0–4 years), whereas the highest IR was identified in older children (10–14 years) (21). American SEARCH for Diabetes in Youth, a multicenter study of American population, also confirmed this phenomenon (an incidence of 33.9/100,000 between 10 and 14 years old, regardless of ethnic group and region of residence). The researchers link this to an increase in insulin resistance during puberty and pathogenic impact of infections (31).

One of the main findings of our study is the general increase in prevalence of AAD at T1D onset. During the study period, the percentage of patients diagnosed with CD and AITD doubled, with the greatest rise between 2017 and 2018. Numerous studies reported that incidence and prevalence of autoimmunity and AID in diabetic patients increase with age and are more common in females (32, 33), what we also confirmed in our research. Although statistically significant differences were noted only regarding AITD and thyroid autoimmunity, for autoimmunity connected to CD, we observed only percentage differences.

The frequency of AITD in a combined population of Europe is calculated as 3% for hypothyroidism and 0.75% for hyperthyroidism (34). Autoimmune thyroid disease affects as much as up to 3% of the pediatric population, so it represents the example of the most common ADs (9). In T1D, patients' reported weighted mean prevalence of hypothyroidism was 9.8%, whereas in hyperthyroidism it was 1.3% according to one of the recent meta-analyses (35), so it is significantly increased compared to general population. What is more, we could speculate that if prevalence of AITD increases during the disease (36), and we noticed incidence >20% at diagnosis in 2018, the prevalence of AITD in children diagnosed in 2018 may in the further course of the disease exceed the peak value of 30% reported by some studies (37).

The presence of thyroid antibodies among new-onset T1D patients occurs to be a common phenomenon, which may be significant for predicting the possible clinical manifestation of AITD at the same time of setting the T1D diagnosis (37). According to a previously conducted study (38), thyroid function at T1D onset is largely affected by metabolic derangement, and the assessment of thyroid antibodies may be valuable in recognizing patients prone to developing hypothyroidism. Our findings provide further evidence that the highest number of patients diagnosed with AITD was observed among teenagers (15–18 years). We also found a solid female predominance in both thyroid autoimmunity and AITD at diagnosis of T1D. Our results are consistent with existing studies that notice thyroid autoimmunity more often in girls (33, 39) and increase by 4.6% in prevalence of hypothyroidism (including subclinical cases) for every 10-year increase of age in T1D and with diabetes duration (15). These findings correlate with overall trends of thyroid autoimmunity incidence in other countries (39, 40) and also in Poland (41). Contrary to our findings, the meta-analysis based on 14 studies shows that the risk of thyroid dysfunction is much higher in children in comparison to adults (42).

Another very interesting finding of our study is the correlation between GADA and thyroid autoimmunity. In our study, we found vital correspondence between the presence of GADA antibodies and both clinically manifested thyroid AD and asymptomatic thyroid autoimmunity. Our results are consistent with previously published data (39, 43). Glutamic acid decarboxylase antibodies are found in ∼70–80% of patients who suffer from T1DM, long before the onset of clinical symptoms, and they remain positive over a long time after. The GADA presence is linked positively to gender (female) and older age at the time of diabetes onset (44). The association between GADA and TPOAb was previously observed. Glutamic acid decarboxylase in patients with T1D not only could indicate higher risk of more severe course of T1D but also could be a marker that takes part in reflecting other endocrine autoimmunity such as thyroid autoimmunity (39).

Deficiency of thyroid hormones, even subclinical, may lead to serious complications. Because of their influence on heart, vessels, and adipose tissue function, they play an important role in atherosclerotic processes (45, 46). Moreover, a possible link was found between subclinical hypothyroidism and cardiovascular risk factors and heart failure in adult patients (47). Despite the fact that similar conclusions have not been confirmed in pediatric patients yet, several studies have found some subtle proatherogenic abnormalities in children with a slight increase in TSH levels, showing improvement after levothyroxine treatment (45). All the facts mentioned above together with the results of our analyses confirm the need for screening and early treatment for AITD among T1D patients.

Celiac disease perception by clinicians has changed remarkably over past 50 years (48), and it became to be known as chronic, food-induced autoimmune disorder that is common and is diagnosed worldwide in patients of any age. Celiac disease keeps increasing in general population over last decades (49, 50). Some studies suggest that accuracy in diagnosis of CD (51) depends on the criteria used for selection of patients for the biopsy (the percentage of patients diagnosed increased

with the percentage of patients qualified for biopsy) (35). Celiac disease, when untreated, could lead to malnutrition and also extraintestinal manifestations of this disease: impaired bone density and stunted growth in children, liver failure, or even infertility (52, 53). Celiac disease, except for the aforementioned complications, may lead to increased risk of occurrence of other disorders. A retrospective study reported that patients who were on a gluten-free diet for a long time developed 50% fewer number of ADs during the period up to about 15 years of follow-up (54). Because of this, screening, possibly early identification and diagnosis are undoubtedly vital. The strong association of CD and AITDs was widely described in literature (55, 56). In patients with T1D, prevalence of CD is higher (4.7%) than in general population (1.4%) (35). Similarly to these results, prevalence of CD was increased among our patients, and the growth trend over the years was also noted.

Nowadays, there is a great effort in research trying to find the possible reasons for increased and multiple autoimmunity in T1D young patients (35). One of the possible explanations could be a common genetic background and defective immunoregulation (44). As for a shared genetic background, we can observe familial aggregation of ADs. It is widely known that some haplotypes of HLA predispose to both T1D and other ADs, i.e., HLA-DQ2, DQ4, and CD (57, 58). Also both T1D and AITD represent similar susceptibility gene polymorphisms, with HLA and non-HLA variants, which may cause such a clustering (59).

The TEDDY Study Group has already assessed a number of environmental candidate triggers, which include probiotics, infections, micronutrient, and microbiome, which could be responsible for recent increase in AD incidence (60). Other possible factors are altering the balance of gut microbiota due to probiotic or antibiotics use (61, 62) and epigenetic changes induced by air pollutants (63).

The increase in incidence of ADs over the last decades is accompanied by the outbreak of obesity. Complex interplay between the metabolic and immune processes is not fully understood, but may interact in developing various disorders (64). However, there are researches describing a visible correlation between obesity and greater prevalence or a worse prognosis of numerous immune-mediated conditions. In our study, the trend in annual changes of SDS-BMI was not considered, unfortunately. Numerous researches have described the properties of white adipose tissue as a crucial site in the dissolvable mediators generation defined as "adipokines" that in majority carry a proinflammatory activity. These adipokines occur to be the link between immune system and adipose tissue (65).

LIMITATIONS OF THE STUDY

The first and foremost limitation to our study is the retrospective design, the improvement over the years in the diagnosis of many conditions, and not testing specifically for other autoimmune conditions. Second is the fact that data were sourced from electronic medical records. Tests screening for ADs other than those mentioned in the study were not performed because of lack of specific guidelines and lack of symptoms manifested by patients accounted for in the study. Controversial finding of our study is that we noticed a lack of simultaneous significant increase in prevalence of laboratory-recognized autoimmunity. It may be caused by growth in the amount of currently unknown factors triggering the development of AD in patients with present autoimmunity. We must also admit that, over the observed years, the diagnostic methods improved, ultrasonography of the thyroid gland was introduced into routine diagnostic method in all T1D patients, and diagnostic criteria for CD changed as well. These factors may have influence on this discrepancy. Further analysis of data gathered by us is required in order to identify factors possibly responsible for the observed phenomenon.

CONCLUSIONS

The IRR of T1D in children increased 4% a year, and the standardized IR increased 1.7-fold over the 9-year observation period. Additional autoimmunity represents a significant comorbidity in patients with new-onset T1D. The number of children diagnosed with AADs that accompany T1D is rapidly growing in all age groups throughout recent years. The most prone group of patients occurred in girls, older children, and patients who tested positive for GADA antibodies. Thus, more attention should be paid on subjects with other coexisting AD since T1D onset, and we hope that our work will contribute to greater emphasis on monitoring this problem. We believe that getting the comprehensive knowledge on this epidemiological problem with great clinical impact will help to establish better interdisciplinary treatment approach.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical University Bioethical Committee (no of approval: APK.002.112.2020). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BG-O made substantial contributions to study design and conception, acquisition, analysis and interpretation of data, and wrote the paper. PP and KZ partially gathered the data, analyzed and interpreted it, and co-wrote the paper. MS prepared the figures, partially gathered the data, analyzed and interpreted it, and co-wrote the paper. MJ-S made substantial contributions to study conception and design, acquisition, analysis, and interpretation of data. AM performed and interpreted statistical analyses. AK, AP, and WŁ were involved in the critical revision for important intellectual content. AB was involved in the design, conception, analysis, and revised the paper. All authors

contributed in discussions, read, and approved 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/fendo.2020.00476/full#supplementary-material

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REVIEW
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Autoimmune Thyroid Disease in Specific Genetic Syndromes in Childhood and Adolescence

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Autoimmune thyroid disease (ATD) is the most frequent cause of acquired thyroid dysfunction, most commonly presenting either as Hashimoto's thyroiditis or Graves' Disease. Hashimoto's thyroiditis is characterized by the presence of thyroid-specific autoantibodies, more commonly anti-thyroperoxidase antibodies in the serum and the typical inhomogeneous echostructure of the thyroid on a thyroid ultrasound examination. Hashimoto's thyroiditis can for a long time be accompanied by normal thyroid function and hypothyroidism can only progressively be established. Graves' disease is much less frequent in childhood and adolescence and presents with overt hyperthyroidism. After the onset of puberty, ATD affects females with a higher incidence than males, while during the prepubertal period there is not such a clear preponderance of affected females. ATD can occur either isolated or in the context of other autoimmune disorders, such as type 1 Diabetes mellitus (T1D), celiac disease, alopecia areata, vitiligo, etc. Especially at the pediatric age, a higher incidence of ATD is also observed in the context of specific genetic syndromes, such as trisomy 21 (Down syndrome), Klinefelter syndrome, Turner syndrome, or 22q11.2 deletion syndrome. Nevertheless, although thyroid dysfunction may also be observed in other genetic syndromes, such as Prader-Willi or Williams syndrome, the thyroid dysfunction in these syndromes is not the result of thyroid autoimmunity. Interestingly, there is emerging evidence supporting a possible link between autoimmunity and RASopathies. In this review article the incidence, as well as the clinical manifestation and accompanied pathologies of ATD in specific genetic syndromes will be presented and regular follow-up for the early identification of the disorder will be proposed.

Keywords: autoimmune thyroid disease, Hashimoto, genetic syndromes, Turner syndrome, Down syndrome, Klinefelter syndrome

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INTRODUCTION

Autoimmune Thyroid Disease in Children and Adolescents

Autoimmune thyroid disease (ATD) is the most common thyroidopathy in children and adolescents. It comprises two main entities, Hashimoto's Thyroiditis (HT) and Graves' Disease (GD) and a wide spectrum of clinical presentations, ranging from Hashimoto's overt or subclinical hypothyroidism, euthyroidism, to Graves' subclinical or overt hyperthyroidism. Conversion of hyperthyroidism to hypothyroidism and vice versa may also occur, implying that these two

disorders may represent opposite sides of the same coin. ATD results from the complex interplay between genetic, environmental and endogenous factors leading to loss of self-tolerance to thyroid antigens. Both cell-mediated and humoral immune responses are implicated in the pathogenesis of ATD. Activation of T- and B cell pathways drives the infiltration of the thyroid by autoreactive lymphocytes and the production of antibodies against three main thyroid antigens, thyroid peroxidase (TPO), thyroglobulin (TG), and thyroid-stimulating hormone receptor (TSHR). While in HT the autoimmune processes lead to apoptosis and destruction of the thyroid follicles, and subsequently hypothyroidism, in GD the immune-mediated activation of TSHR-reactive B cells results in the production of stimulating TSHR antibodies which in turn induce thyroid cell proliferation and function, manifested as hyperthyroidism (1, 2). Around 70% of the risk for developing ATD can be ascribed to genetic factors. It is noteworthy, that thyroid autoantibodies can be found in ∼50% of first-degree relatives of patients with ATD (3). Some of the genes that have been shown to confer susceptibility to ATD include (1) immune modulating genes: Human leukocyte antigen/HLA-DR, cytotoxic T-lymphocyte-associated protein 4/CTLA-4, cluster of differentiation 40/CD40, protein tyrosine phosphatase, non-receptor type 22 (lymphoid) PTPN22 and (2) thyroid specific genes: TG and TSHR (1, 4). Moreover, hormones, mainly estrogens, as also observed during pregnancy, stress, smoking, iodine, infection, drugs (lithium, amiodarone, interferon-alpha), radiation and exposure to environmental toxins may also contribute to the occurrence of ATD in genetically susceptible individuals (1, 5).

The frequency of HT in the pediatric age ranges between 0.3 and 9.6% (around 3%), occurring rarely before the age of 3 years and reaching a peak in early to mid-puberty. There is a strong female preponderance with a female-to-male (F/M) ratio varying across studies between 2:1 and 9.7:1 (6–8). This female predominance is less pronounced in prepubertal children (F/M ratio: 1.6), suggesting the influence of sex hormones in the development of ATD (9). Diagnosis is established by detecting positive serum TPO and/or TG autoantibodies along with a heterogeneous echotexture and diffuse or irregular hypoechogenicity of the thyroid parenchyma on ultrasound scan (1, 6).

Patients with HT are commonly asymptomatic, may present with goiter or be fortuitously diagnosed during investigations for other reasons, such as growth retardation. Moreover, HT can be detected in regular follow-up studies of children that may have an inherent increased risk for ATD, for instance subjects affected by extrathyroidal autoimmune disorders or certain chromosomal abnormalities. At the time of diagnosis, 52.1% of patients are euthyroid, 22.2% have overt and 19.2% subclinical hypothyroidism (SH). The remaining 6.5% of cases present with hyperthyroidism either overt (3.5%) or subclinical (3%) (10). With regards to the natural history of the disease, Aversa et al. evaluated the long-term evolution of thyroid status in 234 children with HT and either euthyroidism or SH at baseline. During a 5 year follow-up, a substantial proportion of patients showed a deterioration of their thyroid function as evidenced by an increase in thyroid-stimulating hormone (TSH) and a decrease in free thyroxine (FT4) levels as well as an increase in thyroid volume in the whole study population. Among patients who were initially euthyroid, 30.6% developed SH and 12.3% overt hypothyroidism. In addition, of those who had SH at baseline, 31.2% progressed to overt hypothyroidism, 3.2% developed hyperthyroidism, and 25% remained in the SH stage. It is worth mentioning, however, that among patients who were initially euthyroid 57.1% remained euthyroid at the end of the follow-up period. Moreover, spontaneous recovery of thyroid function was noted in 40.6% of cases who had SH at baseline (11).

Further data indicate that the presence of goiter and elevated TG autoantibodies at HT diagnosis may predict a deterioration of thyroid function over time (12). Moreover, in patients with HT, elevated TSH levels and TPO antibodies at diagnosis, a progressive increase in TSH during follow-up a well as the concomitant presence of celiac disease were shown to increase the risk of developing hypothyroidism after a 3 year period (7, 13).

Overall, the natural course of SH is worse in patients with underlying HT compared to idiopathic ones (14). With regards to treatment, levothyroxine replacement therapy in patients with SH is generally recommended, if TSH levels are higher than 5 IU/ml in the presence of goiter or positive thyroid antibodies and in all cases where TSH levels exceed 10 IU/ml (1).

GD, the most frequent cause of hyperthyroidism, is uncommon in the pediatric age range, with a prevalence ranging between 1/10.000 in the United States to 1/100.000 in the UK and Ireland. Similarly to HT, GD occurs 4–5 times more often in females than males, but rarely under the age of 4 years and its frequency peaks during puberty. Further to typical manifestations observed in adults, pediatric GD patients may show a decline in their school performance, behavioral changes and acceleration of growth and bone maturation. Antithyroid medications are used as first line treatment, although lower long-term remission rates have been reported in children than in adults, with <30% of patients achieving lasting remission following 24 months of medical therapy, requiring either another course of antithyroid drugs or definitive treatment (either radioiodine with I-131 or thyroidectomy) (1, 15).

The association between ATD and extrathyroid autoimmune diseases has been well-documented. While arthropathies and connective tissue disorders were shown to be the most common coexisting autoimmune disorders in adults with ATD, celiac disease (CD) and type 1 diabetes mellitus (T1D) were the most prevalent ones among ATD children and adolescents. Skin diseases, namely vitiligo, were almost equally represented among adults and children (16–18). Similarly, among GD patients with a mean age of 43 years, the most common non-thyroidal autoimmune diseases (NTAD) were rheumatoid arthritis, followed by vitiligo and pernicious anemia. In contrast, vitiligo, followed by T1D and CD were the NTADs mostly seen in pediatric GD patients (16).

Interestingly, patients with certain genetic abnormalities, namely Turner syndrome (TS), Trisomy 21 or Down syndrome (DS) and 22q11.2 deletion syndrome (22q11.2DS) are prone to develop both ATDs and NTADs. In addition, association with these syndromes may influence not only the clustering of

extrathyroid autoimmune disorders but also the evolution of thyroid function in patients with ATD (16, 19).

In this review, we will present the underlying mechanisms, frequency, and natural course of thyroid function disorders in specific genetic syndromes, providing in parallel recommendations for early identification, follow-up, and monitoring of thyroid disorders in affected individuals.

TURNER SYNDROME (TS)

TS is a rare chromosomal disorder, affecting 1 in 2,000–2,500 live born females. It results from partial or complete loss of one of the X chromosomes and is associated with a constellation of clinical features including short stature, gonadal dysgenesis, dysmorphic features, cardiovascular and renal anomalies, sensorineural hearing loss, skeletal malformations, ophthalmological abnormalities, neurocognitive impairment of variable severity and lymphedema (20, 21). Mortality is increased by ∼3-fold, especially due to cardiovascular diseases, congenital malformations, endocrine, nutritional, and metabolic diseases, resulting in a significant decrease in life expectancy (22).

Furthermore, women with TS are prone to develop autoimmune diseases, the most common being ATD (23–25). Moreover, there is a 4- to 8-fold increased risk of CD over the general population (26, 27). Other associated autoimmune conditions include T1D, inflammatory bowel diseases, alopecia areata, vitiligo, psoriasis, lichen sclerosus, juvenile idiopathic arthritis (JIA), and idiopathic thrombocytopenic purpura (ITP) (23, 25, 27). The risk of developing autoimmune diseases with a male predominance, such as T1D, Dupuytren's contracture, amyotrophic lateral sclerosis, ankylosing spondylitis and reactive arthritis is ∼4-fold increased, whereas the risk for female-predominant autoimmune diseases is increased by 1- and 7-fold (25) (**Table 1**). The frequency of autoimmune disorders increases with age and two or more organ-specific autoantibodies or autoimmune conditions may coexist in the same patient (24, 27).

The pathogenetic mechanisms underlying the increased frequency of autoimmune conditions among women with TS may include X-chromosome genes haploinsufficiency, parental X chromosome origin, excessive production of pro-inflammatory cytokines, decreased levels of anti-inflammatory cytokines and hypogonadism (23, 27, 33–35) (**Table 1**).

Haploinsufficiency of genes on the X chromosome may result in a lack of exposure to self-antigens in the thymus and subsequently inadequate thymic deletion of autoreactive T-lymphocytes and impaired "self" antigen recognition and tolerance (23, 34–36). The X chromosome is known to contain many immune-related genes (36, 37), one of them being FOXP3 (forkhead box P3). FOXP3, a member of the forkhead family of transcription factors is an essential transcriptional regulator for the development and suppressive function of regulatory T cells (Tregs), a subset of CD4+ lymphocytes (35, 38). Of note, Zinn et al. have mapped a possible locus for autoimmune thyroid disease in TS to a critical region of X chromosome, Xp11.2-p22.1, which also contains FOXP3 gene (38, 39). Mutations in the FOXP3 gene cause a rare disorder inherited in males, known as IPEX syndrome (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked) that can be also characterized by ATD (38).

Immune alterations observed in TS women include a decrease in the CD4+ to CD8+ lymphocyte ratio in the peripheral blood, lower IgG levels and percentage of CD4+ lymphocytes, as well as higher percentage of CD8+ T cells and frequencies of effector memory CD4+ T cells compared to controls (27, 33, 38, 40). Furthermore, levels of pro-inflammatory cytokines (IL6 and TGF β1) were found to be increased in women with TS, whereas those of anti-inflammatory cytokines (IL10 and TGF β2) decreased (35).

With regards to Tregs the results are inconclusive. In the study of Lee et al., the Tregs of TS patients displayed impaired ability to suppress the proliferation of autologous effector CD4+T cells compared to controls, despite their higher frequency among CD4+ T cells. The above findings could indicate that the Tregs of TS patients are intrinsically defective at inhibiting the proliferation of effector T cells, and/or that the effector T cells of TS patients are resistant to the suppressive effects of Tregs (38).

On the other hand, Gawlik et al. found that the percentage of Tregs in girls with TS and coexisting autoimmune disease was lower than in healthy controls and TS girls with no autoimmune diseases (33).

PTPN22 encodes a lymphoid-specific phosphatase which acts as a negative regulator of T cells and its polymorphisms have been linked to several autoimmune disorders. In particular, the PTPN22 C1858T polymorphism was associated with autoimmune disease risk in a Brazilian population of TS, however, these findings were not replicated in Hispanic (Mexican) TS patients (41, 42), indicating the influence of different genetic backgrounds on the phenotypic expression of this polymorphism.

It has been shown that the majority (60–80%) of 45,X women retain their maternally derived X chromosome (43–45). However, the frequency of hypothyroidism or thyroid autoimmunity did not differ according to the parental origin of the X chromosome (43, 45). On the other hand, further studies supported that the inheritance of autoimmunity in TS women was preferentially paternally transmitted, since patients were more likely to harbor autoantibodies when their fathers had autoantibodies rather than their mothers. Moreover, HLA-DR7;DQ2 and HLA-DR7;DQ9 haplotypes were associated with autoimmunity in TS patients and were more often paternally transmitted (46).

Interestingly, Bakalov et al. revealed a stepwise increase in the prevalence of HT: from 3.4% in men, to 5.8% in the general US female population, 15% in women with idiopathic 46,XX primary ovarian insufficiency (POI) and 37% in women with TS, suggesting that androgen deficiency may be implicated in the pathogenesis of HT (35). The levels of androgens are reduced in women with 46,XX spontaneous premature ovarian failure and even lower in women with TS. Given the known immunosuppressive role of androgens, higher androgen levels might have a protective effect in men, whereas low androgen levels might be linked to an increased risk of HT in women with ovarian insufficiency (35, 47–49). Indeed,

TABLE 1 | (a) Prevalence of autoimmune thyroid disorders and (b) associated non-autoimmune thyroid disorders in pediatric patients with TS, DS, KS, 22q11.2DS, WS, PWS, NS, and NF1 (c) possible underlying mechanisms involved in the pathogenesis of autoimmunity and (d) extrathyroidal autoimmune disorders associated with these syndromes.

	TS	**DS**	**KS**	**22q11.2DS**
a) ATD (prevalence)	HT (10–42%)* GD (1.7–3%)	HT (13–46%) GD (6.5‰)	HT (5.4–10%) GD (rare)	HT: (5% children, 30% age>17 years) GD (1.8%)
b) Non-autoimmune thyroid disorder	Primary hypothyroidism	CH - Incidence: 1:113–1:141 DS live births - mainly due to thyroid hypoplasia Primary hypothyroidism	Central hypothyroidism Peripheral hypothyroidism	Thyroid gland anomalies/hypoplasia (∼50%) Primary hypothyroidism
c) Possible underlying mechanisms implicated in the pathogenesis of autoimmunity	- X-chromosome genes haploinsufficiency - parental X chromosome origin - excessive production of pro-inflammatory cytokines - decreased levels of anti-inflammatory cytokines hypogonadism	- thymic atrophy and diminished expansion of T and B lymphocytes - altered thymic expression of AIRE - association with MHC class II DQA 0301 allele - altered regulation of pro- and anti-inflammatory cytokines - hyperresponsiveness to IFN	- immunomodulatory role of sex hormones in the immune response - X-linked gene dosage	- absent/hypoplastic thymus, ↓AIRE expression - T-cell lymphopenia, ↓ Tregs - restricted T cell repertoires, abnormal T cell activation, B cell dysregulation, Th1/Th2 imbalance - association with HLA-DR14
d) Associated extrathyroidal autoimmune disorders	- CD: ↑risk 4- to 8-fold - T1D, IBD, alopecia areata, vitiligo, psoriasis, lichen sclerosus, JIA, ITP - ↑ risk 4-fold for AD with a male predominance (T1D, Dupuytren's contracture, amyotrophic lateral sclerosis, ankylosing spondylitis and reactive arthritis)	- In particular: alopecia and vitiligo - CD: ↑ risk >6 years of age - T1D, idiopathic arthritis - Addison disease, chronic autoimmune hepatitis, primary sclerosing cholangitis**	- SLE: ↑risk 14-fold - T1D - Addison's disease, multiple sclerosis, RA, Sjogren's syndrome - JIA, psoriatic arthritis, polymyositis/dermatomyositis, systemic sclerosis, mixed connective tissue disease, antiphospholipid syndrome, ankylosing spondylitis, primary biliary cirrhosis	- JIA, ITP (next most common causes after ATD) - autoimmune hemolytic anemia, autommune neutropenia, psoriasis, vitiligo, CD, pernicious anemia/atrophic gastritis, IBD, urticaria, Raynaud phenomenon, rheumatic fever with chorea, T1D
	WS	**PWS**	**NS**	**NF1**
a) ATD (prevalence)	Rare	Rare	HT (14.3–60%)	HT: 2.5% GD: rare
b) Non-autoimmune thyroid disorder	Thyroid hypoplasia (75%) - Other structural abnormalities: agenesis, hemiagenesis and ectopy Primary hypothyroidism CH (rare) Hyperthyroidism (rare) Central hypothyroidism (rare)	Mainly central hypothyroidism (6.8%) Primary hypothyroidism CH Ectopic thyroid gland	Primary hypothyroidism	Central hypothyroidism Primary hypothyroidism
c) Possible underlying mechanisms implicated in the pathogenesis of autoimmunity			- both under- and overactivities of disparate Ras effectors - both increased and decreased Ras activities may be implicated in lupus-like autoimmunity - linkage of a susceptibility gene for SLE to 12q24, a locus encompassing *PTPN11*, encoding SHP-2 SHP-2 inhibits NK cells activation, cytolytic activity and IFN-γ secretion by NK cells, may mediate inactivation of immunoregulatory receptors and functions as a regulator of NF-κB activation. Increased SHP-2 activity is involved in SLE pathogenesis, modulating T cell proliferation and downstream cytokine production	- loss of neurofibromin resulting in decreased Fas antigen expression which may prevent apoptosis of CD4+ T cells - lymphoproliferative defects, including thymic and splenic hyperplasia, increased numbers of immature and mature T cells *in vivo*, but reduced proliferation in response to TCR and IL-2R stimulation *in vitro*, defective proliferative responses in B lymphocytes and thymocytes were shown in NF1-deficient mice
d) Associated extrathyroidal autoimmune disorders	- CD***	- Autoantibodies against pituitary	- Vasculitis, vitiligo, anterior uveitis, SLE, CD, antiphospholipid syndrome, and autoimmune hepatitis	- Multiple sclerosis, SLE, membranous glomerulonephritis, IgA nephropathy, mixed connective tissue disease, myasthenia gravis, ankylosing spondylitis, JIA, CD, autoimmune hemolytic anemia, bullous pemphigoid, vitiligo, alopecia areata, T1D

TS, Turner syndrome; DS, Down syndrome; KS, Klinefelter syndrome; 22q11.2DS, chromosome 22q11.2 deletion syndrome; WS, Williams syndrome; PWS, Prader-Willi syndrome; NS, Noonan syndrome; NF1, Neurofibromatosis type 1; ATD, autoimmune thyroid disease; HT, Hashimoto's thyroiditis; GD, Graves'disease; CH, congenital hypothyroidism; AIRE, autoimmune regulator; MHC, major histocompatibility complex; IFN, interferon; ↓, decreased; Tregs, regulatory T cells; Th, T helper (cell); HLA, human leukocyte antigen; PTPN11, tyrosine-protein phosphatase non-receptor type 11; SHP-2, Src homology phosphotyrosyl phosphatase 2; NK, natural killer (cells); NF-κB, nuclear factor kappa B; CD4, cluster of differentiation 4; IL-2R, interleukin-2 receptor; TCR, T cell receptor; CD, celiac disease; ↑, increased; T1D, type 1 diabetes mellitus; IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; ITP, idiopathic thrombocytopenic purpura; AD, autoimmune disease; SLE, systemic lupus erythematosus.

**Livadas et al. (28), Aversa et al. (29).*

***Giménez-Barcons et al. (30).*

****Giannotti et al. reported an increased prevalence of CD in WS subjects (31), whereas Stagi et al. found no evidence of increased autoimmunity in their patients with WS, including CD (32).*

Kalantaridou et al. demonstrated lower serum free testosterone concentrations in women with 46,XX spontaneous premature ovarian failure (while off estrogen therapy) compared to control women with normal ovarian function. Interestingly, free testosterone concentrations dropped even lower while these patients were on physiologic transdermal E2 therapy and despite the fact that levels of sex hormone-binding globulin did not alter significantly. A possible explanation for this finding could be that estrogen therapy might have induced a relative androgen deficiency. Given that androgens are secreted by both ovaries and adrenals and LH receptors have been identified in the zona reticularis and the deeper layer of the zona fasciculata of adrenal glands, the induction of E2 therapy might have resulted in lower LH levels and consequently a reduction in the production of testosterone from both the ovary and the adrenals (47). Moreover, the study of Gravholt et al., revealed a 25–40% reduction of circulating androstendione, testosterone, free testosterone, and dihydrotestosterone in TS women while off hormone replacement therapy, compared to age-matched normal women, whereas the level of dehydroepiandrosterone sulfate (DHEAS) was comparable between TS and control subjects. Most women with TS have no ovarian function and this might explain the difference in levels of androgens. On the other hand, the above results could point toward insufficient conversion from DHEAS into androstendione, testosterone, and dihydrotestosterone in TS, given the normal level of circulating DHEAS and thus insufficient extraadrenal and extragonadal 3b-hydroxysteroid dehydrogenase and 17bhydroxysteroid dehydrogenase activity (48).

Taken into account the data of Bakalov et al., showing a higher prevalence of HT in both TS and POI women, it seems that ovarian insufficiency *per se* may be a risk factor for HT, whereas the absence of a normal second X chromosome and the resultant haploinsufficiency for X-chromosome related gene(s) may further increase the risk for thyroid and possibly other autoimmune disorders (35).

The impact of a specific karyotype on the prevalence of thyroid autoimmunity in TS women has been also addressed with conflicting results. Increased frequency of thyroid autoimmunity has been observed among TS patients with isochromosome Xq and isolated Xp deletion (33, 50–52). Common feature of the aforementioned chromosomal abnormalities is the lack of the short arm of the X chromosome (Xp), indicating that haploinsufficiency of immune-related genes located in the Xpter-p11.2 region may predispose to the development of thyroid autoimmunity in women with TS (51). The reported prevalence of positive thyroid autoantibodies in TS women with Xq-isochromosome ranges from 15 to 83% (28). Other studies have failed to find an association between thyroid autoimmunity and specific TS karyotypes (25, 53). Of interest, an increased risk of ulcerative colitis (25) and a higher prevalence of anti-GAD-65 have been documented in TS patients with isochromosome Xq karyotype (24).

The frequency of HT is far higher among TS women than in the general population (29, 54), with the prevalence increasing with age, from 15.4% in patients younger than 10 years to 30.7% in the third decade of life (55) (**Table 1**). Up to ∼50% develop thyroid autoantibodies (50, 56). The frequency of thyroid autoimmunity rises steeply after the age of 13 years (53), but thyroid autoantibodies may appear as early as 5.5 years of age. An estimated 15–40% of patients suffer from hypothyroidism (24, 28, 50, 52, 53, 55), with its annual incidence being 3.2% (57) (**Table 1**). The risk of developing subclinical hypothyroidism has been shown to be highest between the ages of 12 and 14 years (53). With regards to its natural course and hormonal pattern HT in TS is characterized by the following features (1) a milder biochemical picture at diagnosis, given the higher frequency of euthyroidism and the lower TSH levels compared to non-TS subjects. These findings may be related either to the increased physicians' awareness on TS-associated thyroid dysfunction, resulting in an earlier detection of cases or to a milder autoimmune pattern as indicated by the lower TPOAb serum concentrations among TS patients (2) a progressive deterioration of thyroid function over time toward either hypo- or hyperthyroidism. Notably, Aversa et al. reported that the majority (67.7%) of TS patients presenting initially with SH evolved to overt hypothyroidism after a median time interval of 4.9 years (3) a lower frequency of positive family history of thyroid disease (4) more frequent conversion to GD (58).

According to the latest clinical practice guidelines for the care of girls and women with TS, screening for hypothyroidism is recommended at diagnosis and then in annual intervals, with assessment of (free) T4 and TSH levels starting from early childhood throughout life. Thyroid antibodies testing is usually recommended in the presence of elevated TSH levels and/or goiter (53, 59). Taken into consideration that hypothyroidism in TS may occur even before the age of 2 years (53) and could impair growth during childhood and puberty and aggravate the already adverse cardiometabolic profile of these patients, careful endocrinological surveillance is warranted across the lifespan to early detect and appropriately treat thyroid dysfunction and other comorbidities and thus optimize medical management of this population (23, 60) (**Table 2**).

GD, although relatively rare, occurs more frequently in TS girls compared to the pediatric general population with an estimated prevalence of 1.7–3 vs. 1.07%, respectively (28, 29, 61) (**Table 1**). The prevalence rates may vary across studies depending on the patient population ethnicity, age at presentation or the length of follow-up. TS patients with GD present more frequently extrathyroidal associated autoimmune disorders, are older at disease diagnosis and have lower FT4 levels than non-TS subjects. The above findings are in line with previous observations showing an increasing prevalence of ATD with age as well as a higher susceptibility of TS girls to autoimmune diseases. Moreover, regular clinical and laboratory assessment of those patients may enable the detection of thyroid dysfunction at an earlier stage (29, 61). Of interest in patients with TS or Down Syndrome (DS), GD may evolve from antecedent HT more commonly (25.7%) and later during the course of the disease compared to GD patients with no TS/DS (62). The clinical course of the disease does not seem to differ between patients with and without TS, given that the mean methimazole dose required to maintain a euthyroid state during the first cycle of

TABLE 2 | Summary of recommendations on diagnosis and management of thyroid dysfunction in pediatric patients with specific genetic syndromes.

TS	• Screen for hypothyroidism as soon as the diagnosis is established and then in annual intervals, with assessment of (free) T4 and TSH levels starting from early childhood throughout life • Thyroid autoantibodies testing is recommended at first detection of thyroid dysfunction and/or goiter • LT4 substitution therapy should be initiated in patients with OH, and is recommended for those with SH and TSH levels >10 mIU/l and/or positive thyroid antibodies and/or suggestive signs and symptoms (such as goiter, growth deceleration etc.) • Treatment modalities for GD include antithyroid medication, radioactive iodine therapy, or thyroidectomy
DS	• Screen for CH at birth and repeat TSH measurements at 6 and 12 months and annually thereafter. Additional assessment of TSH and FT4 at both 6–8 weeks and 4 months of age may be advised • If TSH levels are mildly elevated, consider reassessment of FT4 and TSH levels in a 2–3 month time, along with measurement of thyroid autoantibodies • Obtain thyroid antibodies at least once every 2 years, from age 1 year and throughout life • In the presence of positive antithyroid antibodies TSH and FT4 levels should be monitored regularly • Treatment with LT4 is advisable in patients with SH and (a) TSH levels >10 μU/ml (b) TSH levels <10 μU/ml in the presence of goiter and/or positive thyroid antibodies. Special considerations should be given in infants and children below the age of 5 years • LT4 therapy should be initiated in all cases that progress to overt hypothyroidism or become symptomatic • A trial off therapy and re-evaluation of thyroid function tests should be offered in those patients who do not require increase in their LT4 dose following treatment initiation or displayed no rise of TSH >10 μU/ml while on treatment. Regular auxological and clinical assessment and life-long monitoring of the thyroid function are recommended for all patients • Patients with GD can be offered antithyroid medication or radioactive iodine. Of note, surgery may not be an optimal choice for DS patients with the GD, as their short necks, craniofacial anomalies and associated airway obstruction may predispose to increased anesthetic and surgical risks
KS	• Measurement of TSH and FT4 levels at diagnosis and annually thereafter is advised. Screening for thyroid antibodies should be considered in case of TSH elevation and/or presence of goiter or periodically in the absence of suggestive clinical or biochemical signs
22q11.2DS	• Obtain TSH, FT4, total T3, and anti-TPO antibodies at diagnosis and annually thereafter • Appropriate treatment should be initiated in case of overt thyroid dysfunction • In case of subclinical thyroid disease, TFTs can be repeated in a 4–6 months' time interval • If subclinical disease persists (and in case of overt thyroid disease), perform a thyroid ultrasound in order to rule out structural anomalies of the gland • Medical treatment for subclinical disease should be offered if TSH levels remain abnormal despite normal FT4 levels in the presence of suggestive signs/symptoms and/or positive TPO antibodies and/or goiter or thyroid hypoplasia. Alternatively, TFTs can be rechecked after another 4–6 months' time interval
WS	• TFTs should be performed at diagnosis, annually for the first 3 years and at 2 years' intervals thereafter • Ultrasonographic assessment of thyroid morphology would be advised as soon as the diagnosis is established and regularly until adulthood • In patients younger than 6 years, or in those with thyroid hypoplasia, close monitoring of the thyroid function in shorter intervals, i.e., at 3–6 months from baseline, and then yearly, should be considered • LT4 treatment should be started in patients with overt hypothyroidism • Ongoing monitoring may be considered for cases with SH. LT4 replacement therapy is advisable, especially in children younger than 3 years, if TSH levels are persistently elevated, in the presence of thyroid hypoplasia and/or suggestive signs and symptoms
PWS	• Obtain TSH and FT4 levels within the first 3 months of life, regardless of the newborn screening result, given that TSH-based screening strategies cannot detect central hypothyroidism • TSH and FT4 should be measured on an annual basis, with consideration of more frequent testing if the patient receives treatment with rGH • Appropriate LT4 therapy should be initiated, if indicated. Taken into account the higher frequency of hypothyroidism during the first 2 years of life, a critical period for both growth and development, special consideration should be given in infancy and early childhood, to ensure adequate thyroid hormone levels
NS and other RASopathies	NS • TFTs including measurement of thyroid antibodies should be performed in children with signs or symptoms of hypothyroidism and every 3-−5 years in older children and adults • Thyroid disorders should be managed as in general population CFC • Obtain FT4 and TSH levels at diagnosis • During follow-up thyroid function should be checked in children with growth failure. Ongoing monitoring of the thyroid function can be decided by the endocrinologist NF1 • It would be advisable to apply the same recommendations on thyroid function monitoring as for NS patients

TS, Turner syndrome; DS, Down syndrome; KS, Klinefelter syndrome; 22q11.2DS, chromosome 22q11.2 deletion syndrome; WS, Williams syndrome; PWS, Prader-Willi syndrome; NS, Noonan syndrome; CFC, Cardio-facio-cutaneous syndrome; NF1, Neurofibromatosis type 1; T4, thyroxine; FT4, free thyroxine; TSH, thyroid stimulating hormone LT4, levothyroxine; OH, overt hypothyroidism; SH, subclinical hypothyroidism; CH, congenital hypothyrodism; GD, Graves'disease; T3, triiodothyronine; anti-TPO, anti-thyroid peroxidase (TPO); TFTs, thyroid function tests; rGH, recombinant growth hormone.

therapy, the initial remission rates and relapse rates following first methimazole cycle discontinuation, remission rates for at least 2 years following withdrawal of the last methimazole cycle, percentages of girls who underwent non-pharmacological therapies and definitive remission rates were similar between the two groups (29, 61) (**Table 2**).

TRISOMY 21 OR DOWN SYNDROME (DS)

DS is the most prevalent chromosomal abnormality affecting 1 in every 787 live births (63). Trisomy of chromosome 21 (non-disjunction) accounts for 95% of cases, while mosaicism or a Robertsonian translocation occurs in the remaining 5% of

children (64). DS is associated with intellectual disability, growth retardation, congenital heart defects, gastrointestinal anomalies, increased risk of hematologic malignancies, hypotonia, hearing loss, ophthalmic and immunologic disorders, dysmorphic features and early-onset Alzheimer's disease (65, 66).

Furthermore, patients with DS exhibit increased susceptibility toward thyroid and non-thyroid autoimmune disorders, in particular alopecia and vitiligo, but also T1D, JIA and CD, the latter presenting more commonly among children older than 6 years of age (66–68) (**Table 1**). Plausible biological mechanisms explaining the predisposition to autoimmunity in DS cases may include: (1) thymic atrophy and diminished expansion of T and B lymphocytes in the first years of life. The T-lymphocyte subpopulation counts gradually normalize, whereas the B- lymphocytopenia persists. After the age of 6 years, DS children display a considerable hypergammaglobulinaemia of the IgA and IgG type, with increased levels of IgG1 and IgG3 and reduced levels of IgG2, IgG4, and IgM. Furthermore, a decrease in CD4+ along with an increase in CD8+ lymphocytes and the percentage of natural killer (NK) cells have been observed. Overall, these immunologic abnormalities may contribute to the increased risk of infections and autoimmune diseases in DS patients (64, 66, 69, 70) (2) altered thymic expression of the autoimmune regulator (*AIRE*) gene. This gene is located on chromosome 21q22.3, mainly expressed in thymic epithelial cells and encodes for a transcription factor that regulates the promiscuous expression of genes encoding tissue-specific antigens, thus playing a key role in the induction and maintenance of central tolerance by eliminating autoreactive T cells. Mutations in *AIRE* cause a rare autosomal-recessive disorder, autoimmune polyendocrine syndrome type 1 (APS-1), also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, with the cardinal features being autoimmune hypoparathyroidism, Addison's disease and chronic mucocutaneous candidiasis. Reduced intrathymic expression of AIRE accompanied by reduced expression of peripheral tissue-restricted antigens has been demonstrated in DS patients. Given that three copies of the gene were expressed in the thymus of those patients, the authors suggested that AIRE expression may be regulated by epigenetic or other post-transcriptional mechanisms in order to overcompensate for the excess of gene dosage (30). In contrast to the above findings, Skogberg et al. found increased expression of AIRE at both mRNA and protein level in the thymus of DS patients, implying that the increased *AIRE* gene dose in DS may alter thymic selection processes, thus contributing to autoimmune disease predisposition (71) (3) the genetic contribution of class II MHC genes. A strong association between the major histocompatibility complex (MHC) class II DQA 0301 allele and hypothyroid autoimmune thyroiditis was identified in DS patients, pointing toward a role of one or more genes on chromosome 21 that may regulate immune function (72) (4) altered activity of enzymes that modulate inflammatory and immune processes by regulating extracellular ATP and adenosine levels and the hydrolysis of acetylcholine (molecules involved in immune responses) resulting in increased pro-inflammatory cytokine levels such as IFN-γ, TNF-α, IL-1β, and IL-6, and decreased levels of anti-inflammatory cytokine IL-10 (73) (5) hyperresponsiveness to interferon (IFN) secondary to increased gene dosage of the four interferon receptors encoded on chromosome 21 (74, 75) (**Table 1**). In this regard, it should be pointed out that treatment with IFNa is associated with thyroid dysfunction via immune mediated and direct thyroid-toxic effects, which may be manifested as either autoimmune (HT or GD) or non-autoimmune destructive thyroiditis (76). Interestingly, *in vitro* studies have demonstrated that both INFα and -β inhibit the TSH-stimulated gene expression of thyroid peroxidase (TPO), sodium/iodide symporter (NIS), and thyroglobulin (TG) as well as T4 release (77). Taken together, it can be postulated that consistent activation of INF signaling may be implicated in the immune dysregulation and thyroid dysfunction associated with DS and may contribute to the increased susceptibility of DS patients to thyroid autoimmunity (74).

Thyroid dysfunction is the most frequently encountered endocrinopathy in DS, affecting 7 to 66% of patients. The spectrum of thyroid abnormalities includes congenital hypothyroidism (CH), isolated hyperthyrotropinemia or SH, primary hypothyroidism and thyroid autoimmunity, such as HT or GD (**Table 1**).

The incidence of CH is estimated between 1:113 and 1:141 DS live births (64, 66), being 28 times more common among DS patients compared to the general population, permanent in 70% and transient in 30% of cases (78, 79) (**Table 1**). Most cases are associated with thyroid hypoplasia, whereas thyroid agenesis, ectopy or goiter are infrequent. In line with the above, Luton et al. examined 13 fetuses with DS and reported that, the thyroid gland was eutopic in all fetuses, without gross anomalies, however, histologically, the thyroid follicles were abnormally small and heterogeneous in size. Moreover, they found TSH levels consistently above the 80th percentile, whereas the FT4 level was below the 50th percentile in most cases (80). Overall the pathogenetic mechanisms underlying CH in DS cases may include: (1) exaggerated response to thyroid-releasing hormone (TRH) stimulation until the third year of life and delayed maturation of the hypothalamic—pituitary—thyroid (HPT) axis (2) peripheral resistance to thyroid hormones resulting in inappropriate TSH secretion (3) inappropriate TSH release, due to a central defect or inadequate dopaminergic control (4) TSH insensitivity and reduced TSH bioactivity, which consequently may result in elevated TSH with low-normal T4 levels (64, 66). While the American Academy of Pediatrics (AAP) recommends screening for CH at birth and repeat TSH measurements at 6 and 12 months and annually thereafter, Pierce et al. have recently demonstrated that among those DS patients who were diagnosed with hypothyroidism beyond the newborn screening period, 11 (7.5% of all acquired hypothyroidism) were diagnosed before the age 6 months (81). Based on these findings, the authors recommended that TSH and FT4 be obtained at both 6–8 weeks and 4 months (81) (**Table 2**). Notably, it has been shown that thyroxine treatment within the first 2 years of life is associated with modest improvements in motor development and growth, however, without improvement of mental or motor development later in life. Nevertheless, it appears to have beneficial effects on

growth outcome especially in children with neonatal plasma TSH concentrations higher than 5 mIU/L (78, 82, 83).

SH, defined as an elevated serum TSH level associated with normal total or free T4 and triiodothyronine (T3) values, is the most frequent thyroid abnormality among DS patients, with a prevalence ranging between 7 and 40% (64). The risk of developing thyroid dysfunction increases yearly by 10%, so that 25% of patients are expected to have thyroid disease by age 7.5 years and up to 50% by adulthood (81). On the other hand, SH before the age of 5 years may be a transient and self-limiting condition in >70% of DS cases. In this regard, the absence of goiter and thyroid autoantibodies have been associated with a greater chance of spontaneous remission (84). In the study of Pierce et al., euthyroidism was restored in 13% of DS patients with a history of hypothyroidism during follow-up, even in the presence of positive thyroid autoantibodies (81). It has been postulated that mild TSH elevation in DS may be an inherent defect of the syndrome reflecting a resetting of the HPT axis. Indeed, Meyerovitch et al. revealed that the 2.5th to 97.5th percentile TSH values in a cohort of DS patients ranged between 1.3 and 13.1 mIU/l, with the 95th percentile value being 8.9 mIU/l, i.e., significantly higher compared to controls. A similar upward shift was also shown in the distribution of FT4 levels (85). For these reasons, it has been supported that SH may be overdiagnosed in DS patients, leading to unnecessary life-long treatment. In addition, SH has not been shown to be a precursor of permanent hypothyroidism (i.e., low T4, increased TSH levels) and the 10 year incidence for onset of definite hypothyroidism has been reported to be low, 13.6% (86).

From another point of view, L-thyroxine treatment for SH in DS patients, whose development and growth are already inherently substantially compromised, is considered a safe and inexpensive treatment with possible benefits on growth and intellectual outcomes, if special attention can be laid to avoid pharmacological hyperthyroidism (64, 66, 82). Moreover, it has been shown that thyroxine treatment during the first 2 years of life was associated with higher FT4 concentrations at age 10.7 years, indicating that early thyroxine treatment may result in an alteration in the set point of the HPT axis that may persist later in life. In addition, the frequency of positive anti-TPO antibodies increased with age from 1% at the age of 12 months, to 6% at age 24 months and 25% at age 10.7 years in the DS placebo group, whereas there was a trend toward a lower percentage of children developing anti-TPO positivity among DS patients who received thyroxine treatment. The above results may indicate a protective effect of early levothyroxine treatment, possibly by reducing TSH levels and thus thyroid autoantigen presentation (87).

Taken together, in cases of mild TSH elevation, repeat testing of FT4 and TSH levels in a 2–3 month time, along with measurement of thyroid autoantibodies titers, should be considered in order to avoid treatment initiation in SH-cases that may be proved transient. With regards to thyroid antibodies screening, the Ireland and UK guidelines recommend testing (including T4 and TSH levels) at least once every 2 years, from age 1 year and throughout life (88). The presence of positive antithyroid antibodies alone does not warrant treatment initiation but TSH and FT4 levels should be monitored regularly in order to early detect conversion from euthyroidism to hypothyroidism. Treatment with levothyroxine is advisable in DS patients with SH and a) TSH levels >10 μU/ml b) TSH levels <10 μU/ml in the presence of goiter and/or positive thyroid antibodies. Moreover, special considerations should be given in infants and children below the age of 5 years. Treatment should be initiated in all cases that progress to overt hypothyroidism (low FT4 and elevated TSH levels) or become symptomatic. A trial off therapy and re-evaluation of thyroid function tests should be offered in those patients who do not require increase in their levothyroxine dose following treatment initiation or displayed no rise of TSH >10 μU/ml while on treatment. Regular auxological and clinical assessment and life-long monitoring of the thyroid function are recommended for all patients (64, 66, 78, 81, 84, 89) (**Table 2**).

Autoimmune hypothyroidism is the most frequent autoimmune disorder in DS. Antithyroid antibodies are found in 13–46% of patients (**Table 1**). Interestingly, almost 50% of them, develop thyroid antibodies positivity at an age younger than 8 years (64, 81). In contrast to the general population, HT in DS is characterized by the following features: (1) younger age at diagnosis (mean: 6.5 years), possibly reflecting the increased awareness of physicians on DS-associated thyroid dysfunction which may result in an earlier detection of HT cases among DS patients (2) lower frequency of positive family history of thyroidopathy and higher frequency of extra-thyroidal autoimmune diseases, suggesting that DS is *per se* associated with an increased risk of developing autoimmune disorders (3) no gender predilection (4) lower prevalence of euthyroidism and increased prevalence of SH at presentation, despite lower anti-TG and anti-TPO levels, suggesting probably a congenital alteration in thyroid gland regulation (5) progressive deterioration of thyroid function over time (median interval of 5.1 years) as the prevalence rates of overt hypothyroidism and hyperthyroidism were shown to increase, whereas the frequency of SH remained unchanged (6) more frequent evolution toward GD (64, 66, 68, 90, 91).

GD occurs more frequently in DS children compared to the general population with an estimated prevalence of 6.5 vs. 1.07%, respectively (68, 92) (**Table 1**). Compared to patients without DS, GD in affected subjects usually presents at a younger age, between late childhood and early adulthood, is more frequently associated with other autoimmune diseases, namely CD, presents a higher rate of positive family history of HT but no gender predominance (92, 93). Furthermore, as already mentioned above, the risk of conversion from HT to GD is higher (64, 90). In the study of Aversa et al., long-term remission could be achieved in all GD patients following the onset of antithyroid medication. Low doses of methimazole treatment were required to maintain euthyroidism, no relapses occurred after treatment withdrawal and no alternative treatments were needed, pointing toward a less severe clinical course of GD in DS (90). Similar findings have been previously reported in a multicenter Italian study by De Luca et al., comprising 28 DS children with GD and 109 children and adolescents with GD but without DS (control group) (93). Lower relapse rates after the first cycle of methimazole treatment withdrawal and

higher persistent remission rates after definitive methimazole withdrawal were observed in DS patients compared to controls. In addition, no DS patient required surgery or radioiodine ablation, compared to 11% of controls who underwent non-pharmacological therapies. Of note, a longer time to achieve remission, but also a trend toward higher remission rate could be observed in GD patients with DS compared to those without in a more recent study (94). Furthermore, none of the DS patients received definitive therapy, compared to 36% of those without DS. On the contrary, Goday-Arno et al. reported that no patient could achieve remission following carbimazole discontinuation in their study population. Radioactive iodine was administered after a mean period of medical treatment of 40 months without clinical remission. Hypothyroidism developed in all treated cases necessitating replacement therapy with levothyroxine (92). At this point it should be noted, that despite the reluctance to offer radioiodine treatment in pediatric patients with GD as second-line therapy, surgery may not be an optimal choice for DS patients with the disease, as their short necks, craniofacial anomalies and associated airway obstruction may predispose to increased anesthetic and surgical risks (64, 92) (**Table 2**).

Of interest, in the study of Aversa et al., one third of GD patients shifted from hyper- to hypothyroidism following methimazole discontinuation. It should be stressed, however, that all DS children enrolled in this study had a previous diagnosis of HT. It can be postulated that the HT-related damage of the thyroid might have overcome the stimulating effects of the TSHR autoantibodies, resulting, thus, in the aforementioned conversion from hyper- to hypothyroidism in a proportion of patients (90).

KLINEFELTER SYNDROME

Klinefelter syndrome (KS) is the most common chromosomal aberration in males, affecting 1 in every 660 men (95). It results from meiotic or mitotic non-disjunction, leading to the presence of one or more extra X chromosomes. Most patients (90%) have the classic 47,XXY karyotype, whereas higher-grade aneuploidies (48,XXXY; 49,XXXXY), structurally abnormal X chromosome (e.g., 47,iXq,Y) or mosaicisms (47,XXY/46,XY) account for the remaining 10% of cases (96). It is characterized by broad phenotypic variability with regards to physical traits, cognitive abilities and comorbidities and remains remarkably underdiagnosed with only around 25–39% of cases receiving a diagnosis postnatally and <10% before puberty (97–99).

Hypergonadotropic hypogonadism, gynecomastia, small firm testes, infertility, sparse facial and pubic hair, tall stature along with impaired psychosocial functioning of variable degree are common features of the condition (99). Moreover, KS is associated with multiple comorbidities, including cardiovascular, cerebrovascular and thromboembolic diseases, osteoporosis, diabetes mellitus, metabolic syndrome, as well as an increased risk of developing breast cancer and extragonadal germ cell tumors (96, 100). In addition, recent studies have demonstrated that KS patients are at increased risk of certain autoimmune disorders, in particular Addison's disease, T1D, multiple sclerosis, acquired hypothyroidism, rheumatoid arthritis, Sjogren's syndrome and systemic lupus erythematosus (SLE), most of which being female predominant (101). Concurrence of KS with other inflammatory rheumatic diseases such as JIA, psoriatic arthritis, polymyositis/dermatomyositis, systemic sclerosis, mixed connective tissue disease, antiphospholipid syndrome, ankylosing spondylitis and primary biliary cirrhosis has been also reported (102) (**Table 1**). Endocrine organ-specific autoantibodies can be detected in 13% of KS subjects and the frequency progressively increases in those with higher-grade aneuploidies and is higher in children than in adults (103). It is worth noting, that KS patients exhibited autoantibodies primarily (i.e., 8.2%) against diabetes-specific autoantigens (104).

Positive thyroid antibodies are found in 5.4–10% of children and 3.3–7% of adults with KS in different studies (103–106). Most cases are euthyroid, whereas GD occurs only rarely in patients with the syndrome (107, 108) (**Table 1**).

The influence of sex hormones as modulators of the immune response and X-linked gene dosage have been implicated in the pathogenesis of autoimmunity in KS (**Table 1**). The immunostimulatory effects of estrogens and the immunosuppressive effects of androgens are well-established. In males with rheumatoid arthritis both low testosterone and increased estradiol have been observed, with the latter being correlated with the degree of inflammation (109, 110). On the other hand, patients with KS often display elevated estradiol levels, which are comparable to those seen in normal menstruating women and androgen levels like those of a preadolescent male (111). Low serum testosterone levels were also found in males with SLE and a recent record linkage study demonstrated an association between testicular hypofunction and SLE (112, 113). Of interest, previous studies on sex hormone metabolism in KS patients with SLE revealed that this was similar to the one seen in women affected by SLE (114). Taken together, the above findings highlight the potential role of sex hormones as contributors to the development of autoimmunity in patients with KS (**Table 1**).

Notably, the risk of SLE among KS patients is 14-fold increased compared to 46,XY males and similar to the risk seen in 46,XX females (115), pointing toward the role of the number of X chromosomes and in particular an X-linked gene dose effect (**Table 1**). Indeed, genes escaping X inactivation may have higher levels of expression in subjects with two X chromosomes (116). The *CD40 ligand* gene, located in the Xq26.3 region, encodes a protein expressed on the surface of activated T cells, which binds to CD40 on the B cell surface and mediates B cell proliferation and immunoglobulin isotype switching, thus playing an important role in adaptive immunity (117). *In vitro* studies reported by Sarmiento et al. showed higher percentage of CD40L-expressing CD3+ T cells and CD40L protein and mRNA expression after activation in females and KS patients compared to males and TS women, indicating that the existence of two copies of the X chromosome may enhance both cell-mediated and humoral immune responses (118). Another gene, *TLR7*, mapped to Xp22.2, encodes a member of the Toll-like receptor (TLR) family, which detects single stranded-RNA and activates innate immune responses, such as the production of inflammatory cytokines and type I interferons (119). Sarmiento

et al. demonstrated also higher Toll-like receptor 7 (TLR7) mRNA levels post-stimulation in healthy females and KS patients than in TS women and males. However, TLR7-mediated IFN-alpha production did not differ between KS patients and healthy males, suggesting that hormonal factors or epigenetic alterations may modulate the innate immune response (118).

Apart from autoimmunity, other mechanisms involved in the development of thyroid dysfunction in KS patients include some degree of central hypothyroidism and peripheral hypothyroidism. A low TSH response to TRH stimulation has been previously observed in KS patients and attributed most likely to the chronic compensatory increase in the production of gonadotropins, leading to reduced availability of the alpha subunit for the formation of TSH (120, 121). Moreover, KS patients displayed lower FT4 and FT4/free T3 (FT3) ratio than control men with no increase in their serum TSH, whereas their FT4 values were in or just below the lower limits of the normal range. The above findings may suggest an impaired hypothalamic-pituitary control of the thyroid function resulting in secondary thyroid insufficiency (120). Similar TSH but lower FT4 levels were also documented in KS patients when compared to non-KS hypogonadal men with similar testosterone levels (105). Further to the above, pubertal KS patients showed similar FT4 and TSH, but lower FT3 levels when compared to age-matched pubertal healthy boys, indicating a mixed form of hypothyroidism in KS boys during the pubertal development: both secondary and peripheral, the latter due to reduced deiodinase activity (106) (**Table 1**). Interestingly, a Danish study aiming to explore the association between KS comorbidities and differential gene expression profiles, revealed that genes involved in 'abnormal thyroid hormone metabolism' were upregulated in KS patients (122).

Taken into account the above data and given the lack of formal recommendations on screening and monitoring of thyroid function in KS, we suggest measurement of TSH and FT4 levels at diagnosis and annually thereafter. Screening for thyroid autoantibodies should be considered in case of TSH elevation and/or presence of goiter or periodically in the absence of suggestive clinical or biochemical signs (**Table 2**).

22q11.2 DELETION SYNDROME

Chromosome 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome with an estimated incidence of 1:4.000 live births worldwide. 22q11.2DS encompasses a heterogeneous group of phenotypically similar disorders, including DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome (CTAF), some cases of autosomal dominant Opitz G/BBB syndrome and Cayler cardiofacial syndrome. It is caused by a (1.5–3.0 Mb) hemizygous deletion at chromosome 22q11.2, which is *de novo* in more than 90% of cases and inherited from a heterozygous parent in ∼10%. The cardinal features of the condition are congenital cardiac defects, mainly conotruncal malformations (ventricular septal defect, tetralogy of Fallot, interrupted aortic arch and truncus arteriosus), palate anomalies, thymic hypoplasia and immunodeficiency, neonatal hypocalcemia, developmental delay, learning disabilities and dysmorphic facial features (123, 124).

Endocrinopathies are identified in 60% of patients (125). In particular, hypoparathyroidism and hypocalcemia are observed in 17–60%, thyroid gland anomalies in 50% and hormonal dysfunction in up to 25.6%, short stature in 41%, growth hormone deficiency in 4%, whereas obesity is present in up to 43.5% of adults with the syndrome (123, 126–128).

Furthermore, 22q11.2DS patients are prone to develop autoimmune disorders, the most common being ATD, JIA, and ITP (129). Coexistence of autoimmune haemolytic anemia, autoimmune neutropenia, psoriasis, vitiligo, CD, pernicious anemia/atrophic gastritis, inflammatory bowel disease, urticaria, Raynaud phenomenon, adult RA, rheumatic fever with chorea and T1D in 22q11.2DS patients has been also reported (129–133) (**Table 1**). Of interest, in the study of Lima et al., 9 of 28 22q11.2DS patients (32%) tested positive for adrenal autoantibodies, however ACTH and cortisol levels were normal in all cases (133). Overall, autoimmune phenomena may occur in 10–30% of affected individuals (129–131, 133).

ATD is common among individuals with 22q11.2DS. Thyroid autoantibodies were found in up to 5% of affected children and 30% of patients older than 17 years (127, 129, 133, 134) (**Table 1**). In the study of Shugar et al., overt thyroid disease was noted in 9.5% of 169 children with the syndrome. Hypothyroidism occurred in 7.7% and hyperthyroidism in 1.8% (**Table 1**). Interestingly, among patients with thyroidopathy the female to male ratio was 2.2, which is lower than the one reported in the general pediatric population. Furthermore, of those with prodromal or subclinical thyroid disease, 42% progressed to overt thyroid disease within a mean follow-up time of 27.6 months, necessitating medical treatment (135). In a previous study conducted in adults with 22q11.2DS the frequency of hypothyroidism reached 20.5% (136).

The association between GD and 22q11.2DS is noteworthy. As mentioned above, the prevalence of hyperthyroidism in the pediatric 22q11.2DS population (1.8%) is far higher than in the general pediatric population (1 in 10.000 children in the United States) (135, 137) (**Table 1**). Moreover, atypical presentation with seizures and very young age at onset (as early as 27 months of age) have been described in pediatric cases of 22q11.2DS, features that are quite unusual especially when taking into account that GD rarely occurs in children younger than 5 years of age (127, 137–139). Hyperthyroidism may occur at any age and affects around 5% of adults with the disorder (136).

A complex interplay between immunologic, genetic, and environmental factors may underlie the association between GD and 22q11.2DS. Possible mechanisms may include the following: (1) Absent or hypoplastic thymus may result in impaired maturation and dysregulation of T cells and thus defective central tolerance allowing self-reactive T cells to escape intrathymic negative selection and trigger autoimmune responses (124). In this regard, abnormal thymic development may lead to decreased AIRE expression and consequently impaired AIRE-mediated intrathymic expression of tissue-restricted antigens

(TRAs) (140). (2) Certain HLA haplotypes, such as HLA-DR14 may increase susceptibility to GD in patients with 22q11.2DS (139, 141). (3) Thymic maldevelopment may result in several immunologic abnormalities. T-cell lymphopenia and decreased absolute counts of CD4+CD25+ natural Tregs have been observed in 22q11.2DS patients especially during the first 4 years of life and might explain the early age of GD onset in some cases (142–144). (4) Furthermore, lymphopenia and restricted T cell repertoires, abnormal T cell activation and associated B cell dysregulation and humoral dysfunction, as well as Th1/Th2 imbalance of the CD4+ T cells may also be implicated in the induction of autoimmunity (130, 141, 145, 146) (**Table 1**).

Further to ATD, thyroid gland anomalies can be also more commonly been observed in 22q11.2DS patients (**Table 1**). Among 28 22q11.2DS children who underwent computed tomography (CT) scans 50% exhibited thyroid gland abnormalities, including an absent isthmus (21%), retrocarotid (14%), and retroesophageal extension (14%) and absence of the left thyroid lobe (one case). Elevated TSH levels were detected in 2/14 patients. Low common carotid artery bifurcations were also noted (147). In a subsequent study, Stagi et al. implied that thyroid hypoplasia may be a feature of 22q11.2DS, given that 46.6% of 30 patients studied had decreased total thyroid volume, with the left thyroid lobe being significantly smaller in all cases. Overt and subclinical hypothyroidism were found in 3.3 and 23.3% of all study population, respectively, and thyroid hypoplasia was noted in the majority of these cases (62.5%). Of note, 71% of patients who presented with thyroid hypoplasia had coexistent congenital heart malformation, compared to 31% of those with a normal thyroid volume (148). Deeper insights into mechanisms involved in the pathogenesis of 22q11.2DS unraveled the major role of T-box transcription factor *(TBX1)* gene. *TBX1*, mapped on the long arm of chromosome 22 at position 11.21, encodes a transcription factor protein essential for the regulation of developmental processes in a number of tissues, derived from the pharyngeal apparatus, such as the development, positioning and size of thyroid gland. Interestingly, Tbx1−/− mice exhibited most of the cardiac and pharyngeal arch anomalies seen in the 22q11DS, including cardiac outflow tract abnormalities, thymic and parathyroid gland hypoplasia and craniofacial anomalies (147–149).

The above findings underline the importance of a meticulous clinical and endocrine evaluation of all 22q11.2DS patients at diagnosis and during follow-up in order to address and appropriately manage associated comorbidities. With respect to thyroid function, baseline screening should include measurement of TSH, FT4, total T3, and TPO autoantibodies. If the results are normal, thyroid function tests (TFTs) should be checked on an annual basis lifelong. When overt disease is biochemically confirmed, appropriate medical treatment should be started. In case of subclinical thyroid disease, thyroid function tests (TFTs) should be repeated in a 4–6 months' time interval. If subclinical disease persists (and in case of overt thyroid disease), a thyroid ultrasound should be performed in order to exclude structural anomalies of the gland (135). Medical treatment for subclinical disease should be offered if TSH levels remain abnormal despite normal FT4 levels in the presence of suggestive signs/symptoms and/or positive TPO antibodies and/or goiter or thyroid hypoplasia. Alternatively, TFTs can be rechecked after another 4–6 months' time interval (**Table 2**).

WILLIAMS SYNDROME

Williams–Beuren or Williams syndrome (WS) is a multisystemic neurodevelopmental disorder caused by a 1.5–1.8 Mb heterozygous microdeletion at chromosome 7q11.23, encompassing 26 to 28 genes. It is a rare disorder, affecting roughly 1 in 10.000 people. Common clinical manifestations include intellectual disability, a constellation of dysmorphic facial features known as elfin facies, connective tissue abnormalities and cardiovascular disease, namely supravalvular aortic and pulmonary artery stenosis. In particular, haploinsufficiency of elastin *(ELN)* gene, located in the deleted region is responsible for the cardiovascular abnormalities of the disorder, whereas hemizygosity of LIM Domain Kinase 1 *(LIMK1)*, mapped to the same area, may contribute to the cognitive impairment associated with the syndrome (150–152).

Endocrine disorders are frequently encountered in patients with WS, including short stature, hypercalcemia, central precocious puberty, hypothyroidism, impaired glucose tolerance and diabetes, dyslipidemia, decreased pubertal growth spurt, GH deficiency, decreased bone mineral density or osteoporosis (150, 153–155).

SH occurs in 15–37.9% of patients, is commonly associated with thyroid hypoplasia and its prevalence decreases with age (156–159) (**Table 1**). In this regard, the frequency of SH in WS children was considerably higher in those younger than 3 years (73.9%) compared to older patients, whereas the vast majority of children aged more than 9 years were euthyroid (158). Selicorni et al. noted the highest incidence of SH in patients under 1 year of age (157). Similarly, Chen et al. demonstrated that the frequency of SH in WS increased during the first years of life, reaching 44.4% among children aged 3–6 years, and declined gradually thereafter (159). The above findings may reflect immaturity of the HPT axis, further supported by an exaggerated, prolonged TSH response to TRH as well as a mildly low biological activity of circulating TSH, which were observed in a 2 month old female infant with WS, thyroid hemiagenesis and elevated TSH levels (160). TSH insensitivity of the thyroid gland has been also proposed to explain HPT axis dysfunction in affected individuals (159). Notably, patients with WS rarely present CH (**Table 1**). However, this could be the initial manifestation that could raise suspicion for the syndrome, in the presence of suggestive clinical features (161, 162).

With regards to the natural history of SH in WS, 13% of patients with normal thyroid function at baseline showed abnormal TSH levels at follow-up. On the contrary, 70% of those who had initially elevated TSH levels, were euthyroid on follow-up evaluation (159). These data point toward a transient, self-limiting condition in most cases, like the one observed in DS patients and the general pediatric population, as well. Overt hypothyroidism is less common (10%) (156) and thyroid autoantibodies are negative in most cases (156–159) (**Table 1**).

On the other hand, thyroid hypoplasia is a frequent feature of WS, presenting in up to 75% of patients (156, 157), with the left lobe being more commonly affected (156). Other structural abnormalities of the thyroid gland may include agenesis, hemiagenesis and ectopy (151, 156, 160, 163) (**Table 1**). From a biochemical point of view, most patients with hypothyroidism had thyroid hypoplasia, indicating that thyroid dysfunction in WS may result from reduced thyroid volume (156, 157). Taken into consideration that the prevalence of SH decreases with age, it can be assumed that the hypoplastic thyroid gland cannot compensate for the increased requirements for thyroid hormones during the very first years of life, resulting in TSH elevation. However, when the need for thyroid hormones decreases with age, the hormonal production by the hypoplastic gland may be sufficient leading to normalization of TSH levels in most cases (157).

Furthermore, thyroid hypoplasia was more frequently seen in older children, pointing toward poor growth of the thyroid gland, possibly associated with genetic defects within the 7q11.23 region (156, 158, 159). Indeed, *BAZ1B* (Bromodomain Adjacent to Zinc Finger Domain 1B), located in the deleted WS-region, has been recently implicated in the thyroid gland developmental defects seen in a percentage of patients with the syndrome. It encodes a tyrosine-protein kinase, which functions as a transcription regulator and plays a key role in chromatin remodeling. The most recent study by Allegri et al. demonstrated that BAZ1B silencing results in reduced cell viability and survival of human thyroid cells, largely due to an increase in apoptotic phenomena and these effects may be mediated through phosphatase and tensin homolog PTEN overexpression (164).

Apart from primary hypothyroidism, other thyroid function abnormalities are rare. In the study of Amenta et al., comprising 50 patients with WS, hyperthyroidism occurred in one case (152). Interestingly, central hypothyroidism and secondary adrenal insufficiency were most recently reported in a 10 month-old boy with WS (165) (**Table 1**).

With regards to concurrent autoimmune disorders, as already mentioned, thyroid autoantibodies are rarely present in patients with WS (152, 166). Of note, Giannotti et al. reported an increased prevalence of CD in WS subjects (9.5% vs. 0.54% in the general pediatric population in Italy) (31) (**Table 1**), whereas Stagi et al. found no evidence of increased autoimmunity in their patients with WS, including CD (32).

The American Academy of Pediatrics currently recommends thyroid function evaluation in WS patients at a minimum: at diagnosis, annually for the first 3 years and at 2 years' intervals thereafter (167). However, in light of the above findings concerning the high frequency of thyroid structural abnormalities associated with WS as well as the higher prevalence of thyroid dysfunction in early childhood, it would be prudent to suggest assessment of both thyroid function and morphology as soon as the diagnosis of WS is made and even if the newborn screening result for CH is negative. Moreover, in patients younger than 6 years, or in those with thyroid hypoplasia, close monitoring of the thyroid function in shorter intervals, i.e., at 3–6 months from baseline, and then yearly, should be considered. Given the higher prevalence of thyroid hypoplasia in older patients, thyroid ultrasound should be performed regularly until adulthood. In case of overt hypothyroidism treatment with levothyroxine should be promptly initiated. SH may be a self-remitting condition, so that ongoing monitoring without treatment initiation may be considered. However, levothyroxine replacement therapy is advisable, especially in children younger than 3 years, if TSH levels are persistently elevated, in the presence of thyroid hypoplasia and/or suggestive signs and symptoms (**Table 2**). In addition, considered the increased cardiovascular risk associated with WS, treatment could be likely of benefit for some of these patients in order to prevent further cardiovascular impairment (157, 159).

PRADER-WILLI SYNDROME

Prader-Willi syndrome (PWS) is a genomic imprinting disorder due to the lack of expression of paternally inherited genes within the chromosome region 15q11-q13. *De novo* paternally derived deletions of the chromosome 15q11-q13 region account for around 75% of cases, maternal uniparental disomy (UPD) for 24% and defects in the genomic imprinting center, chromosomal translocations or rearrangements within 15q11.2-q13 region for 1–3%, respectively. It is considered to be the most frequent form of syndromic obesity, affecting 1:10.000 to 1:30.000 people (168–170). The major clinical features of the disorder include neonatal hypotonia with feeding problems and failure to thrive during infancy, followed by excessive eating and rapid weight gain in early childhood, accompanied by global developmental delay and behavioral problems. Short stature, distinctive facial appearance, small hands and feet, cryptorchidism, and scoliosis are also common findings (169–171). Hypothalamic dysfunction may underlie many components of the syndrome, including hyperphagia, sleep abnormalities, temperature dysregulation, and multiple pituitary hormone insufficiencies, including growth hormone (GH), adrenocorticotropic hormone (ACTH), TSH deficiencies, hypogonadotropic hypogonadism, but also, less commonly, precocious puberty (170, 172, 173). Pituitary gland abnormalities have been reported in more than 60% of cases regardless of the presence or number of pituitary deficiencies. Those may include empty sella, pituitary hypoplasia, small, globular, or displaced posterior pituitary gland or complete absence of the posterior pituitary gland bright spot (172, 173).

The prevalence of hypothyroidism in PWS, mainly of central origin, varies widely from 2.1 to 32%, with only few studies addressing this issue (172–180). In a recent study comprising 339 individuals with PWS (71.7% aged below 18 years), the frequency of thyroid dysfunction was 13.6%: 6.8% had central hypothyroidism (of whom 43.5% were younger than 1 year), 3.8% subclinical hypothyroidism, 1.8% hypothyroidism and 1.2% CH (179) (**Table 1**).

Vaiani et al. examined the thyroid function in infant PWS patients, revealing a frequency of 72.2% of central hypothyroidism during the first 2 years of life. Moreover, patients with thyroid dysfunction displayed lower body length which correlated positively to FT4 levels, indicating that early onset hypothyroidism may impair normal growth (175). In contrast,

studies conducted in older PWS patients reported a far lower prevalence of hypothyroidism (172–174, 176, 177), indicating that the thyroid dysfunction observed at very young PWS children might be associated with delayed CNS development and normalize with further CNS maturation, as these patients get older (175). Despite low T4 and/or FT4 levels in most patients in the study of Vaiani et al., T3 levels were low in just one case. This finding indirectly suggests, that low FT4 levels may be compensated for by an increase in peripheral conversion of T4 to T3 (175). Of note, leptin, produced by adipose tissue in proportion to its mass, increases deiodinase type 2 activity. Interestingly, young, still underweight children were shown to have relatively increased body fat and elevated BMI-adjusted leptin levels (181). In the study of Sharkia et al., PWS children had FT4 levels in the lower half and FT3 above the median of the normal range, findings that could be interpreted within the same context (176). GH replacement therapy may also contribute to the decrease in FT4 concentrations, reflecting either a central inhibition of TSH release due to increased somatostatinergic tone or increased peripheral conversion from FT4 to T3, which could further lead to T3 negative pituitary feedback (178, 180).

Notably, concurrent CH with fetal goiter or due to ectopic, sublingual thyroid gland has been described in association with PWS (182–184) (**Table 1**). Taken into account that signs and symptoms of hypothyroidism and PWS may overlap, the diagnosis of PWS should be considered in cases with CH and severe infantile hypotonia that do not improve, despite appropriate levothyroxine replacement therapy.

Given that endocrine dysfunction in PWS is mainly of hypothalamic origin, thyroid autoantibodies were measured in none but one study, reporting slightly positive TPO antibodies in one out of 21 PWS patients (176). Of interest, autoantibodies against pituitary (APA) were recently detected in 30.9% of 55 PWS patients, a frequency far higher than in healthy controls (**Table 1**). In addition, APA were more common in those with uniparental maternal disomy for chromosome 15 than in those with interstitial deletion of the proximal long arm of paternal chromosome 15. Based on these findings, it can be postulated that autoimmune processes may be involved, at least in part, in the pituitary impairment associated with PWS. However, given that the rate of positive APA did not differ among those with and without pituitary deficiencies, their clinical significance remains to be determined (185).

Based on published reviews and expert recommendations, TSH and FT4 levels should be measured within the first 3 months of life, regardless of the newborn screening result, taken into account that TSH-based screening strategies cannot detect central hypothyroidism. Subsequently, TSH and FT4 should be determined annually, with consideration of more frequent testing if the patient receives treatment with rGH. Appropriate thyroid hormone replacement therapy should be initiated, if indicated. Taken into account the higher frequency of hypothyroidism during the first 2 years of life, as well as, the detrimental effects of untreated hypothyroidism for somatic growth and development, that could be further compromised in PWS patients, particular attention should be paid in infancy and early childhood, to ensure adequate thyroid hormone levels (168, 171, 178) (**Table 2**).

RASOPATHIES: NOONAN SYNDROME AND NEUROFIBROMATOSIS TYPE 1

The RASopathies are a group of neurodevelopmental syndromes caused by germline mutations in genes encoding protein components or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway, a ubiquitous signaling transduction pathway activated by a large number of extracellular stimuli (growth factors, hormones, cell/cell interaction) to regulate essential cellular functions, such as proliferation, survival, differentiation, migration, or metabolism. Ras proteins are small guanosine nucleotide-bound GTPases activated following binding of a growth factor to receptor tyrosine kinases (RTKs), G-protein-coupled receptors, cytokine receptors, and extracellular matrix receptors. They alternate between a guanosine diphosphate–bound inactive state to a guanosine triphosphate (GTP)–bound active state. Ras-GTP can activate several downstream effector pathways namely, phosphatidylinositol 3-kinase (PI3K), RAL guanine nucleotide exchange factor and the RAF kinases, the first MAPK kinase of the pathway. RAS-MAPK signaling dysregulation has profound pathophysiological consequences. Somatic mutations of genes encoding RAS-MAPK components resulting in RAS/MAPK pathway hyperactivation have been found in several types of cancer, whereas germline mutations are causally linked to RASopathies. This term designates a group of clinically related disorders sharing overlapping clinical features, including craniofacial dysmorphism, short stature, cardiac malformations, cutaneous lesions, musculoskeletal, and ocular abnormalities, neurocognitive dysfunction of variable degree, and an increased risk of cancer. RASopathies affect ∼1 in 1,000 live births and include the following conditions (and associated gene mutations): Neurofibromatosis type 1 (NF1) (*NF1* gene), Noonan syndrome (NS) (activating mutations in *PTPN11, SOS1, RAF1, KRAS, NRAS, SHOC2, CBL*), Noonan syndrome with multiple lentigines (NSML) (*PTPN11, RAF1*), capillary malformation–arteriovenous malformation syndrome (CM-AVM) (haploinsufficiency of *RASA1*), Costello syndrome (CS) (activating mutations in *HRAS*), cardio-facio-cutaneous syndrome (CFC) [activating mutations in *BRAF, MAP2K1* (*MEK1*) or *MAP2K2* (*MEK2*)], and Legius syndrome (inactivating mutations in *SPRED1*) (186, 187).

NS is a multisystem genetic disorder characterized by dysmorphic craniofacial features, including a broad forehead, hypertelorism with down-slanting palpebral fissures, ptosis, low-set posteriorly rotated ears with a thickened helix, short or webbed neck, congenital heart disease (most commonly pulmonary valve stenosis, hypertrophic cardiomyopathy and atrial septal defects), short stature, chest deformities, lymphatic dysplasias, ocular abnormalities, cryptorchidism, learning difficulties, short stature, renal anomalies, hearing loss and developmental delay of variable degree (188–190). Furthermore, affected patients have an 8-fold increased risk of developing childhood cancer, including juvenile myelomonocytic leukemia, acute myelogenous leukemia, B-cell acute lymphoblastic leukemia, whereas cases with solid tumors, such as rhabdomyosarcoma and neuroblastoma have been

also reported (190–192). NS occurs in 1:1000 to 1:2500 live births (190) and is the second most frequent syndromic cause of congenital heart disease after Down syndrome (190, 191). The diagnosis is primarily based on clinical grounds, however causative gene mutations can be identified in around 70% of cases (193). The disorder follows mostly autosomal dominant inheritance with a near complete penetrance but a considerable variable expressivity (188, 194). Sixty percentage of affected individuals have *de novo* mutations (193). Around 50% of patients harbor mutations in the *PTPN11*, 10–15% in the *SOS1*, 3% in the *KRAS* and 3–15% in the *RAF1* genes, enhancing the function of the RAS/RAF-MAPK pathway (190). *PTPN11* (tyrosine-protein phosphatase non-receptor type 11) encodes for the non-receptor protein tyrosine phosphatase SHP-2 (Src homology phosphotyrosyl phosphatase 2). The majority of PTPN11 missense, gain-of-function mutations associated with NS primarily impair the activation/inactivation molecular switch of SHP2, resulting in constitutive or prolonged activation of the protein and increased activation of the Ras/MAPK pathway (186, 191).

Endocrine disorders associated with NS include short stature, growth hormone deficiency, neurosecretory dysfunction, and GH resistance, delayed puberty, diminished pubertal growth spurt, cryptorchidism and male gonadal dysfunction (191, 194). The frequency of thyroid autoantibodies in NS has been reported in a few studies, varying between 14.3 and 60%, whereas hypothyroidism occurs less commonly (4–21.4%) (195–199). In the study of Quaio et al., comprising 42 patients with RASopathies, the majority of whom had NS, 17% had subclinical hypothyroidism without thyroid antibodies, 7% were euthyroid having positive thyroid autoantibodies and another 7% presented overt autoimmune hypothyroidism (199) (**Table 1**).

On the other hand, in the study of Svensson et al., the frequency of thyroid antibody positivity did not differ between children with NS and controls. However, it tended to increase at puberty and with age in NS patients. In particular, 30% of those aged 12 years or older but none among those younger than 12 years had positive thyroid autoantibodies (195), suggesting that testing for thyroid autoantibodies should be performed periodically during adolescence in patients with NS.

Interestingly, accumulated evidence points toward a possible link between NS and autoimmune disorders. Scattered cases with NS and coexistent vasculitis, vitiligo, thyroiditis, anterior uveitis, SLE, CD have been anecdotally described in the literature (196, 200, 201). More recently, Quaio et al. revealed the presence of autoantibodies in 52% and autoimmune diseases in 14% of 42 patients with RASopathies, 37 of whom had NS. Those included autoimmune thyroiditis, SLE, polyendocrinopathy (association of autoimmune thyroiditis and CD), antiphospholipid syndrome, vitiligo, and autoimmune hepatitis and all occurred in patients with *PTPN11* mutations (199) (**Table 1**). At this point, it is worth mentioning, that one of the SLE susceptibility loci has been identified at 12q24, a locus encompassing *PTPN11*, germline mutations of which are found in 50% of NS cases. The linkage of a susceptibility gene for SLE to a region implicated in NS lends further support for a possible association between NS and SLE (199, 200, 202).

In this regard, the activity of SHP2 (the protein product of *PTPN11*) was shown to be increased in both lupus-prone mice and in human SLE patients. Of interest, inhibition of SHP2 activity diminished skin lesions, increased life span, reduced lupus-associated organ damage, blocked abnormal T cell proliferation, normalized extracellular signal regulated kinase ERK/MAPK signaling, and decreased production of IFN-γ and IL-17A/F in lupus-prone mice. In addition, SHP2 inhibition reduced the proliferation of cultured human lupus T cells and decreased the production of IFN-γ and IL-17A/F *in vitro*, suggesting integral involvement of SHP2 in human lupus-associated immunopathology (203). Moreover, SHP-2 inhibits activation of human NK cells upon recruitment to killer cell Ig-like receptors (KIR), but may also inhibit both cytolytic activity and IFN-γ secretion by NK cells independently of its role in KIR signaling (204, 205). SHP-2 also functions as a regulator of NF-κB/transcription factor nuclear factor κB activation, which in turn plays a critical role in various biological processes, including immune response, inflammation, cell survival and oncogenesis (206). Given that NK cells and NF-κB are important components of the immune system, gain-of-function mutations of *PTPN11* might play a role in the development of autoimmunity (200). Further to the above, SHP2 may mediate inhibitory receptor tyrosine kinase signaling in immune cells and the consequent inactivation of immunoregulatory receptors might contribute to the development of the autoimmune-like phenotypes (203), as well. Overall, activation of Ras signaling pathway in response to T cell receptor (TCR) stimulation is essential for T cell development, differentiation and activation. Proper regulation of Ras signal transduction plays a critical role in both normal immune responses and the maintenance of tolerance. On the other hand, both increased and decreased Ras activities may be implicated in lupus-like autoimmunity, while both under- and overactivities of disparate Ras effectors have been also linked to autoimmune responses (207) (**Table 1**).

NF1 is an autosomal dominant multisystemic disorder with an estimated incidence of ∼1 in 2,500–3,000 individuals, affecting primarily the bone, the nervous system, soft tissue and the skin. About 50% of cases are caused by *de novo* (spontaneous) mutations. The diagnosis is usually made on clinical grounds, whereas genetic testing may be helpful in cases with unusual presentations or for reproductive decision-making. The presence of two or more of the following clinical features are required to establish a diagnosis of NF1: (1) ≥6 cafe'-au-lait macules >0.5 cm at largest diameter before puberty or >1.5 cm in diameter after puberty (2) axillary or inguinal freckling, (3) ≥2 Lisch nodules (4) ≥neurofibromas or ≥1 plexiform neurofibroma (5) an optic pathway glioma (OPG), (6) a distinctive osseous lesion (sphenoid wing dysplasia, long-bone dysplasia), and (7) a first-degree relative with NF1. NF1 patients have an increased risk of developing both benign and malignant tumors, including OPG, glioblastoma, malignant peripheral nerve sheath tumor, gastrointestinal stromal tumor, breast cancer, leukemia, phaeochromocytoma, duodenal carcinoid tumor, and rhabdomyosarcoma. Associated manifestations may include cardiovascular abnormalities, neurocognitive impairment and craniofacial dysmorphism (186, 208–210).

NF1 is caused by germline loss-of-function mutations in the *NF1* tumor-suppressor gene, located on chromosome 17q11.2 and encoding a large cytoplasmic protein called neurofibromin. Neurofibromin is a GTPase activating protein (GAP) that negatively regulates the Ras signal transduction pathway, by accelerating the conversion of active GTP-bound Ras to inactive GDP-bound Ras. Thus, loss of neurofibromin expression results in hyperactivation of RAS, as well as constitutive downstream MAPK and the mammalian target of rapamycin (mTOR) pathways (208, 211, 212).

Endocrinopathies are frequently observed in patients with NF1 and are usually related to OPGs involving the hypothalamic and sellar region and associated treatment modalities. Those may include short stature, central precocious puberty (CPP), diencephalic syndrome, GH and other pituitary deficiencies, GH hypersecretion and obesity with insulin resistance/impaired glucose tolerance (213, 214). Endocrine disorders were reported in 55.6% of children with NF1 and OPG who did not receive radiotherapy or surgical resection (214). Of note, hypopituitarism or CPP may occur even in the absence of intracranial lesions in NF1 patients. In this regard, GH deficiency was demonstrated in 15 out of 19 NF1 short children who had no intracranial tumors or other recognizable risk factors for short stature (215).

The number of studies reporting an association between NF1 and autoimmune disorders is small but increasing. NF1 cases with multiple sclerosis, SLE, membranous glomerulonephritis, IgA nephropathy, mixed connective tissue disease, myasthenia gravis, ankylosing spondylitis, JIA, CD, autoimmune hemolytic anemia, bullous pemphigoid, vitiligo, alopecia areata, T1D but also autoimmune thyroiditis and GD have been scarcely described in the literature (216–223) (**Table 1**). More recently, Güler et al., conducted a case-control study aiming to assess a possible relationship between NF1 and thyroid disorders. This study revealed that the frequency of autoimmune thyroiditis was not higher compared to the one reported in the general population. In particular, out of 78 NF1 children enrolled, 6.4% had goiter, 2.5% positive antithyroid antibodies, 1.2% SH and 3.8% low TSH levels, possibly related to impaired hypothalamo-hypophyseal-thyroid axis, which normalized after 1 year (224) (**Table 1**). With regards to the latter finding, TSH deficiency has been previously reported in NF1 children in the absence of intracranial tumors (225). It is worth mentioning, that neurofibromin functions as positive regulator of the enzyme adenylyl cyclase and cyclic adenosine monophosphate (cAMP) generation in the brain (213, 226). In particular, neurofibromin was shown to regulate hypothalamic function and pituitary development in the mammalian central nervous system by modulating intracellular cAMP levels. Interestingly, *NF1* inactivation in mice resulted in reduced body weights and anterior pituitary hypoplasia with a 40–60% reduction in the levels of growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH) and TRH mRNA levels compared to wild type controls as well as reduced GH, prolactin and insulin-like growth factor 1 (IGF-1) mRNA levels (212).

The mechanisms underlying a possible association between autoimmune disorders and NF1 have not been elucidated. Studies in mice have shown that loss of neurofibromin results in decreased Fas antigen expression and Fas-ligand mediated apoptosis via hyperactivation of the p21ras-class IA PI-3K signaling pathway. Suppressed Fas ligand expression may prevent apoptosis of CD4+ T cells, which may contribute to the development of autoimmunity (219, 227). Furthermore, NF1-deficient mice exhibited lymphoproliferative defects, including thymic and splenic hyperplasia, increased numbers of immature and mature T cells *in vivo*, but reduced proliferation in response to TCR and interleukin-2 receptor (IL-2R) stimulation *in vitro* (228) as well as defective proliferative responses in B lymphocytes and thymocytes (229) (**Table 1**).

Overall, despite the lack of sufficient evidence, the growing number of reports on the concurrence of NF1 and autoimmune disorders implies a possible link between these conditions rather a coincidence.

Hypothyroidism/CH and thyroid hypoplasia have been scarcely described in the context of CFC, CS, CM-AVM, and Legius syndrome, however, to the best of our knowledge there are no reports on the coexistence of these RASopathies with ATD (230–233).

According to current recommendations for the care of children with NS, thyroid function tests including measurement of thyroid antibodies should be performed in all children with signs or symptoms of hypothyroidism (goiter, fatigue, constipation, poor growth, etc.) and every 3–5 years in older children and adults (194, 234). Thyroid disorders should be managed as in the general population (**Table 2**). Similarly, diagnosis and management guidelines for patients with CFC recommend thyroid function screening (TSH, FT4) at diagnosis, given that thyroid abnormalities can be observed in patients with other RASopathies and autoimmune thyroiditis is frequent in the general population. During follow-up thyroid function tests should be performed in case of growth failure. Only a few cases of hypothyroidism have been described in CFC, therefore the need for ongoing thyroid function testing can be evaluated by the endocrinologist (235) (**Table 2**). On the other hand, health supervision guidelines for NF1 children recommend annual evaluation of growth rate and pubertal development but lack information on thyroid function monitoring (236, 237). Taken into consideration (a) that TSH deficiency may be seen in affected individuals in the absence of intracranial tumors, (b) a possible although not well-documented association between NF1 and ATD, (c) the phenotypic similarities and common underlying pathogenetic etiology with NS and other RASopathies, it would be advisable to apply to NF1 patients the same recommendations on thyroid function monitoring as for NS patients (**Table 2**).

CONCLUSION

In recent years, several research studies have shed light on the complex pathogenesis of genetic syndromes-associated ATD. The presence of chromosomal abnormalities may influence the

phenotypic expression of thyroid autoimmunity, in particular with regards to age of onset of thyroidopathy, evolution of thyroid function and even clustering of extra-thyroidal autoimmune disorders in affected children. While thyroid dysfunction is namely of autoimmune origin in TS, DS, and KS, thyroid hypoplasia and central hypothyroidism account for the majority of cases in WS and PWS, respectively. Patients with 22q11.2DS have an increased risk for developing GD, however, thyroid gland anomalies are also frequently seen in affected individuals. In addition, there is emerging evidence supporting a possible link between autoimmunity and RASopathies. This comprehensive review highlights the need for an early screening, and regular life-long monitoring of the thyroid function in patients with specific genetic syndromes, along with a thorough evaluation that will enable early identification and proper management of both thyroid and extrathyroidal autoimmune disorders and associated endocrinopathies, in order to optimize the health care provided to these patients.

AUTHOR CONTRIBUTIONS

CK-G contributed to the study design, critically revised the manuscript, and approved the submitted version. EK was involved in the literature research and drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Genetic Association Study of IL2RA, IFIH1, and CTLA-4 Polymorphisms With Autoimmune Thyroid Diseases and Type 1 Diabetes

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Autoimmune thyroid diseases (AITDs) which include Graves' disease (GD) and Hashimoto's thyroiditis (HT) as well as type 1 diabetes (T1D) are common autoimmune disorders in children. Many genes are involved in the modulation of the immune system and their polymorphisms might predispose to autoimmune diseases development. According to the literature genes encoding IL2RA (alpha subunit of Interleukin 2 receptor), IFIH1 (Interferon induced with helicase C domain 1) and CTLA-4 (cytotoxic T cell antigen 4) might be associated with autoimmune diseases pathogenesis. The aim of the study was to assess the association of chosen single nucleotide polymorphisms (SNPs) of IL2RA, IFIH1, and CTLA-4 genes in the group of Polish children with AITDs and in children with T1D. We analyzed single nucleotide polymorphisms (SNPs) in the IL2RA region (rs7093069), IFIH1 region (rs1990760) and CTLA-4 region (rs231775) in group of Polish children and adolescents with type 1 diabetes ($n = 194$) and autoimmune thyroid diseases (GD $n = 170$, HT $n = 81$) and healthy age and sex matched controls for comparison ($n = 110$). There were significant differences observed between T1D patients and control group in alleles of IL2RA (rs7093069 T > C) and CTLA-4 (rs231775 G > A). In addition, the study revealed T/T genotype at the IL2RA locus (rs7093069) and G/G genotype at the CTLA-4 locus (rs231775) to be statistically significant more frequent in children with T1D. Moreover, genotypes C/T and T/T at the IFIH1 locus (rs1990760) were significantly more frequent in patients with T1D than in controls. We observed no significant differences between AITD patients and a control group in analyzed SNPs. In conclusion, we detected that each allele T of rs7093069 SNP at the IL2RA locus and G allele of rs231775 SNP at the CTLA-4 locus as well as C/T and T/T genotypes

of rs1990760 SNP at the IFIH1 locus are predisposing in terms of T1D development. Thereby, we confirmed that IL2RA, IFIH1, and CTLA-4 gene locus have a role in T1D susceptibility. The analysis of selected SNPs revealed no association with AITDs in a group of Polish children and adolescents.

Keywords: Graves' disease (GD), Hashimoto's thyroiditis (HT), type 1 diabetes (T1D), genetic susceptibility, single nucleotide polymorphism (SNP), IL2RA, IFIH1, CTLA-4

INTRODUCTION

The underlying cause of autoimmune diseases is the loss of immune tolerance to tissue-specific antigenic peptides which leads to immune response directed against one's own body's cells. Still not completely understood, complex immune mechanisms including the dysfunction of the immune system might be involved in the autoimmune diseases pathogenesis (1). Among the most common chronic autoimmune endocrine disorders in children there are autoimmune thyroid diseases (AITDs) which include Graves' disease (GD) and Hashimoto's thyroiditis (HT) as well as type 1 diabetes (T1D) (2). In children with autoimmune thyroiditis immune reactions are directed against the cells of thyroid gland. In GD the thyrotropin receptor (TSH-R) is activated with antibodies causing the overactivity of the thyroid gland, while in HT humoral and cell-mediated thyroid injury leads to destruction of thyroid cells and hypothyroidism as a consequence (3). In diabetic patients an inappropriate immune reaction results in autoreactive T-cell infiltration and production of tissue specific autoantibodies which cause the destruction and dysfunction of the insulin secreting pancreatic beta cells and insulin deficiency (4). The mechanisms leading to development of these diseases remain unknown, however numerous data indicate that apart from the environmental factors there is a strong genetic susceptibility to the autoimmune diseases (5–8). The relevance of genetic factors is evident from clustering of AITDs or T1D within families, in particular monozygotic and dizygotic twins (9, 10). Many genes might be involved in the modulation of the immune system and some of them were recently found to influence autoimmune endocrine disorders development. Moreover, recent studies have demonstrated that some genetic risk factors for autoimmunity are shared between diseases, contributing to the development of more than one autoimmune disorder (10). Current publications showed association between autoimmune diseases and chromosome 10p15 region for IL2RA (interleukin 2 receptor-α), chromosome 2q33 region for CTLA-4 (cytotoxic T-lymphocyte antigen-4) and chromosome 2q24 region for IFIH1 (interferon induced with helicase C domain 1) (11, 12). The most frequent type of human genome variation are single nucleotide polymorphisms (SNPs) providing powerful tools for a variety of medical genetic studies (13). Although certain polymorphic variants of genes encoding IL2AR, CTLA-4, or IFIH1 have been reported to implicate T1D and ATDs development in adults, there are only few studies focusing on children (14–18).

Interleukin 2 (IL2) is a lymphocytes growth factor playing an important role in modulation of immune homeostasis as an essential self-tolerance regulator (19, 20). Its action is mediated by a quaternary receptor signaling complex (IL2R) containing α, β and a common γ chain receptors (21, 22). Alpha subunit of the IL2 receptor, IL2Rα (also known as CD25), encoded by the interleukin 2 receptor α gene (IL2RA), plays a key role in mediating interleukin 2 immunoregulatory function. The expression of IL2RA has been described at high levels on the surface of the regulatory T cells (Tregs), a population of T cells with an ability to inhibit autoreactive T cells (23). Further studies indicated IL2RA's essential role in sensitizing T cells for induced cell death (22) that is crucial for their function as a suppressor for T cell immune responses to auto-, alloantigens, as well as tumor antigens and antigens deriving from pathogens (24). SNPs of genes influencing Treg function, such as IL2RA, may cause an increased risk of autoimmune disease.

Interferon induced with helicase C domain 1 (IFIH1) also known as Helicard or melanoma differentiation-associated gene 5 (MDA-5), plays an essential role in body immune reactions against viruses. IFIH1 belongs to the family of RNA helicases binding viral RNA (25, 26). IFIH1 protein acts as a detector of viral double strand RNA (dsRNA) and causes the apoptosis of virally infected cells (27). The studies suggest that variants of genes involved in the inflammation responses might have the potential to alter their function and expression (28). Previously established relationships between autoimmune diseases development and viral infection might have a molecular basis provided by genetic variants of IFIH1 (29). The SNPs of IFIH1 could cause the abnormal activation of antiviral defenses signaling leading to the autoimmune disease development.

Cytotoxic T-lymphocyte antigen-4 (CTLA-4), also known as CD152, encodes T cell receptors responsible for the attenuation of immune response. CTLA-4 acts by delivering an inhibitory signal decreasing cytokine production, activation and proliferation of T lymphocytes (30–32). Polymorphic variants of CTLA-4 gene are implicated in dysregulation of immune homeostasis due to an aberrant activation of T-lymphocytes in the periphery which may cause the infiltration of glands leading to their dysfunction and autoimmune disease development. According to the literature, common CTLA-4 polymorphisms have been found to confer susceptibility to T1D, AITDs (12, 33) and other autoimmune disorders (34, 35).

Since it has been suggested that multiple genes are associated with pathogenesis of autoimmune disorders and some autoimmune diseases might share the same genetic background of co-occurrence within individuals and families, the aim of the study was to assess the association of chosen single nucleotide polymorphisms of IL2RA, IFIH1, and CTLA-4 genes in the group

of Polish children with AITDs and in children with T1D. In our study we hypothesized that the same polymorphisms of IL2RA, IFIH1, or CTLA-4 genes might be associated with AITDs and might predispose to T1D development.

MATERIALS AND METHODS

We performed this original research study in the group of 81 HT patients (mean age, 15.2 ± 2.2 years) 170 GD patients (mean age, 16.5 ± 2 years) recruited from the Outpatient Clinic in Bialystok and 194 patients with T1D (mean age, 10.18 ± 3.4 years) recruited from the Outpatient Clinic in Lodz. None of the patients suffered from more than one of these conditions. The qualifying criteria for patients and controls are presented in **Figure 1**. AITDs were diagnosed according to the Polish Endocrinology Association guidelines which correspond with the guidelines of the European Society for Pediatric Endocrinology. The inclusion criteria for patients with AITDs were based on medical history, physical examination, laboratory and ultrasound investigations. GD was diagnosed in children with large goiter, hyperthyroidism in laboratory tests and positive thyrotropin receptor antibodies (TR-Ab). HT patients developed clinical and biochemical symptoms of hypothyroidism and demonstrated presence of anti-TPO and/or anti-TG autoantibodies. T1D was diagnosed according to the Polish Diabetes Association guidelines which correspond with the guidelines of the WHO and was based on clinical symptoms, hyperglycemia, low fasting C-peptide levels and the presence of diabetes autoantibodies (islet cell antibodies - ICA, glutamic acid decarboxylase antibodies – GAD, insulin autoantibodies – IAA, zinc transporter 8 autoantibodies - ZnT8 or antibodies to protein tyrosine phosphatase - IA2). The control group consisted of 110 healthy volunteers (mean age, 16.3 ± 3 years). All controls had no history of HT, GD or T1D, were euthyroid and had no thyroid and diabetes autoantibodies. For the treatment patients with GD received methimazole at a dose of 0.3–1.0 mg/kg/d together with propranolol (0.5–1.0 mg/kg/d) orally. HT patients were treated with L-thyroxine (1 mcg/kg/d) orally. Diabetic patients were receiving insulin in appropriate doses. Before enrolment, all parents of patients and controls and all children over 16 years old gave informed consent. The study protocol was accepted by the Local Ethical Committee at the Medical University of Bialystok and adheres to the Declaration of Helsinki. Additional information regarding the study subjects are shown in **Table 1**.

Blood for analysis was collected in the morning from the basilic vein. Serum levels of thyrotropin (TSH), free thyroxine (fT4), and free triiodothyronine (fT3) were evaluated on electrochemiluminescence "ECLIA" with Cobas E411 analyzer (Roche Diagnostics). Ranges for TSH were: 0.28–4.3 (μIU/l), for fT4: 1.1–1.7 ng/dl and for fT3: 2.3–5.0 pg/ml. Antibodies against TSH-Receptor (TR-Ab), Thyroid Peroxidase (TPO) and Thyroglobulin (TG) were determined using ECLIA with Modular Analytics E170 analyzer (Roche Diagnostics). The positive values were: > 1.75 U/l for TR-Ab, > 34 IU/mL for anti-TPO-Ab and > 115 IU/mL for anti-TG-Ab. The conventional anti-diabetes autoantibodies were detected in serum samples: ICA with immunofluorescence, GAD, IA2, and ZnT8 by ELISA (RSR, USA) and IAA with RIA (CisBiointernational, France and RSR, USA). The positive values for ICA, GADA, IA2 and IAA positivity were 10 Juvenile Diabetes Foundation units, 10 U/ml and 20 U/ml and 7%/0.4 U/ml respectively.

The DNA was extracted with a classical salting-out method from the blood leukocytes. All study subjects were genotyped for SNPs at three loci: IL2RA (rs7093069), IFIH1 (rs1990760), and CTLA-4 (rs231775). TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) was used for all genotyping. For this, polymorphisms fluorogenic TaqMan probes were used. Reactions were performed in a 7900HT fast real-time PCR system (Applied Biosystems) according to the conditions: 10 min at 95 C for starting AmpliTaq Gold activity, 40 cycles of 95 C for 15 s and 60 C for 1 min. A sample without template served as a negative control and it was helpful to detect any false positive signal caused by contamination. All SNPs were analyzed in duplicates.

Median unbiased estimator (mid-p) of odds ratio, the exact confidence interval and associated *p*-value obtained with the mid-p method were used to determine any association between genotype or allele occurrence and patient's status (36). Either parametric or non-parametric methods, according to the normality and homogeneity of variance assumptions, were used to assess whether there are statistically significant differences between groups defined by genotypes and quantitative features. False discovery rate *p*-value adjustment method was applied due to the issue of multiple testing during the *post-hoc* analysis (37). As proposed in (38) measure D' of linkage disequilibrium was used. For all calculations *P*-value of < 0.05 was considered to be significant. The R software (Vienna, Austria) environment was exploited for all calculations (39). Statistical power calculation with respect to the total sample size was calculated with the use of G*Power ver. 3.1.9.6 software (40). Cohen's w was applied as a measure of effect size. Hardy-Weinberg Equilibrium was checked with the utilities of the genetics package (41).

RESULTS

Significant associations were observed between T1D patients and controls in alleles of IL2RA (rs7093069 T > C, $P = 0.013$, OR = 1.59, 95%CI = 1.10–2.34, the power of the test was 0.999, estimated effect size w was equal to 0.231, with the total sample size $n = 598$) (**Figure 2**) and CTLA-4 (rs231775 G > A, $P = 0.016$, OR = 1.50, 95%CI = 1.07–2.10, the power of the test was 0.9935, estimated effect size w was equal to 0.203, with the total sample size $n = 608$) (**Figure 6**). In addition the study revealed T/T genotype at the IL2RA locus (rs7093069) and G/G genotype at the CTLA-4 locus (rs231775) to be statistically significantly more frequent in children with T1D ($P < 0.001$, OR = 8.50, 95%CI = 2.42–58.38, the power of the test 1, estimated effect size w equal to 1.000, with the total sample size $n = 299$ and $P = 0.015$, OR = 2.34, 95%CI = 1.17–4.84, the power of the test was 0.997, estimated effect size w was equal to 0.320, with the total sample size $n = 304$ respectively) (**Figures 3**, **7**). Moreover, genotypes C/T and T/T at the IFIH1 locus (rs1990760) were significantly more frequent in patients with T1D than in controls ($P = 0.011$,

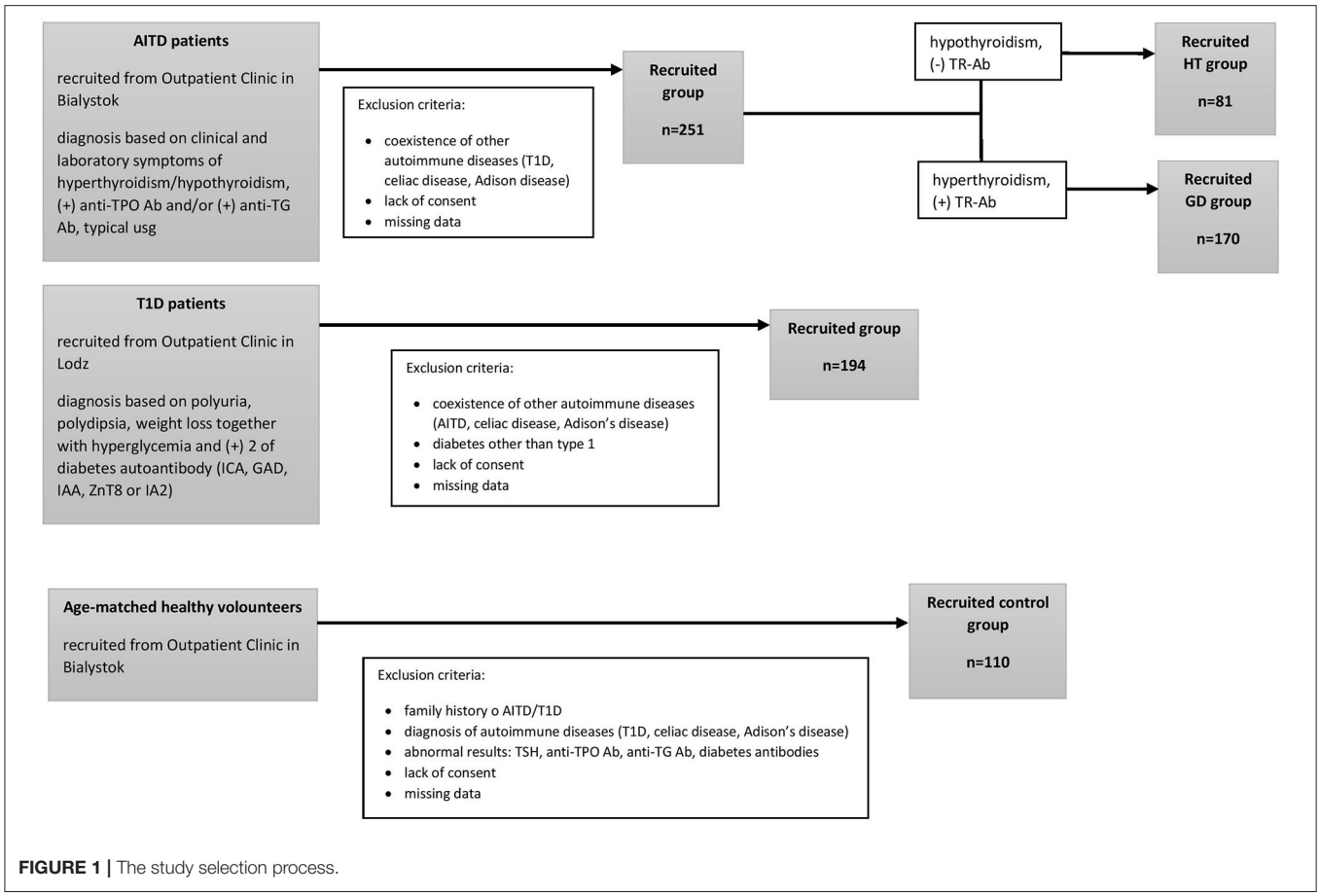

FIGURE 1 | The study selection process.

OR = 3.13, 95%CI = 1.29–8.00 and $P = 0.008$, OR = 3.36, 95%CI = 1.36–8.73 respectively, the power of the test 0.918, estimated effect size w was equal to 0.240, with the total sample size $n = 303$) (**Figure 5**). In contrast there were no significant differences between AITD patients and control group in analyzed SNPs. Further analysis revealed statistically significant differences between GD and T1D patients: T/T genotype at the IL2RA locus (rs7093069) was more frequent in T1D patients ($P = 0.03$, OR = 2.27, 95%CI = 1.06–5.22, the power of the test 1, estimated effect size w equal to 0.449, with the total sample size $n = 354$) (**Figure 3**), as well as T alleles, C/T and T/T genotypes at the IFIH1 locus (rs1990760) were more often in diabetic patients ($P = 0.003$, OR = 1.58, 95%CI = 1.16–2.14, the power of the test 0.998, estimated effect size w equal to 0.203, with the total sample size $n = 728$, $P = 1.69E-06$, OR = 5.82, 95%CI = 2.72–13.69 and $P = 1.74E-05$, OR = 4.99, 95%CI = 2.32–11.80, the power of the test 0.999, estimated effect size w equal to 0.402, with the total sample size $n = 364$, respectively) (**Figures 4**, **5**). Comparing HT children with T1D patients T allele and T/T genotype at the IL2RA locus (rs7093069) were statistically significant more frequent in patients with diabetes ($P = 0.002$, OR = 1.92, 95%CI = 1.25–3.02, the power of the test 0.999, estimated effect size w equal to 0.314, with the total sample size $n = 558$ and $P = 0.002$, OR = 4,96, 95%CI = 1.64–22.39, the power of the test 1, estimated effect size w equal to 0.449, with the total sample size $n = 354$, respectively) (**Figures 2**, **3**). C/T and T/T genotypes at IFIH1 locus (rs1990760) were more frequent in T1D patients than in HT children ($P = 0.001$, OR = 4.67, 95%CI = 1.86–12.33, and $P = 0.004$, OR = 3.78, 95%CI = 1.50–9.99 respectively, the power of the test 0.990, estimated effect size w equal to 0.309, with the total sample size $n = 282$) (**Figure 5**). G alleles, A/G and G/G genotypes at the CTLA-4 locus (rs231775) were more frequent in T1D than in HT group ($P = 0.009$, OR = 1.63, 95%CI = 1.12–2.38, the power of the test 0.999, estimated effect size w equal to 0.246, with the total sample size $n = 566$, $P = 0.003$, OR = 2.42, 95%CI = 1.32–4.45 and $P = 0.02$, OR = 2.29, 95%CI = 1.12–4.81 the power of the test 0.999, estimated effect size w equal to 0.385, with the total sample size $n = 283$, respectively) (**Figures 6**, **7**). There were no violations detected according to Hardy-Weinberg Equilibrium: $p = 0.7369$ for rs1990760, $p = 0.6331$ for rs231775 and $p = 0.3001$ for rs7093069.

DISCUSSION

Variants of IL2RA gene had been recently associated with susceptibility to several autoimmune diseases such as T1D (42), AITDs (16, 43), rheumatoid arthritis (44) or juvenile idiopathic arthritis (45) that implies the possible general effect on predisposition to autoimmunity of this region.

TABLE 1 | Clinical characteristics of patients with T1D, ATD and controls.

	T1D (mean ± SD)	*p**	HT (mean ± SD)	*p***	GD (mean ± SD)	*p****	Controls (mean ± SD)
n (F/M)	194 (88/106)		81 (59/22)		170 (126/44)		110 (50/60)
Age (years)	10.18 ± 3.9	<0.01	15.2 ± 2.2	NS	16.5 ± 2	NS	16.3 ± 3
Weight (kg)	43.4 ± 17.0	<0.01	58 ± 5.28	NS	55.19 ± 2.39	NS	60.9 ± 7.8
Height (cm)	148.5 ± 17.8	<0.01	154.26 ± 4.14	NS	162.19 ± 2.69	NS	160 ± 8
BMI (kg/m^2)	19.0 ± 3.9	<0.01	24.45 ± 1.33	NS	21.1 ± 2.1	0.012	23.78 ± 2.5
HBA1c	11.9 ± 2.9		-		-		-
TSH (mIU/l)	2.19 ± 1.57	NS	9.87 ± 4.37	<0.025	0.37 ± 0.1	<0.01	3.04 ± 0.72
fT4 (ng/dl)	-		1.21 ± 0.03	NS	3.6 ± 1.4	<0.001	1.1 ± 0.17
fT3 (pg/ml)	-		3.08 ± 0.5	NS	7.19 ± 1.65	<0.001	3.79 ± 0.18
TR-Ab (IU/l)[a]	-		0.5 ± 0.32	NS	11.56 ± 2.11	<0.001	0.4 ± 0.2
Anti-TPO Ab (IU/ml)	-		329.91 ± 92.93	<0.001	331.97 ± 58.12	<0.001	26.72 ± 6.8
Anti-TG Ab (IU/ml)	-		620.98 ± 240.34	<0.001	347.49 ± 86.7	<0.001	41.64 ± 12.1
ICA	65% (+)		-		-		-
GAD	79% (+)		-		-		-
IA2	67% (+)		-		-		-
ZnT8	63% (+)		-		-		-
IAA	43% (+)		-		-		-
Treatment	Insulin		L-thyroxine		Methimazole		-

[a] *TR-Ab were analyzed in selected group of patients with HT (n = 43).*
**Statistical significance between patients with T1D and controls.*
***Statistical significance between patients with HT and controls.*
****Statistical significance between patients with GD and controls.*

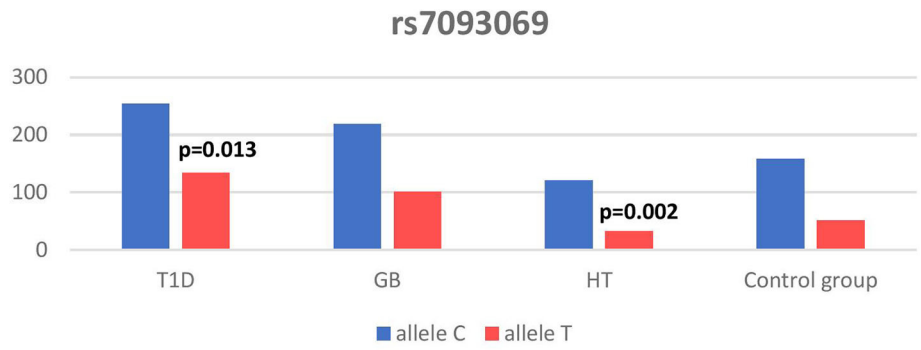

FIGURE 2 | Number of alleles in rs7093069 polymorphism (n) in the IL2RA region.

In our study T allele at the rs7093069 locus of the Il2RA gene was observed to be more frequent in diabetic patients compared to healthy children and HT individuals. Moreover, T/T genotype of analyzed IL2RA SNP was more frequent in T1D patients compared to healthy children as well as AITD children, indicating strong susceptibility of T allele and T/T genotype to diabetes development.

In the literature there are numerous reports demonstrating the association of SNPs in the IL2RA gene with T1D but none of them alone explains the predisposition to the disease. In the meta-analysis of Tang et al. authors analyzed the results of 10 independent studies of polymorphisms in the IL2RA gene. They confirmed rs11594656, rs2104286, and rs41295061 SNPs to be the most associated risk factors for T1D development (46), but the analysis did not investigate rs7093069 locus we analyzed in our study. Further studies of Qu et al. showed two other SNPs in the IL2RA gene that were significantly associated with T1D - rs706778 and rs3118470 (47). Moreover, Kawasaki et al. found in their study SNPs rs706778 and rs3118470 of the IL2RA in patients with T1D to be associated with acute-onset of the disease (24). According to the study of Lowe et al. ss52580101 locus in the IL2RA gene is most associated with T1D, however the authors emphasized the importance of other locus of this region (48). In addition they indicated that SNPs in IL2RA locus associated with T1D influence the soluble form of IL2RA concentration. Klinker et al. demonstrated the association of SNPs in the IL2RA locus to be an important determinant of age at diagnosis time in a group of Finnish type 1 diabetes subjects (49). The study of Fichna et al.

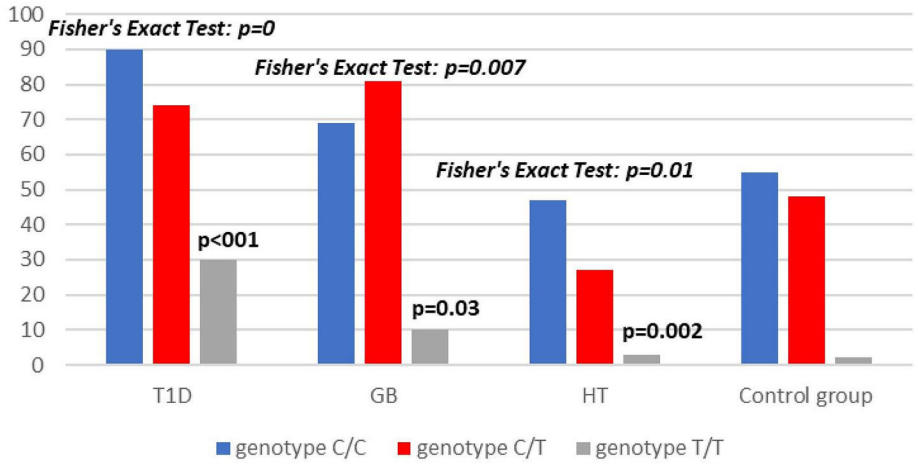

FIGURE 3 | Number of genotypes in rs7093069 polymorphism (n) in the IL2RA region.

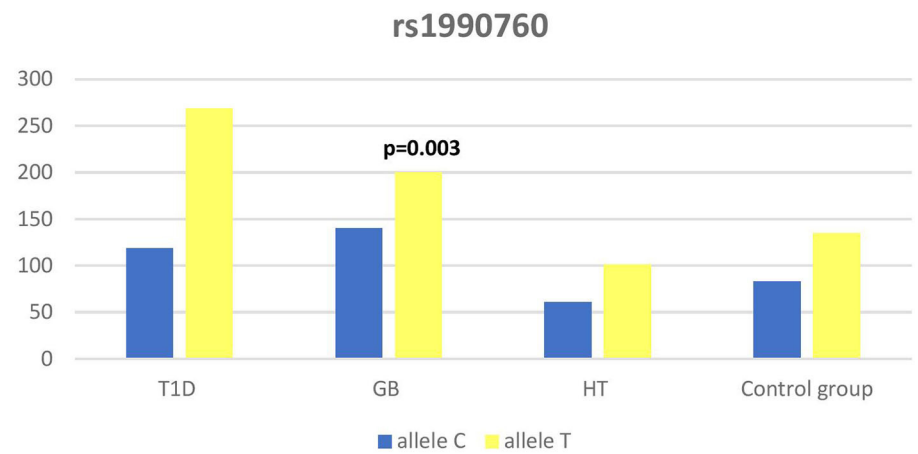

FIGURE 4 | Number of alleles in rs1990760 polymorphism (n) in the IFIH1 region.

in a group of 445 Polish T1D patients and 671 healthy control subjects confirmed the association of Il2RA single nucleotide polymorphism rs11594656 and rs3118470 but not for rs7093069, that was on the contrary to our study (50). In another work of the authors they indicate the association of other polymorphism (rs6822844) with the disease (51).

In our work, rs7093069 polymorphisms in the IL2RA gene did not show any correlation with AITDs in the population of Polish children. However, Chistiakov et al. examined other SNPs in the region and revealed the allele A of rs41295061 SNP to be significantly associated with increased risk of GD in a group of 1474 Russian patients (44). Furthermore, the study revealed that patients carrying two copies of the haploid genotype AA/AA had elevated levels of serum IL2Rα in both GD patients and healthy controls. Brand et al. found an association between GD and healthy control for the ILR2A region for 20 SNPs (among others rs7093069). The pattern of association was similar to that found in T1D patients (16). In our study there was no significant difference between AITD patients and controls in analyzed rs7093069 SNPs but we revealed statistically significant differences between GD and T1D patients in that locus, as T/T genotype was more frequent in T1D patients.

The IFIH1 gene locus has been recently defined as a candidate for susceptibility to autoimmune diseases like vitiligo, T1D and GD (14, 17, 52). Our current study confirms that the rs1990760 in IFIH1 gene is substantially associated with T1D as we found that genotypes C/T and T/T at the IFIH1 locus (rs1990760) is significantly more frequent in patients with T1D than in controls. The genetic predisposition of rs1990760 (A946T) SNP toT1D was first reported in a GWA (53). The data were recently confirmed

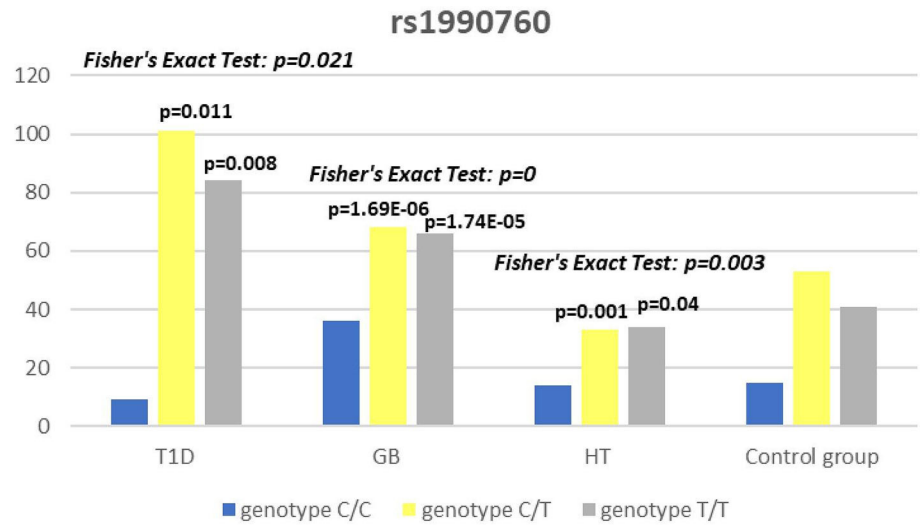

FIGURE 5 | Number of genotypes in rs1990760 polymorphism (n) in the IFIH1 region.

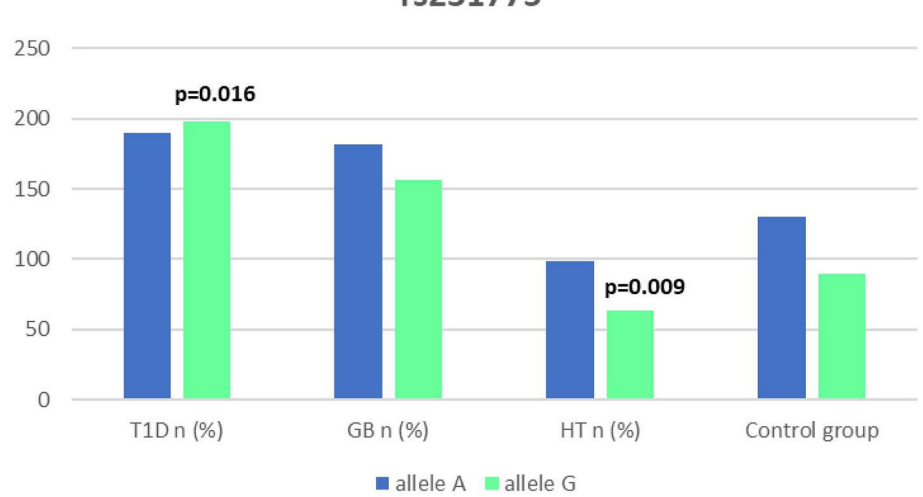

FIGURE 6 | Number of alleles in rs7231775 polymorphism (n) in the CTLA-4 region.

by the other investigators in the multipopulation analyses (54, 55). In the comprehensive meta-analysis by Jermendy et al. revealed that polymorphism in rs1990760 was associated with T1D in both Finnish population (with the high-incidence of the disease) and Hungarian population (with medium-incidence of the disease) and G allele (vs A allele) significantly decreased the risk of T1D (56). Furthermore, in the meta-analysis Cen and colleagues analyzed 19 studies and revealed that the IFIH1 rs1990760 T allele influences susceptibility to T1D and other autoimmune diseases like systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis (57). In a group of Polish adult individuals the association of allele A in rs1990760 locus with susceptibility to T1D was confirmed and the cumulative effect of other polymorphic variants in IFIH1 was observed (58). Genetic association between IFIH1 polymorphism and T1D development might be explained by the link of the disease with prior viral infection. Moreover, Jermedy et al. found in their work that there is a seasonal manifestation of T1D was related to rs1990760 polymorphism as the AA genotype, that predisposes to the disease, was more frequent in patients who developed the disease in summer than in patients with onset in winter indicating that this virus receptor gene might influence T1D manifestation mainly during the summer months (59).

Present work did not reveal any significant differences in allele or genotype frequencies for rs1990760 polymorphism between GD and HT patients in comparison to healthy control subjects in Polish population. In our study T alleles, C/T and T/T genotypes at the IFIH1 locus (rs1990760) were more often in

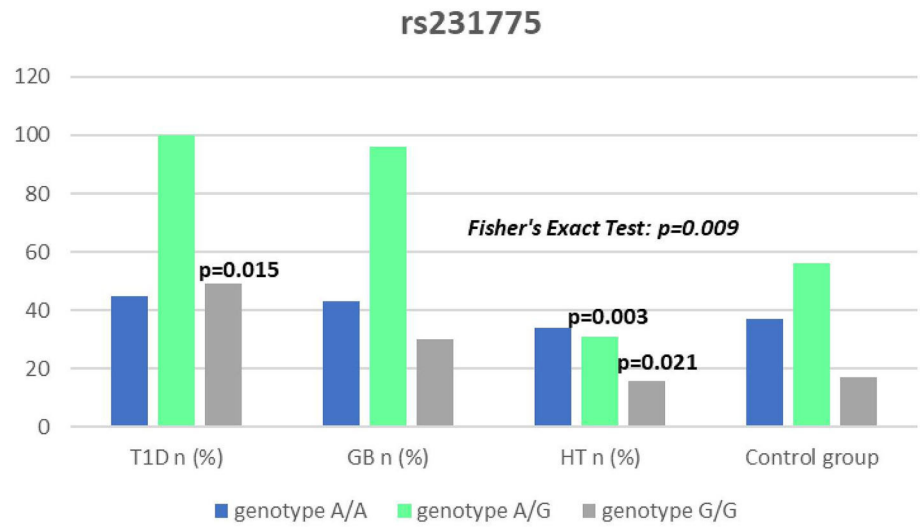

FIGURE 7 | Number of genotypes in rs321775 polymorphism (n) in the CTLA-4 region.

diabetic patients when compared with GD and HT patients. The association with GD and polymorphism rs1990760 (A946T) in IFIH1 gene was observed by Sutherland et al. in the study involving the United Kingdom population (17). In a previous analysis of Polish 142 pediatric patients with AITDs T alleles of rs1990760 were associated with GD in mails. Similarly in HT patients, rs1990760 T alleles were more frequent in males in comparison to healthy subjects (52). In contrast there were no significant differences for rs1990760 IFIH1 polymorphism in German patients with GD and HT in comparison to healthy control in the study of Penna-Martinez et al. (60). Similarly Zhao et al. observed no significant differences in the allele and genotype frequencies for this polymorphism between GD patients and healthy controls in Chinese population (61). In the previous mentioned meta-analysis the results of 19 studies suggested no effect of rs1990760 polymorphism on GD either (57). That stays in agreement with our results and may suggest the possible association between previous viral infection and T1D but not ATIDs development and link the gap between environmental triggers and the development of the T1D in genetically predisposed patients.

It has been proposed that the rs231775 (A49G) polymorphism in exon 1 of CTLA-4 causes a the amino acid replacement (threonine to alanine) influencing the posttranslational modification of CTLA-4, resulting in decreased expression of CTLA-4 on the surface of T-cells. The presence of G allele thus leads to increased activation of T-cell, either autoreactive, causing the autoimmune response, clinically manifested for instance as AITDs or T1D (32, 62). In our study we observed a significantly dominant effect of G allele in rs231775 SNP in CTLA-4 gene in children with T1D in comparison to healthy controls and children with HT. There was also a significantly higher incidence of G/G an A/G genotype in patients with T1D. This might be suggestive of an increasing susceptibility to T1D of G allele in rs231775 SNP. Similar observations result from the recent meta-analysis of Chen et al. The analysis of 76 studies revealed that rs231775 polymorphism was associated with susceptibility to T1D in Caucasians and South Asians, moreover rs231775 polymorphism was also found to be significantly associated with susceptibility to type 2 diabetes (T2D) in East Asians and South Asians (62). The results of another meta-analysis stays in agreement with that indicating that G allele of rs231775 in CTLA-4 increases the risk of T1D development (18).

We did not reveal any statistical differences between any of AITDs children and controls in our study, thus according to our study CTLA-4 gene rs231775 polymorphism seems not to contribute to HT nor GD development in Polish children population. In accordance with our results the study of Narooie-Nejad et al. indicated no association of alleles and genotypes of the A49G in CTLA-4 polymorphism with HT in a group of patients from Iran (63). Similar conclusions come out of the cohort of Japanese and Brazilian patients (64, 65). However, in the study of Fathima et al. the association between rs231775 polymorphism of CTLA-4 gene was reported in patients with AITDs. The study revealed that G allele in rs231775 contributes to an increased incidence in HT and GD (15). Similarly in the study of Tu et al. in Chinese population G allele of rs231775 was revealed to be significantly more often in subjects with GD than in control subjects (66). Ting et al. found in their work that CTLA4 SNPs A49G was associated with GD not only in Chinese adults but also and children (67). In the study of Patel et al. the G allele, GG and AG genotypes in SNPs A49G were also more prevalent among autoimmune hypothyroidism patients in Indian population (68). Interestingly, a significant association with A49G SNP in CTLA4 appeared for GD and HT in a group of 64 pediatric Polish patients. There were also a significantly higher antithyroid antibodies titres associated with T allele in GD, and with G allele in HT patients observed (69).

Our study certainly has some limitations. First, the analysis in our work did not take into consideration the influence of environmental components apart from genetic factors on AITD and T1D susceptibility. We also acknowledge that we could not distinguish whether the associated alleles were causative factors or just markers linked with disease loci. Another limitation of the study is a relatively small sample size.

Genome-wide association (GWA) investigations indicate chromosome regions associated with particular autoimmune diseases. However, the localization of the most likely susceptibility locus requires numerous studies with genotyping in large samples. Here we demonstrated the contribution of polymorphic variants of genes encoding IL2RA, CTLA-4, and IFIH1 to autoimmune diseases predisposition.

In conclusion the rs7093069 T allele and T/T genotype was preponderant in children with T1D in comparison to healthy children as well as AITDs subjects, likewise rs1990760 T allele or T/T genotype had increased frequency in diabetic patients. Moreover, rs231775 G allele, A/G and G/G genotypes were also prevalent in T1D patients when compared with controls and HT. In contrast, there was no association found in rs7093069, rs1990760, and rs231775 polymorphisms with AITD susceptibility in our study. Thus, IL2RA polymorphism (rs7093069), IFIH1 polymorphism (rs1990760), and CTLA-4 rs231775) are associated with T1D susceptibility in Polish children and adolescents.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Bioethical Committee at the Medical University of Bialystok. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AB, AS, WM, and AK contributed conception and design of the study. NW-K organized the database. JG performed the statistical analysis. HB-S wrote the first draft of the manuscript. BS, BG-O, AK, and AŁ wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Infliximab Therapy Could Decrease the Risk of the Development of Thyroid Disorders in Pediatric Patients With Crohn's Disease

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Autoimmune diseases, including autoimmune thyroid diseases (AITDs), may be associated with Crohn's disease (CD). Taking into consideration the role of tumor necrosis factor alpha (TNF-alpha) in the immune-mediated inflammation that underlies both diseases, we evaluated an ultrasound of thyroid gland in pediatric CD patients, naïve, and treated with infliximab (IFX), an anti-TNF-alpha antibody, to assess the risk for AITD and evaluated the usefulness of ultrasonography to diagnose AITD in patients with CD. Sixty-one patients with CD were enrolled in the study, including 36 patients (mean age 14.5 ± 3.5 years) treated with IFX (IFX group) for a mean of 13.9 ± 16.6 months and 25 patients (mean age 14.7 ± 2.3 years) who never received anti-TNF-alpha therapy (control group). An ultrasound examination of the thyroid gland was performed; thyroid function tests and thyroid antibodies were assessed. We found 10-times higher prevalence of decreased thyroid echogenicity in CD and IFX-naive patients compared to IFX-treated group [a significant reduction in thyroid echogenicity in 1/36 (2.8%) patients receiving IFX compared to 7/25 (28%) patients naive to biologic therapy]. The latter showed significantly lower thyroid-stimulating hormone (TSH) levels ($p = 0.034$) and higher levels of thyroid antibodies ($p = 0.042$) in comparison to control. Our data suggest the protective role of IFX therapy in the development of thyroid disorders and indicate the usefulness of thyroid ultrasound to identify the risk of probable AITD in pediatric patients with CD.

Keywords: autoimmune thyroid diseases, Crohn's disease, Ultrasonography, Anti-TNF-alpha, pediatric patients

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INTRODUCTION

Crohn's disease (CD) is one of two main types of inflammatory bowel disease (IBD) that can result in progressive bowel damage and disability. The etiology of CD is multifactorial, and it is considered that chronic inflammation results from complex interactions of environmental factors, an inappropriate immune reaction against an altered microbiome in genetically susceptible individuals (1, 2). Some studies have assessed possible common genetic factors between CD and autoimmune thyroid diseases (AITDs). The role of human leukocyte antigen (HLA) genes such as PTPN22, CTLA4, and CD40 in CD patients has been extensively investigated (3).

However, some studies reported that there were no significant differences in thyroid function tests {serum levels of triiodothyronine [free T3 (fT3)], thyroxine [free T4 (fT4)], and thyroid-stimulating hormone (TSH)} between CD patients and controls, or even the prevalence of thyroid dysfunction was lower in CD patients in comparison to the general population (4–6). The studies identified by the literature search indicated a 2–8% prevalence of thyroid dysfunction (hyper- or hypothyroidism) in the general population, including the populations in iodine-deficient countries (7). Snook et al. (8) reported that the prevalence of hyper- and hypothyroidism in CD amounted to 0.3 and 0.5%, respectively, similar to the control group, which was 0.7% for both. In the study of Yakut et al. (4), the prevalence of both hyper- and hypothyroidism in patients with CD as well as for both control groups was 0% (0/33) and 0% (0/66), respectively. Liu et al. (9) reported that the prevalence of hyper- and hypothyroidism in patients with CD was 0% (0/44) and 2.3% (1/44), respectively. In a study by Pooran et al. (6), the prevalence of hypothyroidism was lower in CD patients [3.8% (8/210)] than in control individuals [8.2% (17/206)], although the prevalence of hyperthyroidism was statistically similar between the groups. In a large population-based study in Canada that included 8,072 IBD patients [3,879 ulcerative colitis (UC) and 4,193 CD patients], the prevalence of Hashimoto thyroiditis (HT) was similar to that in the controls (10).

Taking into consideration the above data, a question appears: How CD therapy influences the diagnosis and clinical outcome of AITD? It is especially interesting nowadays when many patients with CD are treated with infliximab (IFX), a monoclonal anti-TNF-alpha antibody. TNF-alpha plays a role in the pathogenesis of autoimmune diseases, including thyroid diseases (11–13). Therefore, anti-TNF-alpha agents used in CD could modify concomitant autoimmune disease outcome or even may protect against them.

The diagnosis of AITD relies on the presence of circulating antibodies to thyroid antigens in blood and a typical ultrasound pattern of thyroid gland in a patient with proper clinical features and abnormal thyroid hormone levels (14). In the opinion of some experts, an ultrasonography is a more effective tool in the diagnosis and prognosis of AITD than testing for thyroid antibodies circulating in blood. According to data from a large cohort, an abnormal ultrasound pattern allows to diagnose AITD with the probability up to 95% (15–19). The lack of autoantibodies cannot exclude AITD; on the other hand, thyroid antibodies have been detected in healthy populations, also in children (20).

The primary aim of our study was the assessment of the thyroid gland morphology with ultrasonography in IFX-naive and IFX-treated pediatric CD patients. The second goal was to evaluate the usefulness of ultrasonography to assess the risk for probable AITD in pediatric patients with CD.

PATIENTS AND METHODS

We studied 61 patients with CD, treated in the Department of Pediatric, Gastroenterology and Nutrition, without any known thyroid disorder according to their medical history. Thirty-six patients were treated with IFX (IFX group), while 25 patients (control group) have never received any biologic agents. The Local Ethical Committee approved the study (No. 1072.6120.57.2019 of March 28, 2019). Parents and patients over 16 years of age signed an informed consent.

The clinical characteristic of the IFX group is presented in **Table 1**. All patients received biosimilar IFX, a chimeric human–mouse immunoglobulin G (IgG) monoclonal anti-TNF-alpha antibody; 29 patients received Remsima (*Biotec Services International Ltd.*), and seven patients received Flixabi [*Biogen (Denmark) Manufacturing ApS*]. In the IFX group, there were 18 girls (mean age was 14.5 ± 2.3 years) and 18 boys (mean age was 14.4 ± 4.4 years). The mean CD duration in girls was 52.6 ± 31.5 months, and the mean duration of IFX therapy was 43.8 ± 30.1 months. The mean CD duration in boys was 53.6 ± 31.7 months, and the mean duration of IFX therapy was 34 ± 19.2 months. The clinical characteristic of the control group is presented in **Table 2**. In the control group, there were 10 girls (mean age 13.9 ± 2.8 years) and 15 boys (mean age 15.2 ± 1.8 years). The mean CD duration in girls from the control group was 26.3 ± 32.5 months and in boys from the control group was 24.2 ± 22 months. There were no differences regarding the age and body mass index (BMI) between the groups (**Table 3**).

An ultrasound examination of the thyroid gland was performed using a Hitachi Aloka Arietta V70 in supine position with hyperextended neck using a high-frequency linear-array transducer (2–22 Hz) by the same researcher, and in doubtful cases this was followed by verification by a second specialist. Scanning was done in both transverse and longitudinal planes. Real-time imaging of thyroid lesions was performed using both gray scale and color Doppler techniques. Thyroid gland ultrasound examination included measurements of both thyroid lobes in three dimensions and thickness of thyroid isthmus. In addition, echogenicity of the thyroid parenchyma, vascularization of the gland, and presence of focal lesions were examined. Echogenicity of the thyroid gland was assessed using comparing and relationships with surrounding structures: sternocleidomastoid and strap muscles anteriorly; trachea, esophagus, and longus colli muscles posteriorly; and common carotid arteries and jugular veins bilaterally. A significant reduction of thyroid echogenicity was defined as a hypoechoic pattern of thyroid gland in comparison to submandibular gland and neck muscles. A slight reduction in thyroid echogenicity was defined as hypoechoic thyroid parenchymal pattern in comparison to submandibular gland and hyperechoic in comparison to neck muscles.

Thyroid gland function was assessed by measuring serum levels of TSH, fT3, and fT4. Moreover, anti-thyroid peroxidase antibodies (ATPOs), anti-TSH receptor antibodies (TRAbs), anti-thyroglobulin antibodies (aTGs) were measured in diagnostic process of AITD. TSH, fT3, and fT4 levels were measured using direct chemiluminescence assay (Siemens, USA). ATPO, aTG, and TRAb levels were measured using an immunochemical method with isotope label sets (Brahms, Germany). The following reference values were used: TSH

TABLE 1 | The detailed characteristic of the study group—patients treated with anti-TNF alpha.

Patient	Age (years)	Duration of Crohn disease (months)	Duration of anti TNF alpha therapy (months)	Other therapy
1	13–14	59	59	Methotrexate
2	12–13	21	21	Mercaptopurine
3	15–16	40	29	Azathioprine, Mesalazine
4	16–17	27	21	Mercaptopurine, Mesalazine
5	11–12	91	84	Mercaptopurine
6	12–13	25	9	Azathioprine, Mesalazine
7	11–12	11	10	–
8	17–18	59	53	Mercaptopurine, Mesalazine
9	17–18	120	108	Mercaptopurine
10	12–13	65	65	Budesonide, Mesalazine
11	15–16	32	13	Azathioprine, Mesalazine
12	15–16	100	92	–
13	17–18	62	50	Azathioprine
14	11–12	26	23	Azathioprine
15	14–15	16	9	Mercaptopurine, Sulfasalazine
16	12–13	84	52	Mercaptopurine, Mesalazine
17	17–18	38	34	Azathioprine
18	13–14	72	58	Mercaptopurine, Mesalazine
19	17–18	125	34	Mesalazine
20	15–16	70	37	Mercaptopurine
21	16–17	50	23	Methotrexate, Mesalazine
22	16–17	37	35	Mercaptopurine, Mesalazine
23	16–17	36	22	Mercaptopurine, Mesalazine
24	3–4	19	9	–
25	11–12	97	71	Mesalazine
26	17–18	45	14	Azathioprine, Mesalazine
27	17–18	13	8	Mercaptopurine, Mesalazine
28	6–7	45	41	Mesalazine
29	17–18	70	69	Mercaptopurine
30	17–18	13	12	Methotrexate
31	16–17	38	38	Azathioprine, Mesalazine
32	8–9	41	37	Mercaptopurine, Sulfasalazine
33	11–12	63	41	Methylprednisolone
34	17–18	24	23	Methotrexate, Mesalazine
35	17–18	105	66	Mesalazine
36	14–15	74	32	Mesalazine
Mean data ± *SD*	14.5 ± 3.5	53.1 ± 31.2	13.9 ± 16.6	

Patients' age is presented as a range.

0.3–4.0 μIU/ml; fT3 3.0–8.1 pmol/l; fT4 10.0–25.0 pmol/L; ATPO <60.0 IU/ml; TRAb <1.0 IU/ml; aTG <60 U/ml.

Statistical analysis was performed using the Dell Statistica 13.1 64-bit package (StatSoft, Kraków, Poland). Variables are presented as mean with *SD*. Differences between the IFX group and the control group were determined by Student's *t*-test.

RESULTS

In the IFX group, 6/36 patients (5/18 girls, 1/18 boy) had an abnormal echogenicity of thyroid gland parenchyma. In three patients, parenchymal echo pattern was heterogeneous (**Figure 1**); in two patients, it was slightly decreased (**Figure 2**), while in one case, it was decreased significantly (**Figure 3**). The mean CD duration in those patients was 46 ± 21.31 *SD* months (range 21–72 months). The mean duration of IFX therapy was 41 ± 17.9 *SD* months (range 21–59 months). In 8/36 patients (in two boys and five girls, including three with decreased echogenicity of the thyroid gland parenchyma), small colloid cysts located in the lower poles of the thyroid glands were found, and in one boy, two cystic solid lesions in both thyroid lobes (in left lobe 4.5 × 4.3 × 2.1 mm; in the right lobe PP 6.2 × 7.3 × 3.2 mm) were present.

TABLE 2 | The detailed characteristic of the control group—patients not treated with anti-TNF therapy.

Patient	Age (years)	Duration of CD (months)	Treatment
1	10–11	4	Azathioprine, Mesalazine
2	10–11	11	Prednisone, Mercaptopurine
3	9–10	5	Methylprednisolone, Azathioprine, Mesalazine
4	17–18	96	Methotrexate, Mesalazine, Budesonide
5	15–16	30	Methotrexate, Mesalazine
6	12–13	30	Azathioprine, Mesalazine
7	16–17	3	Budesonide, Azathioprine, Mesalazine
8	15–16	7	Azathioprine, Mesalazine
9	16–17	72	Azathioprine, Mesalazine
10	14–15	5	Budesonide, Azathioprine, Mesalazine
11	11–12	60	Azathioprine, Mesalazine
12	15–16	24	Azathioprine, Mesalazine
13	16–17	20	Methotrexate, Mesalazine
14	15–16	43	Methotrexate, Mesalazine
15	11–12	3	Methylprednisolone, Mesalazine
16	15–16	3	Methylprednisolone, Methotrexate, Mesalazine
17	16–17	3	Methotrexate, Mesalazine
18	15–16	27	Methotrexate, Mesalazine
19	15–16	36	Azathioprine, Mesalazine
20	14–15	72	Mercaptopurine, Mesalazine
21	11–12	3	Azathioprine, Mesalazine
22	16–17	36	Mesalazine
23	14–15	3	Mesalazine
24	16–17	6	Methotrexate
25	17–18	24	Azathioprine, Mesalazine
Mean data ± *SD*	14.7 ± 2.3	25.0 ± 26.1	

Patients' age is presented as a range.

In this boy, ultrasound examination was repeated after 6 months, and a reduction of their dimensions and a confirmation of their cystic nature were observed. Most IFX patients (25/36) presented with a normal ultrasound pattern of thyroid gland, and all had the normal vascularization of the thyroid gland.

In the control group, an abnormal echogenicity of the thyroid gland was found in 11/25 patients (5/10 girls, 6/15 boys). In four cases, we found heterogeneous parenchymal echo pattern, and in seven, heterogeneous and significantly hypoechoic parenchymal echo pattern was visible. The mean disease duration in these patients was 26 ± 26.1 *SD* months (range 3–72 months). In 9/25 children (in four girls and five boys, including eight patients with lowered echogenicity of the thyroid parenchyma), small colloid cysts localized in the lower poles of both lobes of the thyroid glands were present. Other patients of the control group (13/25) had a normal ultrasound pattern of thyroid gland and the normal vascularization of the thyroid gland.

Thyroid function tests TSH, fT3, and fT4 were within normal ranges in both groups (**Table 3**). However, TSH levels were significantly lower in the IFX group compared to control. In

TABLE 3 | The presentation of anthropometric data and levels of thyroid tests and thyroid antibodies in patients with Crohn's disease treated (IFX group) and not treated with infliximab (control group).

Group	F/M	Age [year]	Height SDS	BMI SDS	CD duration [month]	TSH [μIU/ml]	fT3 [pmol/l]	fT4 [pmol/l]	Thyroid volumne [ml]	aTPO [IU/ml]	aTG [U/ml]	TRAB [IU/ml]
IFX group $N = 36$	18/18	14.5 ± 3.5	1.16 ±−0.4	1.32 ± 0.12	53.1 ± 31.2	1.31 ± 0.75	5.22 ± 0.82	13.96 ± 1.95	5.12 ± 2.15	<30	<20	0.53 ± 0.19
Control group $N = 25$	10/15	14.7 ± 2.3	−0.34 ± 1.07	−0.6 ± 1.57	25.0 ± 26.1	1.68 ± 0.79	5.33 ± 1.21	17.17 ± 2.46	5,38 ± 1.76	<30	<20	0.69 ± 0.22
P	0.45	0.76	0.84	0.06	<0.001	0.07	0.51	<0.001	0.63	0.97	0.99	0.03

The data are presented as a mean ± SD.

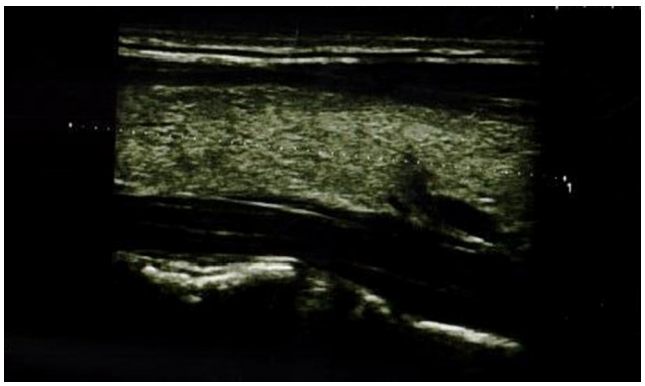

FIGURE 1 | Longitudinal image of thyroid gland with heterogeneous parenchymal echo pattern.

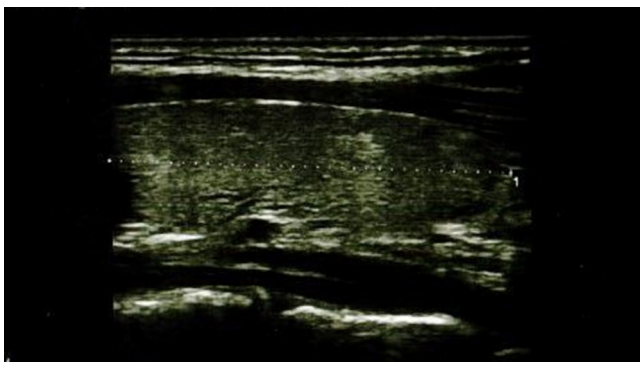

FIGURE 2 | Longitudinal image of thyroid gland with slightly decreased parenchymal echo pattern.

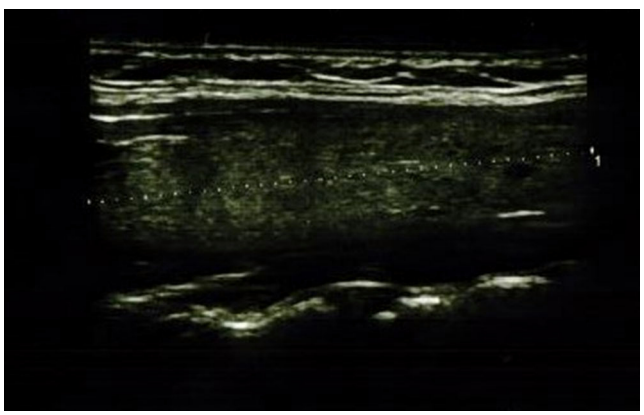

FIGURE 3 | Longitudinal image of thyroid gland with significantly decreased parenchymal echo pattern.

contrary, fT4 levels were significantly higher in the control group than those in the IFX patients. No differences in fT3 levels between the two groups were found. All patients, in both groups, were negative for thyroid autoantibodies (ATPO, aTG). However, all TRAbs were negative in both groups; the titer was significantly higher in the IFX group in comparison to the control group, conversely to TSH levels. There was no difference in volumes of thyroid gland between both groups (**Table 3**). There was no association between abnormal thyroid ultrasound results and TRAb titer levels in the IFX group. In contrary, patients in the control group with heterogenic/hypoechoic thyroid parenchymal pattern have significantly higher TRAb levels compared to the patients with normal thyroid ultrasound (0.79 ± 0.23 vs. 0.59 ± 0.17 IU/ml, $p = 0.042$).

DISCUSSION

Our data could suggest the protective role of IFX therapy in the development of the thyroid disease and the usefulness of thyroid ultrasound to identify the probable risk for AITD in pediatric patients with CD.

Although the development of extraintestinal manifestations or coexistence of autoimmune disorders during the course of IBD is well-known, the coexistence of CD and thyroid diseases is still disputable (21–24). The results of our study show that the prevalence of thyroid abnormalities in CD patients is probably higher, but the outcome is different in comparison to the data from literature regarding the general population; therefore, the diagnostic criteria of thyroid disease used in the general population probably should be modified in this group of patients.

AITD is the most frequent autoimmune disease in the general population, and the most frequent form is HT (14). Most patients with HT have detectable antibodies in the blood. According to the data presented in adult patients with HT, about 81–97% of them have positive ATPO antibodies, and about 50–98% of patients have positive thyroglobulin antibodies (25). There are scarce data regarding this issue in pediatric patients with HT. About 5% of patients with HT based on clinical grounds or by ultrasound appearance have no detectable antibodies. Patients with antibody-negative HT had a milder form of hypothyroidism at the time of diagnosis. This could represent an earlier stage of the disease or simply a less aggressive form of HT (26).

Ultrasound evaluation is recommended as a screening test for patients with a high clinical risk of thyroid disease (27). The indication for thyroid ultrasonography, in spite of a diagnosis of a thyroid nodule, is to evaluate diffuse changes in thyroid parenchyma, including chronic lymphocytic thyroiditis, HT. The characteristic ultrasonography appearance of HT is focal or diffuse glandular enlargement with a coarse, heterogeneous, and hypoechoic parenchymal echo pattern. The presence of multiple discrete hypoechoic micronodules (1–6 mm in size) is strongly suggestive of chronic thyroiditis. Fine echogenic fibrous septae may produce a pseudo lobulated appearance of the parenchyma. Color Doppler may demonstrate slight to markedly increased vascularity of the thyroid parenchyma. Increased vascularity seems to be associated with hypothyroidism likely due to trophic stimulation of TSH (28). In the latter stages of HT, Doppler ultrasound findings are usually of diffuse hypovascularization and sometimes even with no detectable blood flow (29). Small atrophic gland represents end stage of HT. Occasionally, the

nodular form of HT may occur, as well within a sonographic background of diffuse HT or within normal thyroid parenchyma. Moreover, ultrasonography examination may reveal the presence of perithyroidal satellite lymph nodes, especially the "Delphian" node just cephalad to the isthmus (28).

The diagnosis of AITD in patients with CD could be hindered or overlooked because of several reasons. Some signs and symptoms can be mistakenly recognized as signs and symptoms of CD or considered as adverse effects of the therapy for CD. All the more the therapy for IBD could modify the production of thyroid antibodies that are used to confirm the diagnosis of AITD and lead to false-negative results and to exclude of the disease. Wherefore in this group of patients, ultrasound examination seems to be a more useful and effective tool in the diagnosis of AITD or predict the risk in the future in patients with a normal level of thyroid hormones (30–32).

In our study, all 61 patients with CD presented with a normal thyroid function because the levels of TSH, fT3, and fT4 were normal in all cases. Moreover, all participants were negative for thyroid autoantibodies: ATPO, aTG, and TRAb. However, in 17/61 patients (27.8%), we observed a heterogeneous and hypoechoic parenchymal echo pattern of the thyroid gland. Presented data can suggest the predominance of the sensibility of thyroid ultrasound result over biochemical findings in the prognosis of the risk of probable AITD development in patients with CD. Our observations regarding the important role of ultrasonography results in the prediction of probable AITD risk in patients with IBD are in accordance with the results of studies performed in a general population.

Gutekunst et al. (19), on the basis of the results of their study performed in 92 patients with HT (aged 11–81 years), underlined the significance of ultrasound in the diagnosis of chronic lymphocytic thyroiditis. In this study, finally, chronic lymphocytic thyroiditis was confirmed by the results of cytology in 84/92 patients (91.3%). A heterogeneous parenchymal echo pattern appeared in 87/92 patients (94.6%), while antimicrosomal antibodies occurred in 80/92 (87%) patients, among which 16/80 patients (17.4%) had low titers of these antibodies (1:32–1:100) (19).

Pedersen et al. (17) indicated the value of ultrasonography in the prediction of AITD based on the analysis of 3,077 patients, referred to the study because of goiter, thyroid dysfunction, neck discomfort, and/or difficulty in swallowing. Among them, 452/552 patients had diffuse reduction in thyroid echo and were included in the study and compared with 100 control patients with a normal thyroid echogenicity. The authors of this study reported that among 110 patients with a discrete hypoechoic pattern of the thyroid gland, AITD was diagnosed finally in 87/110 patients. But among 342 participants of the study with a significant hypoechoic parenchymal pattern, AITD was diagnosed finally in 312/342. Therefore, the predictive value of a reduced thyroid echogenicity as an indicator of AITD is 79.1% for a slight reduction of thyroid echogenicity and 91.2% for a significant diffuse reduction in thyroid echogenicity. Among participants with a normal ultrasound thyroid result, only seven had finally AITD. To underline the predominance of the value of a diffuse reduction in thyroid echogenicity in the prediction of AITD, in comparison to the role of positive thyroid ATPO antibodies, the authors of the study presented that among 220 patients with a low echogenicity of the thyroid gland and confirmed AITD on the basis of biopsy results, ATPO was positive only in 162/220 patients (73.6%) (17).

In the study of Raber et al. (15) with 451 patients included, abnormal thyroid ultrasound patterns were highly indicative of autoimmune thyroiditis. Positive predictive value of significant reduction of thyroid echogenicity, understood as hypoechoic to submandibular gland and to neck muscles, for the detection of autoimmune thyroiditis was 94% with overt hypothyroidism and 96% with any degree of hypothyroidism. Positive predictive value of the slight reduction in thyroid echogenicity, understood as hypoechoic to submandibular gland, hyperechoic to neck muscles, is 85 and 87%, respectively (15).

Rago et al. (16) presented thyroid ultrasonography as a tool for detecting thyroid autoimmune diseases and predicting thyroid dysfunction in apparently healthy subjects. Among 482 healthy subjects, living in a borderline iodine-sufficient urban area, 41 had thyroid hypoechogenicity, and in this group, 11 had an abnormal thyroid function (seven with positive and four with negative thyroid autoantibodies). None of the 429 participants of the study with normal thyroid echostructure had thyroid dysfunction, although 12 had positive thyroid autoantibodies. Although positive TPO and/or aTG was more frequent (24/482, 5%) in subjects with thyroid dysfunction (7/11) than in those who remained euthyroid during the study (17/471, $\chi^2 = 69.66$, $p < 0.0001$), thyroid hypoechogenicity had a higher sensitivity than the positivity of thyroid autoantibody tests (100 vs. 63.3%) for diagnosing or predicting thyroid dysfunction (16).

The results of our study not only indicate that ultrasound assessment could be a sensitive tool in detecting thyroid abnormality but also suggest that therapy with IFX can modify the clinical course. To our knowledge, this is the first such observation. The only study reported to date regarding the influence of anti-TNF-alpha therapy on the thyroid gland function did not present data on ultrasonography (33). The aim of this cited study was to investigate for the first time the thyroid function in patients with IBD and the potential effect of anti-TNF-alpha therapy. Forty-one patients with IBD, without any known thyroid disorder, were evaluated. Eighteen patients were on anti-TNF-alpha therapy for more than 1 year. From the second group, 12 of 23 patients on conventional therapy (azathioprine plus mesalazine) were put on anti-TNF-alpha and studied 6 months later. Anti-TNF-alpha-treated patients presented with significantly lower fT4 levels, but still within normal ranges, and no differences in TSH and T3 levels. The percentage of patients with positive thyroid antibodies was lower in the anti-TNF-alpha group, but not significantly. After 6 months of treatment with anti-TNF-alpha, fT4 levels were found to be reduced, while no changes in TSH and T3 levels and thyroid autoantibodies were noted. The advantage of this study comparing to ours is the long-term observation. However, the results, based only on biochemical results, seem to be in agreement with our results based on ultrasound of thyroid glands.

We found a significant reduction in thyroid echogenicity in 1/36 (2.8%) patients receiving IFX compared to 7/25 patients

(28%) naive to biologic therapy, although the duration time of CD in the IFX group had been longer in comparison to controls. Therefore, this 10-times higher prevalence of significant reduction in thyroid echogenicity in CD patients without anti-TNF-alpha therapy expressly suggests the preventive role of IFX in the probable development of AITD. Moreover, IFX patients have significantly lower levels of TSH without differences of thyroid volumes and higher thyroid antibody levels in comparison to the control naive group, although both groups did not differ regarding BMI and age. Our observations together with the knowledge from literature about the role of TNF-alpha in the pathogenesis of both AITD and IBD suggest that thyroid ultrasound could be a useful tool in the identification of CD pediatric patients at risk for AITD.

The advantage of our study is the novel observation of the possible preventive role of IFX therapy in the development of thyroid abnormalities probably preceding AITD. On the basis of the presented data, we propose thyroid ultrasound as a useful tool in the identification of the risk for thyroid disease in pediatric patients. All the more thyroid ultrasound is easily accessible, non-invasive, and cost-effective. The main disadvantage of the ultrasonography is that this method is operator dependent. For this reason, in our study, all participants were examined by one physician and verified by a second one, always the same two persons (34). In differential diagnosis of AITD, other diffuse thyroid diseases should be taken into consideration, multinodular goiter, de-Quervain's subacute thyroiditis, and Graves disease, because the sonographic features of these processes may be similar. However, these conditions have different biochemical profiles and clinical presentations. Therefore, always, ultrasound findings should be viewed in relation to clinical and biochemical status of the patient. The most dangerous, but possible, diagnostic pitfall is that diffuse infiltrative vascular thyroid carcinoma like papillary or follicular carcinoma may be mistaken for AITD. Ultrasonography features that suggest malignancy include irregular or nodular enlargement of the thyroid gland, local invasion, and nodal metastases. Sometimes, these features are not visible at once, and such cases require observation with repeated ultrasonography examination (35). Long-term observation is indicated in each case with an abnormal thyroid picture in ultrasonography. In AITD, abnormal ultrasound pictures never normalize and remain for the rest of the patient's life. Moreover, HT is associated with an increased risk of thyroid malignancies like follicular or papillary carcinoma and lymphoma (36). All the more there are data that in patients with inflammatory bowel diseases, focal lesions relating to tumors of the thyroid gland are more common than in the control group (37).

The weaknesses of the presented study are an uneven distribution of girls and boys in both groups and a lack of long-term observation. Female sex has a higher risk of thyroid diseases, so it could influence the results in both groups. However, in our control group with fewer girls than boys, we found more abnormal patterns of thyroid glands just in the boys in comparison to the girls. It would be very interesting how will be the further outcome of thyroid function and its morphology in the presented patients. Therefore, we plan to follow up our patients and repeat the study after 12 months with renewed assessment of thyroid antibodies in both groups.

Summarized, we propose thyroid ultrasound for use as an easily accessible, non-invasive tool to identify the risk of thyroid abnormalities probably preceding AITD in pediatric patients with CD. Because CD treatment especially with TNF blockers could modulate the AITD presentations, the thyroid ultrasonography should be considered before starting IFX therapy, and a long-term follow-up may be necessary in case of abnormal thyroid findings.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because, the data are the property of the patients. Requests to access the datasets should be directed to Jagiellonian University—Medical College.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Jagiellonian University Ethical Committee approved the study (No. 1072.6120.57.2019 of March 28, 2019). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AMW and JS contributed to the concept. AMW and AF contributed to the design, contributed to data collection or processing, contributed to analysis or interpretation, literature search, and contributed to writing. AF, AMW, MS, AW, and KF contributed to the medical and surgical practices. All authors contributed to the final version of the manuscript.

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CASE REPORT
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Unusual Presentation of Polyautoimmunity and Renal Tubular Acidosis in an Adolescent With Hashimoto's Thyroiditis and Central Pontine Myelinolysis

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Background: Hashimoto's thyroiditis is frequently associated with other autoimmune diseases and may include renal involvement.

Case description: A 17-year-old female with previously diagnosed Hashimoto's thyroiditis and vitiligo was admitted to a pediatric intensive care unit with hypokalemic paralysis and acidosis, after having suffered from recurrent muscular weakness for approximately one year. A few days later she developed central pontine myelinolysis. After initial stabilization she was also diagnosed with distal renal tubular acidosis (dRTA) and tubular proteinuria which can occur in Sjögren's syndrome. Extended screening for autoimmune diseases additionally revealed celiac disease. Treatment with Prednisone and substitution of potassium quickly lead to the resolution of proteinuria and dRTA, but unilateral paralysis of the sixth nerve as a result of central pontine myelinolysis was irreversible.

Conclusions: This is the rare case of polyautoimmunity including autoimmune thyroiditis, Sjögren's syndrome, vitiligo and celiac disease in an adolescent with few disease-specific symptoms. The diagnoses were made via a complicating nephritis causing dRTA and proteinuria. Delay in diagnosis lead to permanent neurological damage. This case highlights the need for pediatricians to be aware of rare accompanying diseases and their complications in "common" pediatric autoimmune diseases like Hashimoto's thyroiditis and celiac disease.

Keywords: case report, autoimmune thyroiditis, distal renal tubular acidosis, central pontine myelinolysis, Hashimoto's thyroiditis, Sjögren's syndrome, celiac disease, hypokalemia

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INTRODUCTION

Hashimoto's thyroiditis is the most common autoimmune thyroiditis in children and adolescents and frequently associated with other autoimmune diseases including vitiligo, rheumatoid arthritis, polymyalgia rheumatica, celiac disease, diabetes, and Sjögren's syndrome (1, 2). The presence of more than one well-defined autoimmune disease in one patient is called polyautoimmunity

(3). Various autoimmune disorders can affect the kidneys leading to interstitial nephritis with tubular dysfunction that results in electrolyte loss (distal renal tubular acidosis, dRTA). These autoimmune diseases include Hashimoto's thyroiditis, systemic lupus erythematosus and Sjögren's syndrome (4–7). Celiac disease has also been linked to kidney disease (8, 9). dRTA leads to hypokalemia that can be complicated by hypokalemic paralysis. As a matter of fact, hypokalemic paralysis can be the first manifestation of Sjögren's syndrome (10, 11). A possible complication of hypokalemia of different origins is central pontine myelinolysis, which has repeatedly been reported in the context autoimmune disorders and dRTA (12–15).

Here, we report the case of a female adolescent with Hashimoto's thyroiditis whose polyautoimmunity was unveiled via renal disease leading to severe neurological complications. Two years after being diagnosed with euthyroid Hashimoto's thyroiditis, the girl developed recurrent episodes of muscular weakness and pain that were associated with metabolic acidosis and hypokalemia. Only after developing severe hypokalemia with consecutive central pontine myelinolysis, she was diagnosed with polyautoimmunity that caused interstitial nephritis and dRTA.

CASE DESCRIPTION

A 17-year-old girl presented to the emergency room with muscular weakness and leg pain of ∼2 days duration. For one year she had repeatedly suffered from muscular weakness associated with hypokalemia and metabolic acidosis. Because of the weakness and joint pains, she had presented to doctors of several pediatric subspecialties including neurology, rheumatology, cardiology, and nephrology. Periodic hypokalemic paralysis was primarily suspected, whereas the accompanying acidosis and elevated antinuclear antibody (ANA) titers (1:2560 [< 1:80]) could not be explained then. Three years before the present admission, autoimmune thyroiditis had been diagnosed but required no medical treatment.

Upon admission, she presented with severe hypokalemia (Potassium 1.8 mmol/l) and severe hyperchloremic acidosis (standard bicarbonate 13.3 mmol/l, Chloride 127 mmol/l) with normal anion gap. She was admitted to a pediatric intensive care unit and intravenous potassium substitution was performed with up to 6 mmol/kg/day. Five days after admission, the patient began to suffer from double images, impairment of speech, and general retardation. Magnetic resonance tomography revealed central pontine myelinolysis (**Figure 1**).

She was transferred to the pediatric nephrology department for further diagnostics and treatment. Upon admission, the patient was in stable condition and fully oriented. Neurological symptoms were bilateral palsy of the sixth nerve, dizziness, and slurred speech. She was unable to sit for more than half a minute, stand, or walk, whereas sensitivity and muscle power were not affected. Vitiligo could be observed on knees, chest, and upper back. Serum electrolytes and blood gas analysis were unremarkable.

Due to the persistent metabolic acidosis and high potassium demand, we screened for renal disease. Renin was normal and Aldosterone low. The blood pressure ranged between the 5th and 50th percentile. Alkaline urine (pH 7.0), increased fractional potassium excretion (>20 %), and tubular proteinuria pointed toward tubular dysfunction. Due to the accompanying severe hypokalemia and metabolic acidosis (with normal anion gap), we suspected distal renal tubular acidosis (dRTA). Acquired dRTA frequently results from nephritis due to autoimmune disorders, e.g. systemic lupus erythematodes and Sjögren's syndrome. Supporting these differential diagnoses, central pontine myelinolysis can occur in both conditions. The consecutive renal biopsy revealed chronic and acute tubulointerstitial nephritis (**Figure 2**). Serum markers for Sjögren's syndrome (anti-Ro, anti-La, ANA) were strongly positive, whereas antibodies for systemic lupus erythematodes were negative. Extended screening for autoimmune diseases confirmed Hashimoto's thyroiditis and newly revealed celiac disease, which was verified by biopsy. Even after receiving the diagnosis of celiac disease, our patient denied having any gastrointestinal symptoms. Schirmer's test, ultrasound of the parotid gland and repeated extensive anamnesis could not reveal sicca symptoms. No signs of peripheral neuropathy or central nervous system involvement other than a persistent substance defect caused by central pontine myelinolysis could be found (**Figure 1**).

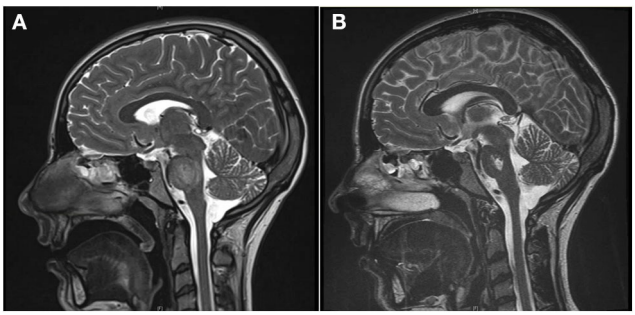

FIGURE 1 | (A) The T2-weighted magnetic resonance tomography shows a diffuse increase of signal intensity and edema in the pons during the acute phase of central pontine myelinolysis. **(B)** Four weeks later, the edema has resolved, but a trident-like substance defect in the pons remains (T2 weighting).

During the hospital stay, oral substitution of potassium hydrogencarbonate (0.5 mmol/kg potassium/day) stabilized electrolyte and metabolic homeostasis. Causal immunosuppressive treatment was initiated with oral Prednisone 60 mg/d and gluten-free diet. Additionally, intensive physiotherapy was initiated. Our patient quickly learned to cope with her neurological deficits that were partially reversible. However, unilateral palsy of the sixth nerve remained, accompanied by double images, intermittent dizziness, and perceived weakness upon physical activity. Four weeks after initiation of treatment, proteinuria was no longer detectable, and prednisone was tapered over the course of 2 months. Substitution of potassium hydrogencarbonate continues and maintains serum electrolytes and standard bicarbonate within normal range to date. **Figure 3** depicts important symptoms, diagnostics, and treatment in a timeline.

DISCUSSION

We describe the unusual case of a patient with polyautoimmunity formed by Hashimoto's disease, celiac disease, vitiligo, and Sjögren's syndrome. Especially autoimmune thyroiditis and Sjögren's syndrome are often associated with polyautoimmunity (16). In our patient, clinical symptoms of the autoimmune diseases were mild or did not exist. This may partially explain the delay in diagnosis that made severe hypokalemia with consecutive central pontine myelinolysis and persisting neurological sequelae possible. Sjögren's syndrome was diagnosed via the complicating nephritis, which results from peritubular infiltration of lymphatic cells and leads to tubular dysfunction. Urine analysis to detect this dysfunction, represented by tubular proteinuria and urine potassium loss (dRTA) gave the crucial cue, as dRTA is frequently caused by Sjögren's syndrome in adults. However, in children Sjögren's syndrome is very rare. Adult population prevalence differs between countries and lies between 0,09 and 1,6% in western European countries—data for children and adolescents are lacking, but believed to be lower (17–20). Recurrent Parotitis is the most common symptom in children with Sjögren's syndrome, whereas adults normally present with sicca symptoms (21, 22). To make diagnosis even more difficult, it is very rare that renal complications are the leading symptoms of Sjögren's syndrome among children. We found only one case report with hypokalemic paralysis revealing Sjögren's syndrome in a 16-year old girl (23).

Hashimoto's thyroiditis was the only pre-existing condition that had been diagnosed, when our patient started consulting doctors about one year before she became critically ill. Autoimmune thyroiditis is frequently associated with other

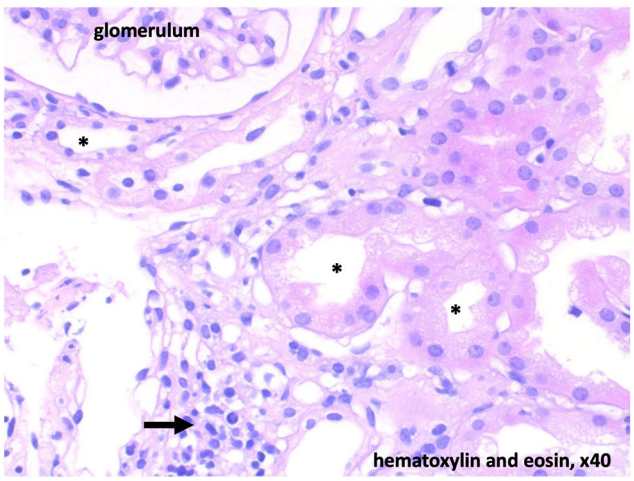

FIGURE 2 | Histology with low-grade chronic and floride tubulointerstitial nephritis. Diffuse interstitial infiltrates (arrow). Acute tubulus damage is light to moderate and potentially reversible. * tubular lumen.

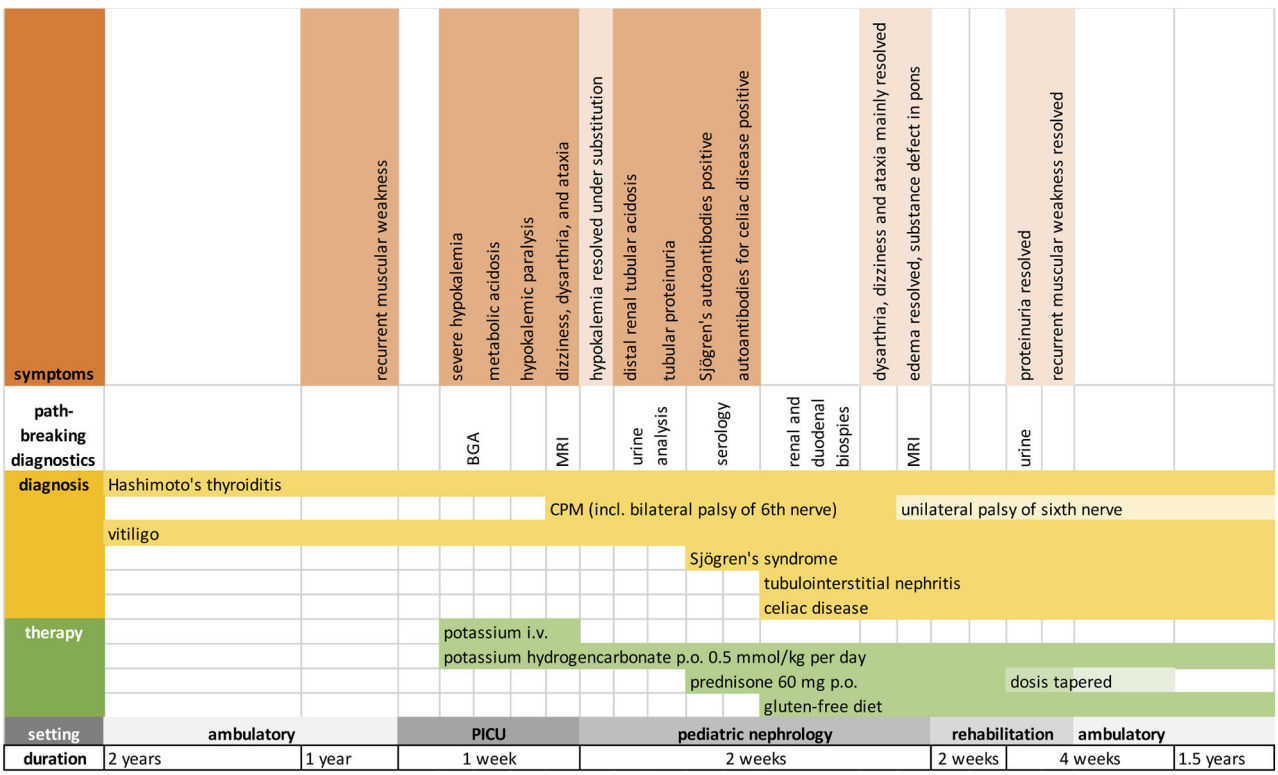

FIGURE 3 | Timeline showing the chronological order of symptoms, diagnoses, and treatment. BGA, blood gas analysis; MRI, magnetic resonance imaging.

autoimmune disorders that show different clustering depending on age at diagnosis (24). Concomitant autoimmune diseases in children are typically type 1 diabetes and celiac disease, whereas adults are more likely to suffer from arthropathies and connective tissue diseases (24). Thus, our patient matches the "pediatric cluster" even though she did not suffer from gastrointestinal symptoms. This fits with the observation that ∼50% of celiac disease is diagnosed in adulthood or adolescence and symptoms in the majority of patients are subtle (2). The most common accompanying autoimmune skin disease in autoimmune thyroiditis in all age groups is vitiligo, which was also present in our patient (24). The coexistence of autoimmune thyroiditis and Sjögren's syndrome was examined by various studies and is attributed to shared pathophysiological mechanisms (25–28). Because of their common genetic and pathophysiological background, it has been suggested that patients with autoimmune thyroiditis who remain unwell despite treatment or develop new unspecific symptoms should be screened for accompanying autoimmune disorders (1).

Initially, our patient did not receive treatment for autoimmune thyroiditis and was unwell for a long period of time. She already presented two autoimmune diseases—Hashimoto's thyroiditis and vitiligo—when she started consulting doctors because of her recurrent weakness. The specialists screened for further autoimmune diseases: e.g., systemic lupus erythematodes was excluded three times and a Schirmer's test (with negative result) was performed months before admission to hospital. Relevant health information was only collected by the family physician or pediatrician in a paper file, making it very difficult to unveil the complexity of this case. The diagnostic approach to generate the suspect diagnosis (urine analysis of proteins and electrolytes and literature search) was simple—concordantly, the treatment required to balance electrolytes and immunosuppression to induce "remission" was mild. This makes it even more tragic that our patient suffered from persistent neurological damage that could have been prevented by timely diagnosis and adequate treatment (immunosuppression to induce remission and substitution of potassium and bicarbonate).

In the future, increased awareness among pediatricians regarding polyautoimmunity and its comorbidities in pediatric autoimmune diseases (e.g., autoimmune thyroiditis, celiac disease, type 1 diabetes, Addison's disease, or atrophic gastritis) may possibly help to detect similar cases earlier. Although the combination and severity of our patient's diseases is extremely rare, pediatricians should be aware of rare diseases and their even more rare complications. A central, national or international database integrating relevant diagnostic information may contribute to a better understanding of complex and rare diseases.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors were involved in the patient's treatment. NB wrote the manuscript and designed figures, IF procured informed consent, follow-up information, and material for figures. IA-A, PH, and RB critically read and redacted the manuscript.

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ORIGINAL RESEARCH
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Analysis of Polymorphisms rs7093069-IL-2RA, rs7138803-FAIM2, and rs1748033-PADI4 in the Group of Adolescents With Autoimmune Thyroid Diseases

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Introduction: The pathogenesis of autoimmune thyroid diseases is complicated and not completely known. Among the causes of thyroid autoimmunity, we distinguish genetic predisposition and environmental factors. Graves' disease and Hashimoto's thyroiditis are associated with a disturbance of immune tolerance of thyroid antigen molecules. The IL2RA gene is located on chromosome 10 and encodes the interleukin 2 receptor (IL2RA), which is expressed by the regulatory T-cells (Tregs) responsible for suppression. It has been shown that this gene and its polymorphism occur in people with various autoimmune diseases (*e.g.* type 1 diabetes mellitus, rheumatoid arthritis, Graves' disease, or multiple sclerosis). The FAIM2 gene is located on chromosome 12 and encodes the molecule involved in the apoptosis inhibition process. The PADI4 gene is located on chromosome 1, and its expression is associated with activation of T-cells, differentiation of macrophages, which leads to increased inflammation.

Aim: The aim of the study was to analyze the polymorphisms of the IL-2RA (rs7093069), FAIM2 (rs7138803) and PADI4 (rs1748033) genes and their correlation to thyroid hormones and anti-thyroid antibodies in pediatric patients with Graves' disease and Hashimoto's thyroiditis compared to the control group.

Material and Methods: The study was performed in 180 patients with GD (mean age 16.5 ± 2), 80 with HT (mean age, 15.2 ± 2.2), and 114 children without any autoimmune diseases (mean age 16.3 ± 3) recruited from the endocrinology outpatient clinic. Three single nucleotide polymorphisms (SNPs): rs7138803-FAIM2, rs7093069-IL-2RA, and rs1748033 PADI4 were determined by TaqMan SNP QuanStudio 12K Flex-OpenArray genotyping with PCR and correlated to thyroid hormones and anti-thyroid antibodies.

Results: Rs7090369-IL-2RA allele T was more frequent in patients with AITDs (33.7% in GD *vs* 28.7% in HT, p = 0.077, OR = 1.52) compared with healthy children (25%). Allele T of that gene predisposes to the occurrence of autoimmune thyroid diseases, especially GD and TT genotype gives a statistically significant 5.2 times higher risk of GD (p = 0.03, OR = 5.26) and increased risk of HT (p = 0.109, OR = 4.46). Allele A rs7138803-FAIM2 is more frequent in patients with GD (p = 0.071, OR = 1.45) and HT (p = 0.028, OR = 1.8). In our data the presence of GG genotype of that gene significantly reduces the risk of autoimmune thyroid diseases (p = 0.05, OR = 0.42). Allele C rs1748033PADI4 and its CC genotype were more frequent in patients with autoimmune thyroid diseases, but it was not statistically significant. The occurrence of CT genotype significantly reduces the risk of HT (p = 0.03, OR = 0.4).

Conclusions: 1). Polymorphisms rs7138803-FAIM2 and rs1748033-PADI4 are more frequent in patients with autoimmune thyroid diseases, more frequent in patients with Hashimoto' thyroiditis, but the occurrence of GG rs7138803-FAIM2 genotype could reduce the risk of thyrocyte apoptosis inhibition. 2). The TT rs7093069-IL2RA genotype may increase the risk of autoimmune thyroid diseases. 3). Analysis of polymorphisms of given genes in clinical practice will allow to determine predisposition to autoimmune thyroid disease development, to find symptoms of thyroid gland dysfunction earlier and to use appropriate treatment.

Keywords: Hashimoto's thyroiditis, Graves' disease, gene polymorphism, FAIM2, PADI4, Il-2RA

INTRODUCTION

Nowadays we observe an increase of newly diagnosed autoimmune thyroid diseases (AITD), like Graves' disease (GD) and Hashimoto's thyroiditis (HT) in different age groups. The pathogenesis of thyroid diseases is still unclear. Significant progress has been made in our understanding of the genetic and environmental triggers contributing to AITD (1). A number of molecular changes occur in genetically predisposed individuals in order to develop clinical symptoms (2, 3).

GD and HT are associated with a disorder of immune tolerance of thyroid antigen molecules. Both are involved with reduced regulatory T-cell (Treg) function. We distinguish a number of different genes encoding proteins included in the structure of the thyroid cell, elements of the immune system and those responsible for modulation of the apoptosis process. Among genetic factors we could divide: genes which coding thyrocyte elements (Tg, TSHR, SEPS1), genes encoding immunomodulators of thyrocyte antigen molecules responsible for the peripheral response (IL-2RA, FOXP3, FCRL3) (4) and responsible for T lymphocyte activation and antigen presentation (CD40, PTPN22, CTLA-4, HLA-B8, DR-3, DR-4, DR-5, PADI) (5, 6), and genes responsible for activating/inhibiting apoptosis (FAIM2).

Gene Il-2RA (interleukin 2 alpha-receptor gene) is located on the short arm of chromosome 10 (p15.1) and coded protein also called CD25, which forms an alpha-receptor chain for interleukin 2 (high-affinity alpha subunit—CD25—of the interleukin-receptor). The molecular weight of CD25 is approximately 55 kDa. This protein plays an essential role in the T lymphocyte response to IL-2, which is the main growth factor for these cells—CD25 expression is important for proliferation, longer life expectancy and T-cell function. CD25 occurs on the surface of maturing T and B lymphocytes; it undergoes transient expression on activated T and B lymphocytes; it constitutively occurs on regulatory T lymphocytes (Tregs), which inhibit activation of autoreactive T lymphocytes. It was shown that the polymorphism of this gene occurs in people with various autoimmune diseases (*e.g.* diabetes mellitus type 1, rheumatoid arthritis, Graves' disease, sclerosis multiplex) (7–13).

Gene FAIM2 (Fas apoptotic inhibitory molecule 2 gene) is located on the long arm of chromosome 12 (q13.12). The molecular weight of FAIM2 is approximately 35 kDa. This gene codes the molecule involved in the apoptosis inhibition process (inhibits Fas-mediated). It regulates apoptosis in neurons by interfering with the activation of caspase-8. It can play a role in cerebellum development. The polymorphism of the molecular coding gene is associated with giant obesity and diabetes mellitus type 2 and increases the risk of cardiovascular diseases (14–19).

Gene PADI4 (Peptydil Arginine Deiminase 4) is located on the short arm of chromosome 1 (p36.13). The molecular weight of PADI4 is approximately 74 kDa. This gene codes the enzymatic proteins responsible for the deamination of arginins. It plays a role in the development of granulocytes and macrophages, leading to inflammation and immune response. It catalyzes the citrilution/deimination of arginine residues of proteins such as histones, thus playing a key role in the histone

code and regulation of stem cell maintenance. It is found in the synovial membrane of people with rheumatoid arthritis (20).

The aim of the study was to analyze the polymorphisms of the IL-2RA (rs7093069), FAIM2 (rs7138803) and PADI4 (rs1748033) genes and their correlation to thyroid hormones and anti-thyroid antibodies in pediatric patients with Graves' disease and Hashimoto's thyroiditis compared to the control group.

MATERIALS AND METHODS

The study was performed in 180 patients with GD (mean age 16.5 ± 2), 80 with HT (mean age, 15.2 ± 2.2), and 114 children without any autoimmune diseases (mean age 16.3 ± 3) recruited from two pediatric endocrinology outpatient clinics. The diagnosis of autoimmune thyroid diseases was based on medical history, physical examination, laboratory, and ultrasound investigations (21, 22). Clinical diagnosis of hyperthyroidism in GD is confirmed by elevated thyroid hormones in serum and suppression of TSH to values close to zero with positive antibodies against receptor for thyroid-stimulating hormone (TRAb = anti-TSH), positive anti-thyroid peroxidase antibodies (aTPO) and anti-thyroglobulin antibodies (aTG) (23, 24). In clinically evident hypothyroidism in HT, serum TSH levels are elevated at reduced concentrations of thyroid hormones (fT4, fT3), usually accompanied by elevated thyroid antibodies (aTPO and/or aTG, rarely blocking anti-TSH). In patients with concomitant nodular goiter, fine-needle aspiration biopsy (FNAB) was also performed, and we excluded thyroid carcinomas. All patients had appropriate therapy for autoimmune thyroid pathology. Patients with GD were treated with methimazole and b-blockers orally. In patients with HT in therapy was used l-thyroxine orally. We excluded other endocrinopathies, extra-thyroid autoimmune diseases or non-endocrine autoimmune diseases. The control group consisted of 114 healthy children with no personal or family history of any AITDs. They were euthyroid and negative for thyroid antibodies. All controls had normal thyroid gland in ultrasonography. Before enrollment, all patients and controls and all children over 16 years old signed informed consents. The protocol for the study was approved by the Local Bioethical Committee at the Medical University of Bialystok.

Assessment of the Thyroid Hormone Concentration and Anti-Thyroid Antibody Titers

Blood for analysis was collected in the morning from the basilic vein. Serum levels of free thyroxine (fT4), free triiodothyronine (fT3), and TSH were determined on electrochemiluminescence 'ECLIA' with Cobas E411 analyzer (Roche Diagnostics). Normal values for fT4 ranged between 1.1 and 1.7 ng/dl, for fT3 between 2.3 and 5.0 pg/ml, and for TSH between 0.28 and 4.3 (μIU/L). TR-Ab, anti-TPO and anti-TG antibodies were measured in all samples using ECLIA with Modular Analytics E170 analyzer (Roche Diagnostics). The positive values for anti-thyroid antibodies were: >1.75 U/L for TR-Ab, >34 IU/ml for anti-TPO-Abs and >115 IU/ml for anti-TG-Abs.

Genotyping

DNA was extracted from the leukocytes deriving from peripheral blood using classical salting-out method. It was performed according to the manufacturer's protocol. The three analyzed SNPs rs 709369 in the IL-2RA gene, rs 7138803 in the FAIM2 gene, and rs1748033 in the PADI4 gene were determinated byTaqMan SNP QuanStudio 12K Flex-OpenArray genotyping with PCR. SNP analysis was performed twice, by the commonly used instructions. As a negative control, we used a molecular grade water.

Statistical Analysis

To assess any relationship between allele or genotype occurrence and patient's status median unbiased estimator (mid-p) of odds ratio (as well as its 95% exact confidence interval), the exact confidence interval (CI) and associated p value obtained with the use of Fisher's exact test, both obtained with the use of the mid-p method were used (25). To determine statistically significant differences between groups defined by genotypes and quantitative features, either parametric or non-parametric methods were used depending on fulfilling the normality and homogeneity of variance assumptions. Due to the issue of multiple testing during the *post-hoc* analysis, false discovery rate p value adjustment method was applied (26). Measure D' of linkage disequilibrium was used as proposed in (27). The p value of <0.05 was considered to be significant for all calculations. The R software environment was exploited for all calculations (28).

RESULTS

Age and anthropometric parameters in the study groups of children with GD, HT compared to the control group did not have statistically significant differences (**Table 1**).

Results for IL-2RA (rs7093069)

Our study shows that rs7093069 T alleles were more frequent in patients with AITDs (33.7% in GD *vs* 28.7% in HT, p = 0.077, OR = 1.52) compared with healthy children (25%) (**Table 2**). Allele T predisposes to the occurrence of autoimmune thyroid diseases, especially GD, and T/T genotype gives a statistically significant 5.2 times higher risk of GD (p = 0.03, OR = 5.26) and increased risk of HT (p = 0.109, OR = 4.46). Comparing both groups of autoimmune diseases: patients with GD and HT allele T, it is more common in patients with GD (33.7 *vs* 28.7%, p = NS). There was no statistically significant correlation between rs7093069-IL-2RA thyroid hormones levels and anti-thyroid antibodies in the studied groups (data not supplied).

Results for FAIM2 (rs7138803)

In the study groups we reported that allele A is more frequent in patients with GD and HT. Allele A was found in 44.7% of

TABLE 1 | Clinical characteristics of patients with Graves' disease (GD) and with Hashimoto's thyroiditis (HT) and control group.

	GD (mean ± SD)	p*	HT (mean ± SD)	p**	Control group
Female/Male	180 (135/45)		80 (59/21)		114 (88/26)
Age (years)	16.5 ± 2	NS	15.2 ± 2.2	NS	16.3 ± 3
Weight (kg)	55.19 ± 2.39	NS	58 ± 5.28	NS	60.9 ± 7.8
Height (cm)	162.1 ± 2.6	NS	154.2 ± 4.1	NS	160 ± 8
BMI (kg/m^2)	21.1 ± 2.1	p < 0.012	24.45 ± 1.33	NS	23.78 ± 2.5
fT4 (ng/dl)	3.6 ± 1.4	p < 0.001	1.21 ± 0.03	NS	1.1 ± 0.17
fT3 (pg/ml)	7.19 ± 1.65	p < 0.001	3.59 ± 0.69	NS	3.79 ± 0.18
TSH (mIU/L)	0.37 ± 0.1	p < 0.01	6.45 ± 3.27	p <0.02	3.04 ± 0.72
Anti-TSH (U/l)	11.56 ± 2.11	p < 0.001	0.5 ± 0.32***	NS	0.4 ± 0.2
aTG (IU/ml)	347.49 ± 86.7	p < 0.001	389.8 ± 245.34	p <0.001	41.6 ± 12.1
aTPO(IU/ml)	431.97 ± 58.12	p < 0.001	531.5 ± 460.93	p <0.001	26.72 ± 6.8
Treatment	methamizole/ b-blocker		l-thyroxine		none

NS, no statistical significance.
p, statistical significance between patients with GD and controls.*
*p**, statistical significance between patients with HT and controls.*
*anti-TSH***, anti-TSH antibodies levels were analyzed in a selected group of HT patients. (n = 43).*

TABLE 2 | Genotype and allele frequencies for IL-2RA gene polymorphism (rs7093069) in groups with Graves' disease (GD) and with Hashimoto's thyroiditis (HT) compared to control group.

Group	Patients with GB (n = 180)	p (95%CI)/OR	Patients with HT (n = 80)	p (95%CI)/OR	Control group (n = 114)
Allele C	175 (66.2%)	NS	67 (71.2%)	NS	114 (75%)
REF		p = 0.077;			
T	89 (33.7%)	OR = 1.52	27 (28.7%)	p = 0.05 OR = 1.2	38 (25%)
Genotype					
CC	51 (38.6%)	NS	23 (48.9%)	NS	38 (50%)
CT	73 (55.3%)	NS	21 (44.6%)	NS	38 (50%)
TT	8 (6.1%)	p = 0.03 OR = 5.26	3 (6.3%)	p = 0.109 OR = 4.46	0 (0%)

P, p-value; REF., reference. NS, p-value > 0.05. OR, odds ratio. 95% CI, 95% confidence interval for odds ratio.

patients with GD (p = 0.071, OR = 1.45), in 50% of patients with HT (p = 0.028, OR = 1.8), and in 35.6% of healthy children (**Table 3**). In our data the presence of GG genotype significantly reduces the risk of autoimmune thyroid diseases (p = 0.05, OR = 0.42). There were no significant differences in the frequency of A and G alleles and their genotypes when comparing the groups of patients with autoimmune diseases (data not included). A statistical correlation between rs 7138803 FAIM2 gene polymorphism and aTPO antibodies in patients with GD was found (p < 0.05). There was no statistically significant correlation between rs7138803 FAIM2, thyroid hormone levels, and other anti-thyroid antibodies in the studied groups (data not supplied).

Results for PADI4 (rs1748033)

Allele C rs 1748033 PADI4 and its CC genotype were more frequent in patients with autoimmune thyroid diseases, but it was not statistically significant. The occurrence of CT genotype significantly reduces the risk of HT (p = 0.03, OR = 0.4) (**Table 4**). Allele T and its CT genotype occur more frequently in this group of patients with GD, but it is not a significant correlation. The positive correlation of rs1748033 PADI4 with anti-TSH antibodies in patients with GD (p = 0.05) and positive correlation of this gene with aTPO antibodies in patients with HT were found (p = 0.001). It is worth noting that there were no statistically significant correlations between gene polymorphisms and thyroid hormone levels in patients with autoimmune thyroid diseases (data not supplied).

DISCUSSION

In recent years, many reports have been published confirming the various genes in the development of autoimmune thyroid diseases. There are still very few reports of children assessing chosen genes as a risk autoimmune endocrine disorder (2, 3, 5).

An important development of autoimmune diseases is the disturbed immune system. Physiologically correct investigation and removal of the "foreign" antigen ensure the safety of the owned tissues and organs. Autoreactive lymphocytes are cloned in thymus, while in peripheral organs of the immune system, tolerance is achieved by energy or active suppression with Tregs regulatory (suppressor) lymphocytes. IL-2-cytokine is the most important growth factor for T lymphocytes. Moreover, this cytokine has a positive effect on the immune response because, after stimulation of T lymphocyte, it induces the appearance of molecules on its surface that enable apoptosis of this cell. Many

TABLE 3 | Genotype and allele frequencies for FAIM2 gene polymorphism (rs7138803) in groups with Graves' disease (GD) and with Hashimoto's thyroiditis (HT) compared to control group.

Group	Patients with GB (n = 180)	p (95%CI)/OR	Patients with HT (n = 80)	p (95%CI)/OR	Control group (n = 114)
Allele A	126 (44.7%)	p = 0.071	51 (50%)	p = 0.028	57 (35.6%)
REF		OR = 1.45		OR = 1.8	
G	156 (55.3%)	NS	51 (50%)	NS	103 (64.3%)
Genotype					
AA	45 (21.27%)	NS	12 (23.5%)	NS	9 (11.2%)
AG	66 (46.8%)	NS	27 (53%)	NS	39 (48.7%)
GG	45 (31.9%)	p = 0.05	12 (23.5%)	p = 0.02	32 (40%)
		OR = 0.42		OR = 0.28	

P, p-value; REF., reference. NS, p-value >0.05. OR, odds ratio. 95% CI, 95% confidence interval for odds ratio.

TABLE 4 | Genotype and allele frequencies for PADI4 gene polymorphism (rs1748033) in groups with Graves' disease (GD) and with Hashimoto's thyroiditis (HT) compared to control group.

Group	Patients with GB (n = 180)	p (95%CI)/OR	Patients with HT (n = 80)	p (95%CI)/OR	Control group (n = 114)
Allele C	155 (74.8%)	NS	78 (76.5%)	NS	107 (69.5%)
REF					
T	69 (25.2%)	NS	24 (23.5%)	NS	47 (30.5%)
Genotype					
CC	78 (56.9%)	NS	34 (66.7%)	NS	39 (50.6%)
CT	49 (35.8%)	NS	10 (19.6%)	p=0.03	29 (37.7%)
				OR=0.4	
TT	10 (7.3%)	NS	7 (13.7%)	NS	9 (11.7%)

P, p-value. REF., reference. NS, p-value >0.05. OR, odds ratio. 95% CI, 95% confidence interval for odds ratio.

studies have confirmed the association of IL-2 level in serum with many autoimmune diseases, for example, diabetes mellitus type 1 (7), pediatric atopic dermatitis (8), alopecia areata (9), autoimmune thyroid diseases (10), or multiple sclerosis (11). There are only few studies evaluating alleles and individual genotypes of the IL-2 gene in particular diseases (12). It has been shown that the alleles T in rs11938795 Il and Gin rs6822844 Il 2 were significantly associated with a higher risk of colorectal carcinoma in adults (13). Our study has showed that rs7093069 T alleles were more frequent in patients with AITDs compared with healthy children. Genotype TT gave a statistically significant 5.2 times higher risk of GD and increased risk of HT.

As it turns out, there are various immunomodulators that strictly control cellular functions. For example, the citrullination is a process in which arginine is deminated to citrulline. It is catalyzed by a group of hydrolyses called arginine protein deiminases (PADs), from which, till now, five isoforms have been identified. Hypercitrullinating, as a result of increased expression or activity of PAD, is associated with autoimmune diseases such as rheumatoid arthritis, lupus, Alzheimer's disease, ulcerative colitis, multiple sclerosis, and certain cancers (29). PADI4 is found in various cells, including granulocytes, lymphocytes, monocytes and macrophages (30). One of the theories of the autoimmune process is the production of antibodies against citrulline proteins. Peptidylarginine deiminase 4 (PADI4) catalyzes the citrullines of histones and thus regulates the maintenance of stem cells. This process results in extracellular neutrophil traps (NETs), which as citrullinated proteins, are the target of autoantibodies in the treatment of inflammation and arthritis (31). PADI4 was found in the differentiation of macrophages and its role in inflammatory reactions (32). So far, there are no studies evaluating the polymorphism of the gene encoding PADI4 in humans with autoimmune diseases of the thyroid gland. We found that patients with allele C rs1748033 PADI4 and its CC genotype predisposes to the occurrence of autoimmune thyroid diseases.

Apoptosis plays an important role in the pathomechanism of autoimmune thyroid diseases. It is one of the forms of programmed cell death. In Hashimoto's thyroiditis, cytokines released by macrophages and Th1 lymphocytes induce mass regulation by the increase of expression of CD95 molecules on the surface of thyroid cells. This stimulation activates the programmed death of these cells when Fas ligand is present on their surface as a result of self-destruction mechanism. In Graves' disease, Th2 lymphocytes are infiltrated and IL-4 and IL-10 are produced, causing the expression of anti-apoptotic molecules and also, their resistance to apoptosis through CD95. Thyroid cells, both Graves' disease and Hashimoto's disease, showed a strong expression of Fas ligand together with a receptor for Fas. As a result of combining the Fas receptor with its ligand, intracellular areas are initiated, which activate cascades responsible for cell death. There are many molecules that influence the apoptosis process. One of them is the FAIM2 molecule. Many studies suggest that the polymorphism of this gene is associated with obesity and the development of type 2 diabetes (14). A multicenter study of more than 13,000 children aged 2–18 with BMI above 95 percentile years confirmed the occurrence of this gene polymorphism in obese children (15). The variant of gene

FAIM2-rs7138803 is associated with BMI z-scores in children over 6 years (16). On the other hand, the Mexican researchers have not confirmed that kind of association in obese children without metabolic disorders (17). It is known that the obesity gene, the apoptotic suppressing molecule Fas 2 (FAIM2), is regulated by nutritional status, and the promoter FAIM2 methylation levels are significantly related to overweight. An interesting study was conducted by Chinese researchers, who studied the influence of different lifestyles on methylation changes in obese and lean children. They have showed that lifestyle (17) might have an impact on FAIM2 (18), but it has been independently associated with dyslipidemia (19). The polymorphisms of this gene in people with thyroid diseases are till now unknown. In patients with HT, thyroid cells are destroyed as a result of cytotoxic action of T lymphocytes and increased process of apoptosis. Even though in our study we reported that allele A of gene FAIM2-rs7138803 is statistically significantly more frequent in patients with HT, the presence of GG genotype significantly could reduce the risk of autoimmune thyroid diseases. Because of the small group, this requires further research.

There are no studies of these genes' polymorphisms in adolescents with autoimmune thyroid diseases in the literature. To sum up, analysis of polymorphisms of presented genes in clinical practice could allow for determining the predisposition to autoimmune thyroid disease development, to find symptoms of thyroid gland dysfunction earlier, and to use appropriate treatment, but it still requires a lot of research to improve the knowledge on this topic.

CONCLUSIONS

1. Polymorphisms rs7138803-FAIM2 and rs1748033-PADI4 are more frequent in patients with autoimmune thyroid diseases, more frequent in patients with Hashimoto' thyroiditis, but the occurrence of GG rs7138803-FAIM2 genotype could reduce the risk of thyrocyte apoptosis inhibition.
2. The TT rs7093069-IL2RA genotype may increase the risk of autoimmune thyroid diseases.
3. Analysis of polymorphisms of given genes in clinical practice will allow for determining predisposition to autoimmune thyroid disease development, for finding symptoms of thyroid gland dysfunction earlier, and for using appropriate treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of his article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee of Medical University in Bialystok. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kins.

AUTHOR CONTRIBUTIONS

The authors equally participated in the creation of the work. All authors contributed to the article and approved the submitted version.

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