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Genetic Susceptibility to Joint Occurrence of Polycystic Ovary Syndrome and Hashimoto's Thyroiditis: How Far Is Our Understanding?

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Polycystic ovary syndrome (PCOS) and Hashimoto's thyroiditis (HT) are endocrine disorders that commonly occur among young women. A higher prevalence of HT in women with PCOS, relative to healthy individuals, is observed consistently. Combined occurrence of both diseases is associated with a higher risk of severe metabolic and reproductive complications. Genetic factors strongly impact the pathogenesis of both PCOS and HT and several susceptibility loci associated with a higher risk of both disorders have been identified. Furthermore, some candidate gene polymorphisms are thought to be functionally relevant; however, few genetic variants are proposed to be causally associated with the incidence of both disorders together.

Keywords: autoimmune thyroid disease, Hashimoto's thyroiditis, polycystic ovary syndrome, genetic variants, association studies, susceptibility loci

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age (1). Similarly, thyroid dysfunction, including Hashimoto's thyroiditis (HT) as the principal autoimmune thyroid disease (AITD), is frequently observed among young women (2, 3). Both PCOS and HT can result in a wide range of metabolic syndrome features, including weight gain, impaired glucose tolerance, insulin resistance (IR), and dyslipidemia, which can lead to obesity, diabetes, or cardiovascular disease over a lifetime (4–6). Moreover, both disorders are major causes of infertility, a medical problem of a growing prevalence that is associated with strong physical, emotional, and socioeconomic consequences.

There is growing evidence that there may be mutual interaction between PCOS and HT. More importantly, combined occurrence of both diseases is associated with a higher risk of severe metabolic and reproductive complications than either PCOS or HT alone, and the severity of disease symptoms depends on the duration of the thyroid dysfunction (7–10). In addition, co-occurrence of thyroid autoimmune disease is associated with poorer response to treatment in infertile women with PCOS (11). The strong genetic influence on the development of these two diseases is well documented. Many candidate gene variants and susceptibility loci have been identified that are

associated with a higher risk of PCOS or HT; however, the mechanisms underlying the close relationship between these two diseases, and common components to their genetic backgrounds, remain uncertain. Here, we comprehensively review a wide range of reports on genetic discoveries related to PCOS, HT, and their combined occurrence.

POLYCYSTIC OVARY SYNDROME

PCOS is a heterogeneous endocrinopathy that affects 5–20% of women of reproductive age (12). It is characterized mainly by clinical and/or biochemical hyperandrogenism (HA), ovary dysfunction (OD), often reflected by chronic oligo- or anovulatory menstrual cycles, and polycystic ovarian morphology (PCOM) on pelvic ultrasound; for diagnosis, two of these three criteria must be present (13). Consequently, four main PCOS phenotypes have been proposed: phenotype A, with HA, OD, and PCOM; phenotype B, with HA and OD but without PCOM; phenotype C, with HA and PCOM but without OD; and phenotype D, with OD and PCOM but without HA; with phenotypes A and B comprising an estimated > 40% of cases (14, 15). PCOS is characterized by symptoms of hypothalamic-pituitary-ovarian axis dysfunction, including increased hypothalamic gonadotropin-releasing hormone (GnRH) pulse frequency and GnRH quantity, as well as an elevated ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH), which contribute to excessive ovarian androgen production and ovulatory dysfunction (16, 17).

Depending on the phenotype, POCS is associated with variable degrees of reproductive and metabolic dysfunction. Dyslipidemia occurs with a particularly high prevalence of up to 70% (14, 18). Also, defects in insulin activity and secretion, which may lead to IR, hyperinsulinemia, and impaired glucose tolerance, are observed very frequently (19, 20). PCOS is a low-grade inflammatory state (21); women with PCOS have higher levels of inflammatory markers such as C reactive protein (CRP), tumor necrosis factor α (TNF-α), and interleukin 6 (IL6), independent of associated obesity (22). Over a lifetime, PCOS is associated with a higher risk of menstrual irregularities, hirsutism, anovulatory infertility, spontaneous abortion, obesity, and obesity-linked co-morbidities such as nonalcoholic fatty liver disease and metabolic syndrome, type 2 diabetes mellitus, and cardiovascular pathologies, in addition to endometrial adenocarcinoma (20, 23–27). PCOS reduces quality of life, resulting in more frequent depressive episodes and suicide in women affected by this disease (28).

The etiology of PCOS is not fully elucidated. It is a complex disorder that might be represented by a common clinical phenotype of different processes. An IR or intrinsic ovarian dysfunction, possibly stimulated by an imbalance of female sex hormones, are considered inciting factors, which primarily results in increased androgen biosynthesis and secretion by the ovary (29, 30). Resulted hyperandrogenism can be further modulated by impaired insulin action (14). Insulin can also directly stimulate androgen production by amplifying theca cell responses to LH (31).

Genetics of Polycystic Ovary Syndrome

PCOS is a complex, multifactorial disorder with a strong heritable component, as indicated by familial clustering and twin studies, which accounts for as much as 72% of the risk of syndrome development (32, 33). Until recently, the genomic approach to PCOS research was dominated by candidate gene association studies, including more than 100 genes whose alterations may be functionally involved in PCOS development. These genes are mainly related to the insulin signaling pathway and IR, chronic inflammation, reproductive hormone disorders, and biosynthesis of androgens; such as those encoding insulin (INS), INS receptor (INSR), INSR substrates (IRS), methylenetetrahydrofolate reductase (MTHFR), IL family members, TNF-α, toll-like receptor 2 (TLR2), fat mass and obesity-associated protein (FTO), paraoxonase 1 (PON1), calpain 10 (CAPN10), peroxisome proliferator-activated receptor γ (PPAR-γ), FSH receptor (FSHR), LH/choriogonadotropin receptor (LHCGR), LH-β, sex hormone-binding globulin (SHBG), androgen receptor, transforming growth factor β (TGF-β), fibrillin 3 (FBN3), and vitamin D receptor (VDR) (17, 33–37). Nevertheless, the vast majority of candidate gene variants associated with PCOS in previous studies have not been replicated, indicating the possibility of substantial genetic heterogeneity across various ethnic populations or different genetic architectures underlying specific PCOS phenotypes (38–49).

Genome-Wide Association Studies for Polycystic Ovary Syndrome

To more deeply investigate genetic predisposition to PCOS, several genome-wide association studies (GWAS) have been carried out (**Table 1**) (50–54, 59, 66). The first two studies were conducted in China, and together identified 11 susceptibility loci located within or near genes implicated in: 1) gonadotropin activity, ovulation, and erectile dysfunction [*FSHR* and *LHCGR*, both at 2p16.3, and the zinc aminopeptidase gene (*C9ORF3*) at 9q22.32]; 2) insulin signaling and diabetes mellitus [the thyroid adenoma-associated protein (*THADA*) at 2p21, high mobility group AT-hook protein (*HMGA2*) at 12q14.3, and *INSR* at 19p13.3]; 3) organ growth control, cell proliferation, and apoptosis [yes-associated protein (*YAP1*) at 11q22.1 and zinc finger protein 217 (*ZNF217*) at 20q13.2]; 4) endosomal membrane trafficking and exocytosis of gonadotropins [DENN domain-containing protein 1A (*DENND1A*) at 9q33.3 and RAS-related protein (*RAB5B*) at 12q13.2]; and 5) modification of chromatin structure [TOX high mobility group box family member 3 protein (*TOX3*) at 16q12.1] (51, 52). Subsequently, associations between the single nucleotide polymorphism (SNP) rs13425728 in *LHCGR* with higher levels of testosterone, triglycerides, and low density lipoprotein (LDL) were detected among Hui Chinese subjects (56), and the two SNPs rs10818854 and rs10986105 in *DENND1A* were associated with increased HOMA-IR and anti-Müllerian hormone (AMH) levels in Arab women with PCOS (68).

Later, two GWAS were conducted in Korean populations (50, 53). In the first, three novel SNPs in the glycogen synthase 2 (*GYS2*) gene on chromosome 12p12.2 were identified as being associated with PCOS through investigation of individuals with obesity-related condition (53); however, none of these variants

TABLE 1 | GWAS and replication studies for PCOS susceptibility.

Locus	Nearby genes	dbSNP ID[a/]		GWAS population [references]	Replication study population [references]	
					Replicated	Not replicated
1p22	OLFM3	rs11164278	*downstream*	KRN (50)		
2p16.3	**LHCGR**	rs13405728	*intron*	CHN (51, 52)	KRN (50, 53); EUR (54, 55); CHN (56–58)	EUR (59–63)
2p16.3	**FSHR**	rs2268361	*intron*	CHN (52)	KRN (50); EUR (54, 59, 64)	CHN (58, 65)
		rs2349415	*intron*	CHN (52)	CHN (65)	EUR (64); CHN (58)
2p21	**THADA**	rs13429458	*intron*	CHN (51, 52)	KRN (50, 53); EUR (59); CHN (57)	EUR (55, 60–64); CHN (56)
		rs12478601	*intron*	CHN (51)	KRN (53); EUR (54, 55, 63)	EUR (60, 64)
		rs12468394	*intron*	CHN (51)	KRN (53); EUR (54, 55, 63, 64)	EUR (60)
		rs7563201	*intron*	EUR (59, 66)		
2q34	ERRB4	rs1351592	*intron*	EUR (59)		
		rs2178575	*intron*	EUR (66)		
3q26.33	PEX5L	rs7652876	*upstream*	KRN (50)		
4p12	GABRB1	rs1159315	*intron*	KRN (50)		
4q35.2	TRIML1/TRIML2	rs7666129	*upstream*	KRN (50)		
5q31.1	RAD50	rs13164856	*downstream*	EUR (59, 66)		
8q24.2	KHDRBS3	rs10505648	*upstream*	KRN (50)		EUR (54)
8q32.1	GATA4/NEIL2	rs804279	*between*	EUR (54, 66)		
9p24.1	PLGRKT	rs10739076	*upstream*	EUR (66)		
9q21.32	RASEF	rs11536913	*upstream*	KRN (50)		
9q22.32	**AOPEP (C9orf3)**	rs3802457	*intron*	CHN (52)	EUR (54); CHN (58, 65)	EUR (59)
		rs4385527	*intron*	CHN (52)	EUR (54, 55)	KRN (50); CHN (58, 65); EUR (64)
		rs10993397	*intron*	EUR (54)		
		rs7864171	*intron*	EUR (66)		
9q33.3	**DENND1A**	rs2479106	*intron*	CHN (51, 52)	ASN (67)	KRN (50); CHN (56, 58); EUR (55, 59–64, 67); ARB (68, 69)
		rs10818854	*intron*	CHN (51)	KRN (53); ASN (67, 70); ARB (68); EUR (54, 55, 60, 63, 67, 70)	ARB (69); CHN (58)
		rs10986105	*intron*	CHN (51)	KRN (53); ASN (67); ARB (68); EUR (54, 55, 60, 64, 67)	ARB (69)
		rs9696009	*intron*	EUR (66)		
11p13	HIPK3	rs4755571	*3'UTR*	KRN (50)		
11p14.1	FSHB	rs11031006	*downstream*	EUR (54, 59)	CHN (71)	
		rs11031005	*downstream*	EUR (66)		
11q22.1	**YAP1**	rs1894116	*intron*	CHN (52)	KRN (50); EUR (54, 55, 59)	CHN (65)
		rs11225154	*intron*	EUR (59, 66)		
11q23.2	ZBTB16	rs1784692	*intron*	EUR (66)		
12p12.2	GYS2	rs7485509	*intron*	KRN (53)		
		rs10841843	*intron*	KRN (53)		
		rs6487237	*intron*	KRN (53)		
12q13.2	**RAB5B/SUOX**	rs705702	*5'UTR*	CHN (52)	KRN (50); EUR (54, 55, 59)	CHN (58, 65)
12q13.2	ERBB3	rs2271194	*intron*	EUR (66)		
12q14.3	HMGA2	rs2272046	*intron*	CHN (52)	EUR (59)	EUR (54, 64); CHN (58, 65)
12q21.2	KRR1	rs1275468	*upstream*	EUR (59)		
		rs1795379	*upstream*	EUR (66)		
16q12.1	**TOX3**	rs4784165	*downstream*	CHN (52)	KRN (50); EUR (59)	EUR (54); CHN (58, 65)
		rs8043701	*downstream*	EUR (66)		
16p13.3	SOX8	rs500492	*upstream*	KRN (50)		
19p13.3	INSR	rs2059807	*intron*	CHN (52)	EUR (64)	KRN (50); EUR (54, 55, 59); CHN (58, 65)
20q11.21	MAPRE1	rs853854	*intron*	EUR (66)		
20q13.2	ZNF217	rs6022786	*upstream*	CHN (52)	KRN (50)	EUR (54, 59); CHN (58, 65)

Bolded are loci replicated in at least two replication studies. Study populations: ARB, Arabic; ASN, Asian; CHN, Chinese; EUR, European; KRN, Korean.
[a/]SNP identifier based on NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp/).

reached the genome-wide significance threshold. In the second study, eight new susceptibility loci were suggested, including a main association signal at rs10505648 on chromosome 8q24.2, close to KH RNA-binding domain-containing, signal transduction associated 3 (*KHDRBS3*), which is associated with regulation of telomerase activity and variant splicing (72), and seven moderate signals at 1p22, 3q26.33, 4p12, 4q35.2, 9q21.32, 11p13, and 16p13.3 (50). In addition, eight of the Chinese susceptibility loci were replicated in Korean women (**Table 1**).

Two GWAS were recently conducted in populations of European ancestry, confirming the association of the three Asian susceptibility loci (*YAP1, THADA*, and *C9ORF3*), as well as identifying five novel loci corresponding to the epidermal growth factor receptor (*ERBB4/HER4*) gene at 2q34, double strand break repair protein (*RAD50*) at 5q31.1, the *GATA4/NEIL2* region encoding zinc finger transcription factor and endonuclease VIII-like 2 at 8q23.1, FSH β polypeptide (*FSHB*) at 11p14.1, and *KRR1* at 12q21.2, which encodes a ribosome assembly factor (**Table 1**) (54, 59). The same rs11031006 variant in the *FSHB* region, which was identified independently in both European GWAS, was associated with lower level of FSH and higher levels of LH and the LH/FSH ratio, suggesting that it may act by affecting gonadotropin secretion (54, 59). Together with the Chinese findings implicating *FSHR* variants, these results strongly suggest an etiologic role for gonadotropins in PCOS development. Additionally, the identified associations point to gonadal development, ovarian folliculogenesis, and follicular maturation, as well as repair of DNA damage, as processes involved in PCOS pathogenesis in European woman.

Recently, a large-scale genome-wide meta-analysis including 10,074 PCOS cases and 103,164 controls of European ancestry (66), confirmed 11 previously reported loci and identified three additional novel susceptibility loci: *ZBTB16* (zinc finger and BTB domain-containing 16) at 11q23.2, which is involved in cell cycle progression, control of the early stages of spermatogenesis, and endometrial stromal cell decidualization (73, 74); *MAPRE1* (microtubule associated protein RP/EB family member 1) at 20q11.20, which is involved in the regulation of microtubule structures and chromosome stability, and may participate in follicle development; and the plasminogen receptor (*PLGRKT*) gene at 9p24.1, which contributes to regulation of inflammatory responses and matrix metalloproteinase activation (**Table 1**). These newly identified loci provide additional evidence for the involvement of neuroendocrine, metabolic, and reproductive factors in PCOS. Additionally, the data suggest that the genetic architecture underlying the different PCOS phenotypes does not differ in terms of common susceptibility variants (66).

Post-Genome-Wide Association Studies for Polycystic Ovary Syndrome

A number of gene polymorphisms discovered in Chinese populations, including *LHCGR*, *FSHR*, *THADA*, *DENND1A*, *C9ORF3*, *YAP1*, and *RAB5B/SUOX*, have been confirmed in at least two replication studies or meta-analyses for different Caucasian populations, and can be considered general PCOS susceptibility variants (**Table 1**) (55–58, 60–65, 67–71). However, GWAS have identified < 10% of the estimated heritability of PCOS (36), and there is relatively little overlap between the gene loci identified by candidate gene studies and those found by GWAS, likely because polymorphisms in gene coding regions are usually rare genetic variants that have a large impact on disease risk, whereas GWAS is designed to focus primarily on common variants with a low impact on genetic risk.

The greatest challenge for genomic studies is to identify causative mechanisms and determine the functional relevance of loci identified as risk factors by GWAS. A pathway-based approach has been proposed to increase the power to determine the biological meaning of GWAS results. Application of meta-analysis gene-set enrichment of variant associations (MAGENTA) to the PCOS GWAS dataset revealed oocyte meiosis and regulation of insulin secretion by acetylcholine and free fatty acids as biological pathways significantly associated with the syndrome (75). Among significantly associated genes, the *INS* gene was indicated in all three pathways. However, some caution should be taken with regard to these results as the analysis included variants of lower significance level than generally accepted for GWAS.

The genetic loci identified by GWAS are often named according to the nearest gene. While the nearby location of a gene does not necessarily mean that it is an effector gene at a given locus, several putative susceptibility genes located within GWAS-discovered risk loci for PCOS were found to be abnormally expressed in women with this condition. PCOS subtypes have been proposed based on phenotypes, expression quantitative trait loci, and biological pathway analyses, in which genes related to gonadotropin levels, metabolic mechanisms, or inflammation and its consequences, have independent impacts on the risk of syndrome development (76). Similarly, expression analysis of genes from 11 GWAS-identified PCOS risk loci revealed that different mechanisms may be involved in the pathogenesis of clinically diverse PCOS subtypes in relation to obesity, since *LHCGR* is over-expressed in non-obese women and *INSR* is under-expressed only in obese women with PCOS. A causal role was hypothesized for SNPs in the *LHCGR, INSR*, and *RAB5B* regions, suggesting that they may affect gene expression directly or indirectly through epigenetic mechanisms (77). Also, lower expression levels of the *RAB5A* gene in granulosa cells of obese patients with PCOS were observed relative to those in obese women without the syndrome, which may explain the high FSHR levels and FSH-FSHR signaling pathway disorders in PCOS (78). In turn, *HMGA2*, another gene identified by GWAS as a candidate, is expressed at high levels in granulosa cells of women with PCOS, where the *HMGA2/IMP2* pathway is suggested to be responsible for granulosa cell proliferation (79).

One of the most extensively studied GWAS-identified PCOS candidate genes is *DENND1A*, which encodes a clathrin binding protein (80). The PCOS risk SNP, rs10986105, in *DENND1A* was also found to increase the risk of hyperandrogenism in women without PCOS (60). DENND1A variant 2 (DENND1A.V2), a truncated splice isoform of DENND1A with a unique C-terminal sequence, is up-regulated in PCOS theca cells, and compelled expression of V2 in normal theca cells results in increased

expression of genes encoding cytochrome P450 isoforms (*CYP17A1* and *CYP11A1*) and higher androgen production (81, 82). Recently, a functional network, including miR-130b-3p, DENND1A.V.2, LHCGR, RSB5B, and the signaling pathways they target, was proposed to potentially mediate PCOS-related hyperandrogenemia. The functional significance of this network is strongly supported by the discovery of the co-localization of LHCGR, DENND1A.V.2, and RAB5B proteins in theca cells (82), as well as the fact that decreased expression of miR-130b-3p, which is predicted to target DENND1A, correlates with increased V2 mRNA levels and androgen synthesis in PCOS theca cells (83).

Despite the clear and strong influence of genetic factors in the pathogenesis of PCOS, common genetic risk loci identified to date explain only a small percentage of estimated PCOS heritability. Therefore, next-generation sequencing studies to investigate entire genomic regions have been conducted to determine whether rare genetic variants with large effect size may contribute to PCOS pathogenesis. Potentially deleterious variants in *AMH* were identified using whole-genome and targeted sequencing of *AMH* in a case-control PCOS study (84). Seventeen PCOS-specific rare *AMH* coding variants resulted in significant reduction of AMH-mediated signaling in dual luciferase reporter tests. Therefore, *AMH* mutations in PCOS are suggested to result in decreased AMH-mediated inhibition of *CYP17* expression and androgen biosynthesis, leading to syndrome-specific hyperandrogenism. Furthermore, the missense variant rs104893836 in the first exon of the GnRH receptor gene (*GNRHR*) was detected by the whole-exome sequencing (85). Functional analyses revealed significantly reduced GnRH binding by the resulting variant GnRHR containing a Gln106Arg substitution; however, since this is a fairly common variant affecting the first extracellular receptor loop, its effect *in vivo* is likely to be mild (86). It is worth noting that an increase rather than a decrease in GnRH signaling is a hallmark of PCOS. Recently, whole-genome sequencing revealed several rare non-coding variants in *DENND1A* associated with reproductive and metabolic traits in PCOS families, suggesting their contribution to disease pathogenesis and providing additional evidence for the central role of DENND1A in PCOS (87).

AUTOIMMUNE THYROID DISEASE

AITD is the most common autoimmune disorder, affecting 5%–20% of the female population of fertile age (88). Its prevalence varies depending on age, geographical origin, and iodine intake (2). AITD is classified as an organ-specific autoimmune disorder mediated by T cells (89). An autoimmune attack, directed against components of the thyroid gland, may lead to clinically heterogeneous conditions, manifested by either thyroid hormone excess (hyperthyroidism), as in the case of Graves' disease (GD), or reduced hormone production (hypothyroidism), a typical feature of HT, on which this review will mainly focus.

HT is considered the principal autoimmune disease among young women and the most frequent form of AITD, affecting 4%–9.5% of the adult population (90); its occurrence is eight times more common in women than in men (88). As a result of autoimmunity to self-antigens, approximately 60%–80% of patients with HT have serum antibodies against thyroglobulin (Tg) and 90%–95% have antibodies against thyroid peroxidase (TPO) (91, 92). HT is characterized by infiltration of lymphocytes and chronic inflammation of the thyroid gland, which promotes T cell-induced apoptosis of thyroid follicular cells. The Fas/Fas ligand (FasL) cascade is the main signaling pathway leading to apoptosis of thyroid cells in response to infiltration by pro-inflammatory cytokines, such as interferon (IFN)-γ, TNF-α, and IL1β (93). It is proposed that the mechanism of apoptosis leading to the characteristic thyroid destruction in HT may be a consequence of abnormal Fas/FasL regulation and decreased expression of the apoptosis regulator Bcl-2 (94, 95).

The progressive destruction, and finally fibrosis, of the glandular parenchyma often leads to hypothyroidism. AITD is considered the most frequent cause of hypothyroidism, although it can go unnoticed for years, without overt thyroid dysfunction. Subclinical hypothyroidism (SCH), defined as a serum thyroid-stimulating hormone (TSH) above the defined upper limit of the reference range, in combination with a normal level of free thyroxine (fT_4), is more common than overt hypothyroidism, in which fT_4 levels are reduced below the normal lower limit and TSH levels are further increased (96). HT has clinically heterogeneous presentation, from the presence of thyroid antibodies but normal thyroid function, to SCH or overt hypothyroidism. The prevalence of hypothyroidism among women with HT increases with age, and in most of cases it eventually develops despite patients initially being euthyroid (97). The diagnostic criteria for HT are based on detection of elevated levels of circulating anti-thyroid antibodies (anti-TPO and/or anti-Tg) and a typical hypoechogenic pattern of the thyroid gland on ultrasound examination (3). Although the presence of thyroid antibodies is a marker for thyroid damage, they are unlikely to play a role in the pathogenesis of HT (98).

Hypothyroidism may be associated with serious pregnancy complications, such as spontaneous abortion, preterm delivery, and placental abruption, as well as reduced fertility, ovulation disorders, insufficient endometrial thickness, and excessive, irregular menstrual bleeding (99–101). A recently published meta-analysis showed that SCH significantly increases the risk of miscarriage before 20 weeks of pregnancy and that early treatment can reduce the miscarriage rate (102). In hypothyroid states, lower levels of several hormones related to the ovarian axis, including SHBG, 17-β-estradiol (E_2), testosterone, and androstenedione, were observed (103). As a result of increased secretion of thyrotropin-releasing hormone (TRH), prolactin levels can increase, while LH and FSH concentrations remain normal (104). Underactive thyroid is also associated with the formation of ovarian cysts, which were normalized after T_4 replacement (105, 106).

Metabolic changes are also common in hypothyroidism, particularly dyslipidemia and IR, and the severity of these disorders correlates with TSH concentration, even within its normal range (107–109). A link between autoimmunity and

obesity has been noted, with leptin as a factor linking the two conditions (110). Being overweight during childhood is positively associated with anti-TPO antibody levels in women at 60–64 years-of-age (111). Since thyroid hormones have a wide impact, the symptoms of thyroid dysfunction can be systemic, affecting almost every physiological system, in addition to significant reproductive or metabolic defects (3). Nevertheless, the pathogenesis of HT remains largely unknown.

Genetics of Autoimmune Thyroid Disease

As in the case of PCOS, a strong genetic predisposition to AITD has been established, suggesting that over 70% of the susceptibility to the development of antibodies directed against thyroid antigens can be attributed to genetic factors (112). Among first-degree relatives of patients with HT, the risk of developing HT is increased by 20- to 30-fold; in 60% of first-degree relatives of patients with HT, anti-thyroid antibody positivity is observed (27). Also, the high concordance of HT in monozygotic twins (55%) strongly supports a genetic component in AITD predisposition (113).

Candidate gene case-control studies identified several putative susceptibility variants associated with AITD development or progression. These included variants in genes encoding proteins related to inflammation, modulation of immune responses, or specific for the thyroid, such as: human leukocyte antigen (HLA) class I and class II, forkhead box P3 (FOXP3), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), cluster of differentiation 40 (CD40), protein tyrosine phosphatase, non-receptor type 22 (PTPN22), selenoprotein S (SEPS1), IL4, IL2 receptor α (IL2RA), VDR, Tg, TSH receptor (TSHR), signal transducer and activator of transcription 3 (STAT3), and STAT4, with *HLA-DR3* carrying the highest risk (2, 114–120). Among non-HLA genes, *CTLA4* and *PTPN22* were most consistently identified as predisposing to both HT and GD (121–124), while the *TSHR* locus appears to be specific for GD, but not HT, suggesting some genetic differences between these two types of AITD (125, 126); however, similar to PCOS, candidate gene screening for HT susceptibility mainly generated controversial and non-replicable findings (127–132). Limited understanding of disease pathogenesis and the consequent lack of comprehensive datasets on candidate genes, small sample sizes, and low statistical power, were some of the main limitations of candidate gene studies for both disorders. The era of GWAS and subsequent replication studies have brought further data on genetic susceptibility to HT.

Genome-Wide Association Studies and Post-Genome-Wide Association Studies for Autoimmune Thyroid Disease and Thyroid Function Related Traits

Several GWAS for AITD (HT or GD), hypothyroidism, positivity for anti-thyroid (anti-TPO or anti-Tg) antibodies, and thyroid function parameters (including TSH or fT_4 levels), have been undertaken (**Table 2**) (133–135, 139–146, 148, 154, 155, 157–160). Some GWAS-identified AITD susceptibility loci have been replicated in different populations (**Table 2**) (136–138, 147, 149–153, 156); however, HT is rather poorly represented among GWAS. Numerous studies have been performed using small cohorts or patients with HT and GD grouped together (128, 161). In total, six GWAS included separate groups of patients with HT (134, 140, 146, 147, 154, 159), while three included patients with hypothyroidism, a typical feature of HT (133, 135, 158). Together, 16 putative HT susceptibility loci have been identified; however, the most convincing evidence for associations is limited to *HLA* (6p21), *CTLA4* (2q33.2), *PTPN22* (1q13.2), and *FOXE1* (9q22.33) variants. Additionally, two loci, *TPO* (2p25.3) and *ATXN2* (ataxin2; 12p24.12), are convincingly associated with the presence of anti-TPO antibodies, and three loci, *CAPZB* (capping actin protein of muscle Z-line subunit beta; 1p36.13), phosphodiesterase *PDE8B* (5q13.3), and *PDE10A* (6q27), are associated with TSH levels (**Table 2**).

A genetic overlap was observed between susceptibility loci identified in GWAS for AITD or hypothyroidism and GWAS for thyroid function, including *FOXE1*, *CAPZB*, and *PDE8B*. *FOXE1*, also known as TTF-2 (thyroid transcription factor 2), is associated with hypothyroidism, and with TSH and fT_4 levels, as well as with thyroid cancer (133, 143, 158, 162). This factor is involved in thyroid gland development and differentiation (163, 164). FOXE1 regulates *TG* and *TPO* transcription by binding to response elements in their promoter regions (165), and is necessary for synthesis of thyroid hormones. Both PDE8B and CAPZB also have strong links to thyroid function. *PDE8B* encodes a phosphodiesterase that catalyzes the hydrolysis of cAMP and is primarily expressed in the thyroid gland (166). It is proposed that PDE8B may affect TSH release from the pituitary and mediate the effects of TSH in the thyroid by altering cAMP levels (155). A role for PDE8B in cAMP-dependent generation of triiodothyronine (T_3) and T_4 has also been proposed (143). Similarly, CAPZB, a member of the F-actin-capping protein family, is highly expressed in thyroid tissue. As a protein associated with cytoskeleton remodeling and assembly of cytoplasmic microtubules, it may contribute to the disturbance of thyroid follicular architecture commonly observed in AITD.

Interestingly, three independent GWAS connected *VAV3* (vav guanine nucleotide exchange factor 3) variants with HT, hypothyroidism, and TSH levels (133, 140, 141). VAV3 activates Rho and Rac GTPases and is important for both thyroid and immune function (167). VAV3 is also necessary for B-cell receptor endocytosis and antigen presentation by major histocompatibility complex (MHC) class II molecules (168).

Notably, the MHC region, *CTLA4*, and *PTPN22*, which were identified by GWAS as most significantly associated with HT, were previously described as AITD susceptibility loci, based on a systematic review of candidate genes (128). All of these loci are related to autoimmune responses and involved in antigen presentation and T cell receptor signaling.

Human Leukocyte Antigen Alleles

The highly polymorphic MHC region of chromosome 6p21, encoding the HLA glycoproteins, is the most intensively studied region of the genome in the search for associations with development of AITD. Numerous *HLA* alleles have been identified as genetic risk factors for AITD; however, the data

TABLE 2 | GWAS and replication studies for AITD and thyroid dysfunction traits susceptibility.

Locus	Nearby genes	dbSNP ID[a/]	GWAS		Replication study	
			Population [references]	Phenotype	Population [references]	Phenotype
1p13.2	**PTPN22**	rs2476601 *(1858 C/T)*	USA-EUR (133)	hypothyroidism	EUR (135, 136)	hypothyroidism
			UK (134)	HT+GD	POL (137)	HT, GD
		rs6679677	USA-EUR (133)	hypothyroidism		
		rs3811021			POL (138)	HT
1p13.2	MAGI3	rs1230666	EUR-CAU (139)	anti-TPO level		
1p13.3	VAV3	rs4915077	USA-EUR (133)	hypothyroidism		
		rs7537605	JAP (140)	HT		
		rs12126655	KRN (141)	TSH level		
1p36.13	**CAPZB**	rs1472565	USA-EUR (133)	hypothyroidism		
		rs10799824	ICE (142)	TSH level	USA-EUR (143)	TSH level
		rs10917469	UK (144)	TSH level		
		rs6683419			CHN (145)	TSH level
2p16.3	FSHR	rs12713034	CRT (146)	anti-TPO/Tg-positivity		
2p25.1	TRIB2	rs1534422	UK (134)	HT+GD		
2p25.3	**TPO**	rs11675434	EUR-CAU (139)	anti-TPO-positivity, anti-TPO level	CRT (147)	HT
					CRT (148)	anti-TPO/Tg-positivity
		rs2071403	KRN (141)	anti-TPO-positivity		
		rs11211645			POL (138)	HT
2q33.2	**CTLA4**	rs231775 *(A49G)*	UK (134)	HT+GD	POL (149); IND (150)	HT, GD
		rs3087243 *(CT60)*	EUR (135)	hypothyroidism	EUR (136)	hypothyroidism
					JAP (151); CHN (152)	HT, GD
		rs231779	USA-EUR (133)	hypothyroidism		
2q36	IGFBP5	rs13015993	USA-EUR (143)	TSH level		
		rs6435953			CRT (153)	anti-Tg level
3q13.12	DUBR	rs561030786	CRT (146)	anti-TPO/Tg-positivity		
3q21	KALRN	rs2010099	EUR-CAU (139)	anti-TPO level		
3q27.3	LPP	rs13093110	UK (134)	HT+GD		
4q27	GPR103	rs7679475	NHW (154)	HT		
		rs1513695	NHW (154)	HT		
4q31.2	NR3C2	rs10032216	USA-EUR (143)	TSH level		
		rs11935941			CRT (153)	HT
4q32.3	TRIM61 TRIM60	rs12507813	CRT (146)	anti-TPO-positivity		
5q13.3	**PDE8B**	rs4704397	ITN (155)	TSH level	USA-EUR (133)	hypothyroidism
					CRT (156)	HT
		rs6885099	ITN (155)	TSH level	KRN (141); USA-EUR (143)	TSH level
		rs2046045	ICE (142)	TSH level		
5q21.2	RP11-138J23.1	rs13190616	CRT (146)	anti-TPO/Tg-positivity		
6p21.3	**HLA class I**	rs2517532	USA-EUR (133)	hypothyroidism	CRT (157)	HT
		rs2516049	USA-EUR (133)	hypothyroidism		
6p21.3	**HLA-DPB2**	rs733208	KRN (141)	anti-TPO-positivity		
6p21.3	**HLA-DRB1**	rs17886918			POL (138)	HT
6p21.3	**HLA-DQB1**	rs3210176			CRT (157)	HT
6p21.3	IP6K3	rs791903	CRT (157)	HT		
6q15	BACH2	rs72928038	UK (134)	HT+GD		
		rs10944479	EUR-CAU (139)	anti-TPO-positivity		
6q27	DLL1	rs4710782	CRT (148)	anti-Tg level		
6q27	PDE10A	rs2983521	ITN (155)	TSH level		
		rs3008043	ICE (142)	TSH level		
		rs753760			USA-EUR (143)	TSH level
7q31.31	ANKRD7 LSM8	rs6972286	CRT (146)	anti-Tg-positivity		
8q12.1	XKR4	rs2622590	CHN (145)	TSH level		
9p24	GLIS3	rs1571583	USA-EUR (143)	TSH level	CRT (153)	anti-TPO level
		rs10974423				
9q21.2	GNA14	rs75201096	CRT (157)	HT		
9q22.33	**FOXE1**	rs7850258	USA-EUR (158)	hypothyroidism	EUR (136)	hypothyroidism
		rs965513	USA-EUR (158)	hypothyroidism	CRT (157)	HT
			ICE (142)	TSH level		

(Continued)

TABLE 2 | Continued

Locus	Nearby genes	dbSNP ID[a/]	GWAS		Replication study	
			Population [references]	Phenotype	Population [references]	Phenotype
		rs925489	USA-EUR (158)	hypothyroidism	USA-EUR (133) CHN (145)	hypothyroidism TSH level
9q31.1	GRIN3A	rs4457391	CRT (148)	anti-TPO/Tg level		
		rs1935377	CRT (148)	anti-TPO level		
9q32	WHRN	rs4979402			CRT (156)	anti-Tg level
11q21	FAM76B	rs4409785	UK (134)	HT+GD		
12q24.12	SH2B3	rs3184504	USA-EUR (133)	hypothyroidism	CRT (156)	HT, anti-TPO level
12q24.12	**ATXN2**	rs653178	EUR-CAU (139)	anti-TPO-positivity	CRT (148)	anti-TPO/Tg level
		rs10774625			CRT (147)	HT, anti-TPO-positivity
14q13	MBIP	rs1537424	USA-EUR (143)	TSH level	CRT (153)	HT, anti-Tg level
14q31	CEP128	rs327463	CHN (159)	HT, GD		
15q14	RASGRP1	rs7171171			CRT (147)	HT, anti-TPO-positivity
16q24	IRF8	rs16939945	CRT (160)	anti-TPO/Tg-positivity		
17q21.33	CA10	rs756763	CRT (146)	anti-Tg-positivity		
17q25	SDK2	rs12944194	CRT (157)	HT		
18p11.32	YES1	rs77284350	CRT (160)	anti-TPO/Tg-positivity		
19p13.2	INSR	rs4804416	USA-EUR (143)	TSH level	CRT (153)	anti-Tg level

Bolded are loci replicated in at least two replication studies. Study populations: ASN, Asian; CAU, Caucasian; CHN, Chinese; CRT, Croatian; EUR, European; ICE, Icelandic; IND, Indian; ITN, Italian; JAP, Japanese; KRN, Korean; NHW, non-Hispanic White; POL, Polish; EUR-CAU, EUR-Caucasian ancestry; USA-EUR, USA-European ancestry.
GD, Graves' disease; HT, Hashimoto's thyroiditis; Tg, thyroglobulin; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone.
[a/]SNP identifier based on NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp/).

on *HLA* haplotypes in HT are less definitive than that in GD. Although, some *HLA* alleles associated with HT are common to GD, others appear to be unique to each disease (119, 169, 170), suggesting that *HLA* genotypes may contribute, at least in part, to differences in HT and GD immunopathogenic mechanisms.

Among the *HLA* class I alleles, *HLA-A*02:07* was associated with HT susceptibility in a Japanese population (169, 170). Of note, this allele was the strongest and most significantly associated susceptibility allele in HT, in contrast to GD, where alleles from the MHC class II region were more strongly associated (169). Furthermore, it was estimated that approximately 58% of patients with HT carried at least one of four *HLA* class I alleles (*HLA-A*02:07*, *B*35:01*, *B*40:02*, or *B*40:06*) (169). Conversely, in Caucasian populations, only a few studies have suggested associations between *HLA* class I alleles and HT risk (171, 172). Hence, it is hypothesized that ethnic differences may play a role in the immunological mechanisms involved in thyrocyte destruction (169).

Numerous associations between *HLA* class II alleles and HT have been demonstrated (particularly in Caucasian populations), including *DQA1*03*, *DQB1*02*, *DQB1*03*, *DRB1*03*, and *DRB1*04* alleles, or *DR3*, *DR4*, and *DR5* haplotypes (173–176). In Japanese population, *DRB1*04* and *DRB4*01* were suggested as susceptibility alleles, while *DQA1*01* and *DQB1*06* were protective (169). However, all of these studies included relatively small cohorts and generated inconsistent results. Some of these previous observations have been confirmed in a large group of UK Caucasian patients with HT; *DRB1*04*, *DQB1*03:01*, and *DQA1*03:01* showed the most significant predisposing effect, while *DQA1*01:02*, *DQA1*02:01*, and *DQB1*06* had the most significant protective effect. Overall, a strong predisposing association between *DR4* or *DR8* haplotypes and HT has been found, as well as a borderline association with *DR3*, whereas protective effects were detected for *DR13*, *DR7*, and *DR15* (119). Similarly, *DRB1*04:05*, *DQB1*02:01*, *DQB1*03:02*, and *DQA1*03:01* allele frequencies were higher in Greek patients with HT, while those of *DRB1*07* were lower, than in the control group (177).

In recent studies including non-Caucasian populations, the *HLA-DRB4*53:01* allele was identified as associated with HT susceptibility in Japanese patients (170) and the *DR8* haplotype in Koreans (178). Additionally, *HLA* alleles in the *HP-2* haplotype (*HLA-A*33:03-C*14:03-B*44:03-DRB1*13:02-DQB1*06:04-DPB1*04:01*) and the *HP-2* haplotype itself were associated with protective effects against GD and HT in a Japanese population (169, 170), with OR = 0.36 for HT (169). Furthermore, the *HLA-DRB1*03* allele was shown to be predisposing in Kayseri Turkish patients with HT, in contrast to *DRB1*01*, which was associated with a protective effect (179). Moreover, in an Indian population, *DRB1*12* and *DRB1*10* exhibited the strongest predisposing and protective effects, respectively, among different *HLA-DRB1* alleles (150); however, in contrast to previous reports, a decreased frequency of the *DRB1*03* allele was observed in HT patients in this study.

Due to its crucial role in the presentation of peptide antigens to T cells, particular attention has been paid to the molecular structure of the peptide binding pocket in HLA class II molecules. Arginine at position 74 of the HLA-DRβ1 chain (DRβ1-Arg74) was identified as a critical pocket amino acid signature that confers susceptibility to both GD and HT (180, 181). Conversely, the presence of glutamine at position 74 of the DRβ1 chain conferred a protective effect. It was suggested that a specific HLA-DR peptide binding pocket structure may predispose to AITD by enabling presentation of certain autoantigens, such as Tg, TPO, or TSHR pathogenic peptides. Indeed, the non-synonymous *TG* variant, W1999R, can interact

with HLA-DRβ1-Arg74, and together these two variants confer a high risk for AITD (182). Recently, among Polish patients, HLA-DRB1 with phenylalanine at position 67 (encoded by the rs17886918T variant) was significantly associated with HT (138).

Cytotoxic T-Lymphocyte-Associated Protein 4

GWAS identified several *CTLA4* genetic variants as being associated with AITD (both HT and GD) or hypothyroidism, and these associations were widely replicated in various ethnic groups (**Table 2**). The most consistent associations with HT were a SNP at position 49 (A49G, rs231775) in the CTLA4 leader peptide, resulting in an alanine to threonine substitution, and a SNP located near to the 3' UTR (CT60, rs3087243) in both the Caucasian and Asian populations (**Table 2**). Several subsequent meta-analyses have confirmed these associations (183–187). *CTLA4* is suggested to contribute to AITD susceptibility by interacting with other loci (188) and a strong synergy has been demonstrated for the *CTLA4* and *HLA* genes in AITD (189). In an Indian population, associations with both susceptibility (for a combination of the G allele of *CTLA4*, A49G, and the *HLA-DR5* allele) and protection (for a combination of the A allele of *CTLA4*, A49G, and the *HLA-DR3*, *DR10*, and *DR15* alleles) have been established (150).

CTLA4 is a transmembrane protein expressed on activated T cells that functions as a negative regulator of their activation by competing with CD28 for binding to the ligand B7 on antigen-presenting cells (190). Therefore, it is likely that polymorphisms that reduce *CTLA4* expression or activity may cause excessive activation and proliferation of T cells, predisposing to autoimmunity (114, 191). Consistent with this, SNPs in *CTLA4* are risk variants for various autoimmune disorders (192); however, it is unclear which *CTLA4* variants are causative and by what mechanism they confer susceptibility to autoimmunity.

Protein Tyrosine Phosphatase, Non-Receptor Type 22

Similar to CTLA4, PTPN22 is a powerful inhibitor of T cell activity; it belongs to a family of protein tyrosine phosphatases expressed in both mature and immature B and T cells. PTPN22 binds to the SH3 domain of the C-terminal Src kinase (Csk), thereby suppressing kinases that mediate T cell signaling (193). PTPN22 also functions as a negative regulator of T cell activation through its interaction with the Grb2 adaptor molecule (194).

After the HLA system, *PTPN22* polymorphisms may be the most important genetic risk factor for autoimmune diseases (195). Variations in *PTPN22* are associated with various autoimmune disorders, including AITD (191). The most extensively studied and widely confirmed is the association of the C1858T missense mutation (rs2476601), which results in a substitution of arginine (R) to tryptophan (W) at position 620 (R620W) of the encoded protein. The *PTPN22* C1858T variant is associated with an increased risk of both HT and GD (196, 197); however, significant differences in this association have been observed across various ethnic groups. A significant association between the *PTPN22* C1858T polymorphism and susceptibility to AITD was demonstrated specifically in Caucasians, but it was hardly detected in non-Caucasian populations (198–201). It is suggested that this could be due to the ancestral effects and different prevalence rates of rare variants in the studied populations. Indeed, the susceptibility allele (T) of rs2476601 is generally extremely rare in Asian and African populations (198, 202). Also, a decreasing frequency of this allele was observed from the north to the south of the Europe (203). It is suggested that AITD susceptibility in different ethnic populations may be related to distinct risk loci. Notably, an extremely rare predisposing variant of *PTPN22* (missense A77G mutation) has recently been identified in a Chinese HT pedigree using whole-exome sequencing (204).

Although the exact mechanism by which the R620W variant predisposes to autoimmunity remains largely unknown, PTPN22 with a tryptophan residue at position 620 (Trp^{620}) binds Csk less efficiently than the variant with an arginine at this position (205). Consequently, the capacity of PTPN22 to downregulate T cell responses may be reduced, thereby increasing susceptibility to autoimmunity. By contrast, PTPN22 Trp^{620} is more active and a more potent negative regulator of T cell signaling, suggesting a role for R620W in positive selection of autoreactive T cells (206). Furthermore, PTPN22 Trp^{620} was associated with increased frequencies of thymically-derived T regulatory (Treg) cells and with increased expression of programmed cell death protein (PD-1) on both Treg and T effector cells (207). Currently, the role of the *PTPN22* C1858T polymorphism in autoimmunity is suggested to be alteration of both the innate and adaptive immune responses (206, 208).

Overall, current knowledge of HT genetics is rather limited. The majority of identified associations relate to general immune-regulatory genes involved in the development of central and peripheral tolerance and antigen presentation, which are important for achieving the proper balance between an adequate immune response against foreign antigens and maintaining autoantigenic tolerance. Moreover, all susceptibility loci identified to date together account for only a small proportion of the heritability of HT (209). It is estimated that < 5.5% of total HT variance can be explained by common genetic variants (138, 157), indicating that a substantial number of HT predisposing factors remain to be discovered.

JOINT PREVALENCE OF POLYCYSTIC OVARY SYNDROME AND AUTOIMMUNE THYROID DISEASE

Considering the similar elements in the pathogenic mechanisms and high prevalence rates of both AITD and PCOS among women of reproductive age, the interesting question of whether thyroid dysfunction is significantly more frequent in women with PCOS arises. An increasing number of studies indicate a higher prevalence of AITD, and particularly hypothyroidism, in patients with PCOS (**Table 3** and relevant references therein). In most of these studies, although not all, higher levels of anti-TPO and/or anti-Tg antibodies were observed, exceeding the upper limit of the normal range in an average of 22.3% of patients with PCOS (ranging from 4.8% to 37.9%, depending on the study), compared with an average of 8.5% in healthy women (range 3.3%

TABLE 3 | Joint prevalence of SCH and AITD in women with PCOS.

Study year (ref)	Compared groups	*N*	Population	TSH and thyroid hormone level	Prevalence of SCH; TSH cut-off value	Anti-TPO and anti-Tg antibody levels	Hypoechoic thyroid (goiter)	Prevalence of AITD; AITD criteria
2004 (210)	PCOS vs. age-matched women without PCOS	175 vs. 168	German	TSH 2.0 ± 1.0 vs. 1.4 ± 0.6 mIU/L $p<0.001$; TSH level above upper limit 10.9% vs. 1.8% $p<0.001$; fT_4 level - NS		Anti-TPO and/or anti-Tg positivity 26.9% vs. 8.3% $p<0.001$	42.3% vs. 6.5% $p<0.001$	20.6% vs. 6.5% $p<0.001$; Anti-TPO and/or anti-Tg positivity and hypoechoic thyroid
2011 (211)	PCOS vs. age-matched healthy women	84 vs. 81	Turkish	TSH, fT_3 and fT_4 levels - NS	TSH > 4.5 mIU/L	Anti-TPO and anti-Tg l evels - NS	Thyroid volume and heterogeneity of thyroid parenchyma - NS	PCOS alone was not associated with AITD; Higher thyroid volume ($p=0.001$), anti-TPO ($p=0.005$) and anti-Tg (p=0.003) levels in MS
2012 (212)	PCOS vs. age-matched healthy women	78 vs. 350	Iranian	TSH level - NS		Higher anti-TPO median level $p=0.04$;Anti-Tg level - NS	Goiter 62.3% vs. 35.7% $p<0.0001$	
2013 (213)	PCOS vs. age and BMI-matched women without PCOS	80 vs. 80	Indian	TSH 4.55 ± 2.66 vs. 2.67 ± 3.11 mIU/L $p<0.05$; Higher fT_3, fT_4 levels $p<0.001$	22.5 vs. 8.75% $p<0.05$; TSH > 4.25 mIU/L	Anti-TPO 28.04 ± 9.14 vs. 25.72 ± 8.27 IU/ml $p=0.035$	12.5% vs. 2.5% $p<0.001$; Goiter 27.5% vs. 7.5% $p<0.001$	22.5% vs. 1.25% $p<0.05$; Anti-TPO positivity
2013 (7)	PCOS infertile vs. infertile controls	151 vs. 155	Italian	TSH 2.17 ± 1.19 vs. 1.82 ± 1.1 mIU/L $p<0.009$; fT_3, fT_4 levels - NS	33.7% vs. 23.2% $p<0.05$; TSH > 2.5 mIU/L			
2013 (214)	PCOS vs. age-matched healthy women	113 vs. 100	Italian	TSH level above the normal range in 43.3% (13/30) of PCOS patients with AITD	SCH in 43.3% (13/30) of PCOS patients with AITD, remaining have normal thyroid function	Anti-TPO and anti-Tg positivity in 53.3% (16/30) of PCOS patients with AITD; Anti-TPO positivity in 33.3% (10/30) of PCOS patients with AITD	93.3% (28/30) of PCOS patients with AITD	27% vs. 8% $p<0.001$; At least two of three criteria: anti-TPO and/or anti-Tg positivity, TSH levels above the normal range and hypoechoic thyroid
2014 (215)	PCOS euthyroid vs. healthy women	56 vs. 30	Syrian	TSH and fT_4 levels - NS	TSH > 4.2 mIU/L	Anti-TPO positivity 19.6% vs. 3.3% $p<0.05$; Anti-Tg positivity 21.4% vs. 3% $p<0.05$; Anti-TPO 39.9 ± 59.5 vs. 18.9 ± 11.2 IU/ml $p=0.013$; Anti-Tg level - NS		28.6% vs. 3.3% p<0.05; Anti-TPO and/or anti-Tg positivity
2015 (216)	PCOS vs. age-matched women without PCOS	73 vs. 60	Turkish	TSH and fT_4 levels - NS		Anti-TPO and anti-Tg positivity - NS	Thyroid nodules frequency and thyroid gland volume - NS	32.5% vs. 23.3% - NS; Anti-TPO and anti-Tg positivity and/or hypoechoic thyroid
2015 (6)	PCOS vs. women without PCOS	65 vs. 65	Brazilian	TSH 2.9 ± 1.8 vs. 2.2 ± 1.2 mIU/L $p=0.013$;	16.9% vs. 6.2% $p<0.05$; TSH > 4.5 mIU/L	Anti-TPO and anti-Tg positivity - NS	26.8% vs. 15.4% $p=0.052$	43.1% vs. 26.2% $p=0.04$; At least two of three

(Continued)

TABLE 3 | Continued

Study year (ref)	Compared groups	*N*	Population	TSH and thyroid hormone level	Prevalence of SCH; TSH cut-off value	Anti-TPO and anti-Tg antibody levels	Hypoechoic thyroid (goiter)	Prevalence of AITD; AITD criteria
				fT_3 level p=0.002; fT_4 level - NS				criteria: anti-TPO and/or anti-Tg positivity, TSH levels above the normal range and hypoechoic thyroid
2015 (217)	PCOS vs. age-matched healthy women	64 vs. 68	Slovak	TSH and fT_4 levels - NS	Hypothyroidism 10.94% vs. 13.24% - NS; TSH > 4.5 mIU/L	Anti-TPO positivity 18.75% vs. 7.35% p=0.045; Anti-Tg positivity - NS		18.75% vs. 10.29% - NS; Anti-TPO and/or anti-Tg positivity and/or hypoechoic thyroid
2015 (218)	PCOS vs. age-matched healthy women	142 vs. 52	Argentine	TSH 3.4 ± 2.8 vs. 1.8 ± 0.9 mIU/L p<0.001; fT_4 level - NS	30.3% vs. 1.9% p<0.001; TSH ≥ 4.2 mIU/L	Anti-TPO positivity 19% vs. 13.5% - NS		36.6% vs. 13.5% p<0.001; Anti-TPO positivity and/or SCH
2015 (219)	PCOS vs. age and BMI-matched healthy women	86 vs. 60	Turkish	TSH median level 2.9 vs. 1.8 mIU/L p=0.037; TSH level above the normal range 26.7% vs. 5% p=0.001;fT_3 and fT_4 levels - NS	TSH > 4.25 mIU/L	Anti-TPO positivity 26.7% vs. 6.6% p=0.002; Anti-Tg positivity 16.2% vs. 5% p=0.039; Higher anti-TPO median level p=0.017; Higher anti-Tg median level p=0.014		22.1% vs. 5% p=0.004; Anti-TPO and/or anti-Tg positivity and hypoechoic thyroid
2016 (220)	PCOS vs. age and BMI-matched healthy women	100 vs. 100	Chinese	TSH 5.11 ± 22.2 vs. 2.9 ± .2 mIU/L p<0.001; fT_3 level p=0.03; fT_4 level - NS	27% vs. 8% p=0.0002; Overt hypothyroid 3% vs. 0% p=0.01; TSH > 4.25 mIU/L	Anti-TPO 76.2 ± 23.4 vs. 20.14 ± 12.4 IU/ml p<0.001	34% vs. 7% p<0.01; Goiter 25% vs. 2% p=0.02	25% vs. 2% p<0.001; Anti-TPO and hypoechoic thyroid
2016 (221)	PCOS vs. age-matched women without PCOS	55 vs. 51	Indian	TSH, fT_3 and fT_4 levels - NS	TSH > 6.2 mIU/L	Anti-TPO 49.54 ± 136.9 vs. 22.63 ± 38.5 IU/ml - NS; Higher anti-Tg level p=0.004		37.7% vs. 15.6% - NS; Anti-TPO and/or anti-Tg positivity
2017 (222)	PCOS vs. age-matched women without PCOS	90 vs. 90	Indian	TSH and fT_4 levels - NS	6.6% vs. 5.6% - NS	Anti-TPO 25.8 ± 2.9 vs. 14.6% ± 2.3 5 IU/ml p<0.009		25% vs. 5.6% p<0.05; Anti-TPO positivity
2017 (223)	PCOS vs. age-matched healthy women	97 vs. 71	Turkish	TSH and fT_4 levels - NS	TSH > 5. 33 mIU/L	Anti-TPO positivity 32.0% vs. 15.5% p=0.019; Anti-Tg positivity 16.5% vs. 5.6% p=0.051	Thyroid nodules 29.9% vs. 15.5% p=0.043	40.2% vs. 15.5% p=0.001; Anti-TPO and/or anti-Tg positivity and/or hypoechoic thyroid
2017 (224)	PCOS vs. normo-ovulatory, age-matched controls	144 vs. 48	Chinese	TSH 2.72 ± 1.5 vs. 2.14 ± 0.98 mIU/L p=0.003; fT_3, fT_4 levels - NS	TSH > 4.78 mIU/L	Anti-TPO positivity 15.28% vs. 6.25% - NS; Anti-Tg positivity 13.2% vs. 12.5% - NS		
2018 (225)	PCOS vs. age-matched healthy women	184 vs. 106	Turkish	TSH and fT_4 levels - NS	TSH > 4.94 mIU/L	Higher anti-TPO and anti-Tg mean levels p<0.001; Anti-TPO positivity 37.9% vs. 11.1% p<0.001;Anti-Tg		37.9% vs. 11.1% p<0.001; Anti-TPO positivity

(Continued)

TABLE 3 | Continued

Study year (ref)	Compared groups	*N*	Population	TSH and thyroid hormone level	Prevalence of SCH; TSH cut-off value	Anti-TPO and anti-Tg antibody levels	Hypoechoic thyroid (goiter)	Prevalence of AITD; AITD criteria
						positivity 15.3% vs. 5.1% p=0.013		
2018 (226)	PCOS vs. women without PCOS	827 vs. 804	German		All PCOS were euthyroid; TSH >2.5 mIU/L			23% vs. 9.2% p<0.05; At least two of three criteria: anti-TPO and/or anti-Tg positivity, TSH levels above the normal range and hypoechoic thyroid
2020 (10)	PCOS vs. age-matched healthy women	210 vs. 343	Korean	TSH and fT_4 mean levels - NS	TSH > 4.1 mIU/L	Anti-TPO positivity 4.8% vs.7.6% - NS	9.3% vs. 12.3% - NS	PCOS 6.2%; Anti-TPO positivity and/or hypoechoic thyroid

Study year [ref]	Examined group	*N*	Population	TSH level	Prevalence of SCH;TSH cut-off value	Metabolic and hormonal parameters
2013 (96)	PCOS	168	Brazilian	SCH 6.1 ± 1.2 vs. euthyroidism 2.3 ± 1.0 mIU/L	11.3%; TSH > 4.5 mIU/L	Higher LDL in SCH p=0.04
2014 (227)	PCOS	75	Indian	SCH 6.89 ± 5.52 vs. euthyroidism 1.89 ± 0,78 mIU/L p=0.006	25.5%; TSH > 3.75 mIU/L	Lower free testosterone in SCH p=0.006
2014 (5)	PCOS	428	Chinese	SCH 5.94 ± 0.53 mIU/L	14%; TSH > 5 mIU/L	In SCH higher TC p=0.049; higher LDL p=0.001; lower HDL p=0.051; higher dyslipidemia p=0.014
2018 (228)	PCOS	137	U.S.		21.9%; TSH > 2.5 mIU/L	In SCH higher fasting glucose p=0.03; higher HOMA-IR p=0.03

AITD, autoimmune thyroid disease; fT_3, free triiodothyronine; fT_4, free thyroxine; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; LDL, low-density lipoprotein cholesterol; MS, metabolic syndrome; NS, no significant change or similar level; PCOS, polycystic ovary syndrome; SCH, subclinical hypothyroidism; TC, total cholesterol; Tg, thyroglobulin; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone.

to 15.3%). Also, decreased echogenicity of the thyroid gland, a characteristic ultrasound pattern typical of HT, was observed more frequently in women with PCOS than in those without the condition (mean, 29% *vs.* 9%, respectively). The prevalence of SCH at TSH levels between 4.2 and 10 mIU/L ranged from 11.3% to 30.3% (mean, 20.3%) among patients with PCOS, which was more than 3 times higher than that reported for women without this condition (mean, 6.2%; range, 1.9% to 8.75%). Altogether, the estimated prevalence of AITD among women with PCOS was nearly threefold higher than that in healthy women (mean, 28% *vs.* 10%, respectively), although it should be noted that the diagnostic criteria for AITD differed among studies (**Table 3**).

In women with PCOS, compared with those without the condition, higher TSH levels, anti-thyroid antibody positivity rate, and prevalence of thyroid disorders, particularly HT, have been demonstrated in three independent meta-analyses conducted to date (9, 229, 230). Based on six studies, the combined OR of SCH risk for women with PCOS (compared with healthy women) was 2.87 (95% confidence interval (CI), 1.82–9.92; $p < 10^{-6}$), assuming a TSH cut-off level of > 2.5 mIU/L, and 3.59 (95% CI, 2.25–5.73; $p < 10^{-6}$) when limiting TSH to ≥ 4 mIU/L (229). Similarly, two meta-analyses indicated a significant association between PCOS and the presence of AITD: one included six studies (OR = 4.81; 95% CI, 2.88–8.04; $p < 10^{-5}$) (9) and the other included 13 studies (OR = 3.27; 95% CI, 2.32–4.63; $p < 10^{-4}$) (230). The higher risk of AITD among women with PCOS also persisted after geographical stratification of the study populations (230).

Possible Cross-Connections Predisposing to Joint Occurrence of Polycystic Ovary Syndrome and Autoimmune Thyroid Disease

Although the association between PCOS and AITD is generally uncontested, its cause remains unclear. Both syndromes share a number of common clinical and pathological features; however, whether mutual interrelationships are present, or whether one condition predisposes an individual to another disorder, remains speculative (8). Some evidence suggests that PCOS may have an autoimmune background. Abnormally elevated levels of systemic autoimmune markers such as anti-histone, anti-double stranded DNA (anti-dsDNA), and anti-nuclear antibodies, which are considered classic features of autoimmune disease, have been observed in women with PCOS (231, 232); however, the presence of anti-ovarian antibodies in PCOS remains controversial (231).

The most obvious association between PCOS and HT is the increased metabolic risk of obesity, IR, and dyslipidemia (27, 233). Overweight and obese patients with PCOS showed a higher tendency toward thyroid dysfunction; TSH levels > 2.5 mIU/L were significantly more common in patients with BMI > 25 kg/m^2 (56%) than in those with BMI ≤ 25 (25.8%, $p < 0.005$) (7). Patients with PCOS and AITD were more obese by an average of 2 kg/m^2 (226). Higher TSH levels (as well as a higher frequency of nodular goiter and thyroid volume) were observed in patients with PCOS, and these parameters correlated with IR (7, 223). Furthermore, fasting glucose and homeostasis model assessment (HOMA)-IR levels among patients with PCOS and SCH were higher than those in euthyroid PCOS patients, independent of BMI (10, 228, 234). Notably, ethnic diversity among euthyroid PCOS patients and patients with PCOS and SCH was suggested with respect to IR and lipid profiles (227). By contrast, SCH is not an independent risk factor for PCOS among obese women of reproductive age (235).

In particular, combined occurrence of PCOS and SCH increases the risk of impaired lipid profiles. Compared with euthyroid PCOS patients, PCOS patients with elevated TSH levels show a trend toward higher triglyceride and LDL cholesterol levels, as well as lower high-density lipoprotein (HDL) cholesterol levels (5, 96, 220). A positive correlation was found between TSH and LDL cholesterol levels, with the optimal TSH cut-off point for elevated LDL cholesterol risk defined as 4.07 mIU/L (5). Furthermore, two recent meta-analyses covering 12 and nine studies demonstrate that the presence of SCH in women with PCOS is associated with an increase in metabolic disorders, particularly dyslipidemia, which affect triglyceride, LDL, HDL, and total cholesterol levels (234). Taken together, current data suggest that the combined effect of PCOS and HT is associated with a higher risk of more pronounced metabolic disorders than either of these syndromes alone.

It has been hypothesized that increased IR in obesity and secretion of pro-inflammatory mediators can lead to elevated TSH levels through one of two pathways: decreased deiodinase-2 activity or increased levels of leptin hormone, which act directly to stimulate increased TRH secretion by the hypothalamus (110, 236). In addition, increased leptin, as a result of weight gain, can mediate autoimmunity by preferential up-regulation of autoreactive T cells and down-regulation of Treg cells, mediating a suppressive effect on the immune system (8). It is suggested that PCOS exacerbates development of SCH, likely *via* obesity and IR (237). Moreover, hypothyroidism may aggravate IR.

One possible explanation for the high prevalence of HT in PCOS assumes that changes in the fetal thymus, and resulting alterations in immune tolerance, may predispose to combined PCOS and HT in adulthood (27). Estrogen or adrenal steroids, such as corticosterone, injected into female mice early in life (before the final stage of thymus development) results in anovulation and follicular cyst formation (238). It is also suggested that estrogen can damage the thymus during its development, and that the resulting absence of Treg cells is a prerequisite for formation of ovarian cysts (238). Similar to PCOS, hypothyroidism can result in ovarian cyst formation (8) and, notably, they are normalized in response to T_4 replacement (106). $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells protected against autoimmunity in a murine model of HT (239). The transcription factor, FOXP3, which is involved in Treg cell formation and consequently *FOXP3* expression, is dependent on production of the cytokine TGF-β (239). Since lower levels of TGF-β1 are reported in HT than in healthy women (240), lower levels of Treg cells are also expected. TGF-β1 inhibits proliferation, differentiation, and apoptosis of T cells, as well as increases the growth of naive T cells (241); hence, it is thought to be involved in development of autoimmunity. Furthermore, TGF-β signaling appears to be important for the fetal origin of PCOS and folliculogenesis (242).

The impact of sex hormones on development of autoimmunity appears obvious given the clear predominance of women among patients affected by autoimmune diseases; the ratio of women to men is 15:1 in the case of AITD (243). Estrogen can stimulate the immune system and increase Treg cell formation (244). In fertile, non-pregnant women, the number of $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells increases in the late follicular phase of the menstrual cycle and decreases in the luteal phase, which correlates with E_2 levels (245). Unlike estrogen, progesterone levels correlate inversely with dendritic cell secretion of pro-inflammatory cytokine IL6, which in turn inhibits *FOXP3* expression and Treg cell generation (246, 247). Furthermore, progesterone may suppress $CD4^+$ T cell proliferation and Th1 responses (248). In women with normal menstrual periods, the immunosuppressive action of progesterone and androgens counteracts the stimulating effect of estrogen (248, 249), while in women with PCOS, a decrease in progesterone levels is usually detected due to irregular menses and oligo- or anovulatory cycles (250). Also, a significantly higher E_2 level and estrogen-to-progesterone ratio were observed in anti-TPO-positive compared with anti-TPO-negative women with PCOS (219). The resulting imbalance between estrogen and progesterone is thought to be associated with an excessive inflammatory response that promotes autoimmune disorders in PCOS (8). Consistent with this assumption, patients with PCOS exhibit low-grade inflammation, characterized by elevated levels of CRP and independent of obesity (251). PCOS is characterized by an excess of androgens; however, it is speculated that the suppressive effect of androgens on the immune system at the levels observed in PCOS is probably insufficient to prevent autoimmunity (27). In addition, lower testosterone levels, a lower free androgen index, and less severe hyperandrogenemia are observed in patients with PCOS and HT relative to those with PCOS alone (226), although, the results have not been confirmed in patients with PCOS and SCH (234, 252).

Low levels of vitamin D are associated with both PCOS and HT (253, 254) and provide another possible link between these syndromes and autoimmunity. Vitamin D is considered to be protective against autoimmune diseases (255). A strong association has been reported between the severity of vitamin D deficiency and HT, as well as anti-thyroid antibody and thyroid hormone levels (256). Nevertheless, concerns remain that the effects of vitamin D may not be direct, but rather secondary to estrogen dysregulation (257). Consistent with this hypothesis, low levels of 25 hydroxyvitamin D_3 ($25(OH)D_3$) are associated with AITD in pre-menopausal, but not post-menopausal, women (258). A genetic variant of *CYP27B1* hydroxylase, which is responsible for production of an active form of vitamin D from $25(OH)D_3$, is associated with HT (259). Furthermore, polymorphisms in the *VDR* gene causing a decrease in vitamin D levels are associated with both HT and several metabolic syndrome features in women with PCOS (27). Moreover, levels of $25(OH)D_3$ are significantly lower in women with PCOS and AITD than those without AITD (253). Among overweight and obese persons, a significantly higher frequency of vitamin D deficiency was observed in patients with HT than without HT (69% vs. 52%, $p = 0.042$) (260), suggesting that low vitamin D level is not merely a marker of obesity.

Genetic Predisposition to Combined Polycystic Ovary Syndrome and Hashimoto's Thyroiditis

As stated above, a strong genetic impact on inheritance and susceptibility to disease development is present in both PCOS and HT. Although a functional relevance of several candidate gene polymorphisms has been suggested, no common genetic background has been established. To date, only a few genetic variants have been proposed to be causally associated with the joint incidence of both disorders. The most convincingly described are three genetic polymorphisms that can contribute to both PCOS and HT. These are polymorphisms in *FBN3*, a gene related to TGF-β activity and Treg cell levels; *CYP1B1*, a gene involved in E_2 metabolism; and *GNRHR*. In addition, there are two susceptibility loci identified in GWAS that are common for both diseases (**Tables 1** and **2**): *FSHR* (2p16.3) and *INSR* (19p13.3); however, no functional overlap between the two diseases has yet been confirmed.

The *CYP1B1* gene encodes an enzyme belonging to the multi-gene-encoded cytochrome P450 enzyme family, which oxidizes E_2 to 4-hydroxyestradiol (261). *CYP1B1* is expressed at a level 3 times lower in PCOS ovaries than in control ovaries (262). The pathogenic polymorphism, L432V (rs1056836), in *CYP1B1* is associated with serum T_4, fT_4, and fT_3 concentrations among patients with PCOS (263). Although none of the studied polymorphisms in this gene are associated with disease risk, suggesting that CYP1B1 may not have a causative role in the etiology of PCOS, the *CYP1B1* L432V polymorphism provides a potential link between PCOS and HT.

GnRH is a key hypothalamic peptide that, after binding to a specific receptor, GnRHR, stimulates a release of LH and FSH from pituitary gonadotropic cells. Increased levels of LH and a higher frequency of LH pulses (264), as well as a higher prevalence of increased TSH levels (8), were observed in women with PCOS. GnRH was shown to enhance the release of TSH (265). Furthermore, a 3'-UTR polymorphism in the *GNRHR* gene, rs1038426, was found to affect *GNRHR* expression, with a variant allele-dose effect, and was associated with the concentration of serum TSH as well as insulin secretion and insulin sensitivity in women with PCOS (266). An association between TSH and fasting insulin levels and insulin sensitivity was also reported (267). While *GNRHR* polymorphism did not contribute to the risk of PCOS (266), it links gonadotropin action-related dysfunction with IR and possibly also with thyroid function disorders in PCOS. Several PCOS risk variants were identified in the *INSR* gene, although replication studies produced mostly inconsistent results. The most convincing seems to be an association of rs2252673 in intron 11, identified by the entire gene examination with a tagging approach (268), and silent SNP rs1799817 at exon 17, encoding the tyrosine kinase domain of the INSR (80); especially among lean women with PCOS. The intronic *INSR* variants were also independently identified by the GWAS for PCOS and for the level of TSH (**Tables 1** and **2**); however, the causal variant of this loci and the impact on *INSR* expression remained unknown. Nevertheless, pronounced IR and defects in insulin secretion are commonly associated with PCOS. In women with PCOS, an increase in

beta-cell function was observed when compared to the age and BMI matched controls, which was correlated with the intensity of IR (269).

A strong association was observed in candidate gene studies between linked to each other missense *FSHR* gene variants: Thr307Ala (rs6165) and Asn680Ser (rs6166), and intensity of some PCOS clinical traits. The Ser^{680} allele was associated with higher serum gonadotrophic hormones concentrations and higher frequency of hyperandrogenism presence (44). Longer follicular phase and lower E_2 levels after exogenous FSH stimulation were also observed, suggesting lower sensitivity of this *FSHR* variant (270). Several other *FSHR* polymorphisms have been shown to correlate with ovarian function, including SNPs identified by GWAS in Han Chinese population (**Table 1**) and by fine mapping of 2p16.3 region in a population of European ancestry (271); however, they seem to be associated with FSH levels and the PCOM phenotype rather than with disease risk (33, 47). Recent GWAS among patients with HT identified SNP rs12713034 in the *FSHR* gene that is associated with the presence of anti-thyroid antibodies (146). In addition, hypothyroidism has been found to decrease FSH and E_2 levels and alter FSHR-mediated expression of *CYP51*, a key enzyme involved in sterols and steroids biosynthesis during folliculogenesis and oocyte maturation, which is regulated by FSH (272); thus providing a further link between the functions of the ovaries and thyroid gland.

FBN3 is a member of the fibrillin/LTBP (latent TGF-β binding protein) family of ubiquitously expressed extracellular matrix proteins that regulate the bioavailability and activity of TGF-β, providing binding sites for its sequestration (273). The *FBN3* genetic variant, D19S884 allele 8 (A8), a dinucleotide repeat microsatellite marker in intron 55, is the variant most strongly associated with PCOS susceptibility at the 19p13.2 locus (274). A rare missense variant (Asp911Val) in *FBN3* was also found by the whole-exome sequencing approach (275). Although the results were not consistent, further studies suggest that D19S884 is likely a causal variant for PCOS susceptibility (275, 276). It was suggested that the A8 allele may affect splicing of *FBN3* transcript (277). Women with PCOS carrying the A8 allele had significantly lower circulating TGF-β1 levels, and higher inhibin B and aldosterone levels, as well as higher levels of fasting INS and HOMA-IR than women with PCOS without the A8 allele (277). It is hypothesized that women with PCOS and the A8 variant, and therefore lower TGF-β levels, are more susceptible to HT than those without this allele (27). Some findings suggest that expression of the *FBN3* gene in fetal ovaries may predispose to PCOS development in later life, supporting the existing hypothesis of the fetal origin of PCOS (278).

As outlined in this review, our current knowledge of the genetic basis of the joint occurrence of PCOS and HT leads us to

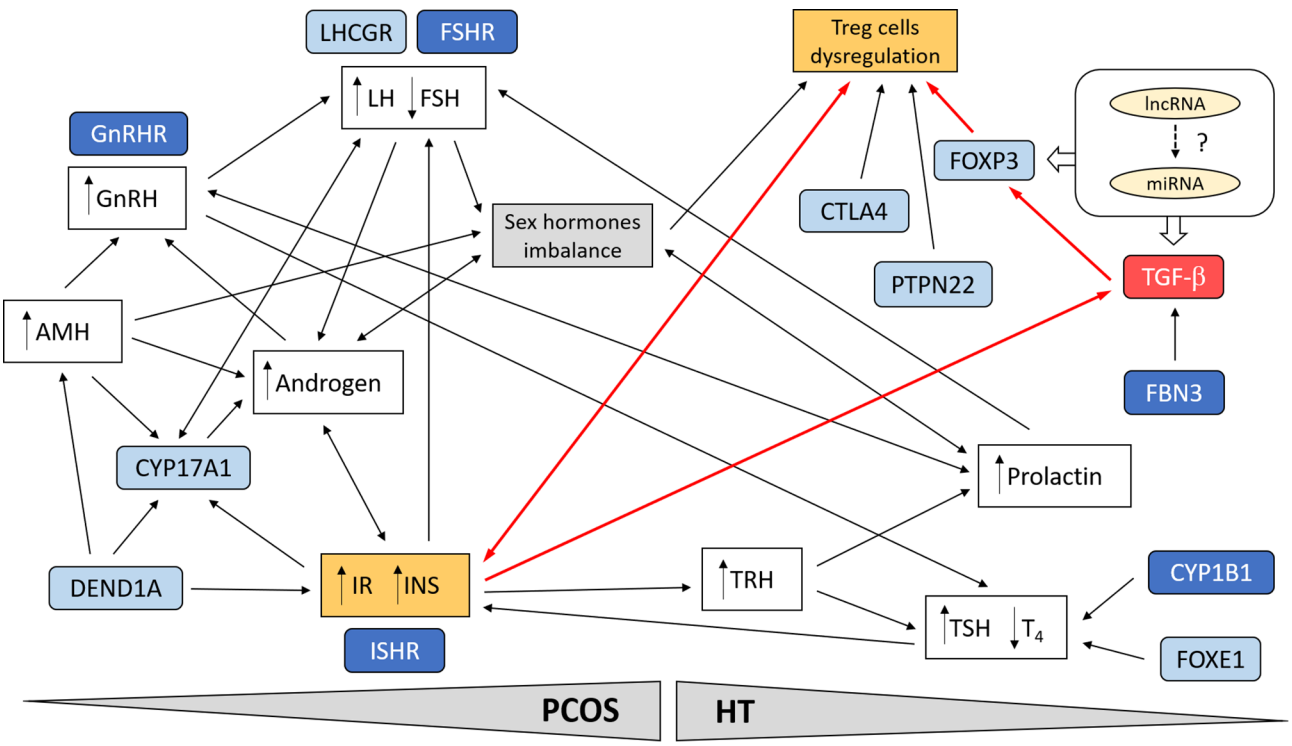

FIGURE 1 | Schematic presentation of possible cross-linkages between PCOS and HT. Involvement of the most important genetic factors and molecular pathways. Treg cell dysregulation emerges as a critical point in the genetic and functional network linking the two diseases. In PCOS, genetic variation emphasizes the contribution of both hormonal imbalance (gonadotropins, androgens, and female sex hormones) and metabolic factors (IR and INS secretion), often interacting through a feedback loop. Suppression of TGF-β signaling pathway in combination with IR may lead to dysregulation of Treg cells and promotion of autoimmunity in women with PCOS (red line connections). Possible interaction of ncRNA (miRNA, lncRNA) with TGF-β signaling. The most important genes are indicated in blue; darker blue designates susceptibility genes for both diseases.

believe that there may be no strong shared genetic variants associated with the risk of both diseases. Rather, it is a specific combination of risk factors for individual diseases that predisposes them to occur together. Moreover, due to the complex background of both disorders, this combination may be specific not so much to the risk of the disease itself as to the expression of its individual phenotypes. In line with this assumption, it has been recently indicated that the use of combined polygenic and phenotypic risk prediction may improve the accuracy of PCOS diagnosis (279).

As shown in **Figure 1**, which simplifies the possible cross-linkages between PCOS and HT, the multi-directional link seems to be the best explanation for the predisposition to joint occurrence of both diseases. Treg cells dysregulation emerges as a critical point in the genetic and functional network connecting the two diseases. In PCOS, genetic variation emphasizes the contribution of both hormonal imbalance (gonadotropins, androgens, and female sex hormones) and metabolic factors (IR and INS secretion), often interacting through a feedback loop (230, 248). It can be assumed that the favorable hormonal and metabolic background in women with PCOS may predispose them to thyroid autoimmunity and aggravate the disease symptoms through the HT susceptibility factors (8, 219).

The best documented genetic association between PCOS and HT relates to the TGF-β signaling pathway. Factors involved in this pathway are clearly good candidate susceptibility genes for both syndromes, since they have key roles in the immune system, hormone regulation, inflammation, cell proliferation, tissue differentiation, apoptosis, and related metabolic consequences such as IR. In PCOS, the inflammation of visceral adipose tissue resulting in chronic release of pro-inflammatory cytokines is a major contributor to IR (280). Treg cells suppress pro-inflammatory effects of autoreactive T cells (281). Depletion of $CD4^+CD25^+Foxp3^+$ Treg cells and increased inflammation in visceral adipose tissues was found to contribute to IR in HT (282). We are hypothesizing that the suppression of TGF-β signaling pathway in combination with IR may lead to Treg cells dysregulation and promotion of autoimmunity in women with PCOS (**Figure 1**). Beside *FBN3*, no members of the TGF-β signaling have been shown to be among the top GWAS associations for PCOS or HT. However, rs4803457 polymorphism in *TGFB1* gene was associated with PCOS susceptibility in candidate gene studies in Chinese Han women (273). The genetic variation in transcription factor *FOXP3* has also been reported in candidate gene studies for AITD (2). FOXP3 is a crucial regulator of Treg cells differentiation and function and its expression is induced by TGF-β, which activity, in turn, is regulated by FBNs (33).

In recent years, there has been an increase in evidence of the role of non-coding RNA (ncRNA) in the development of various diseases. Differentially expressed microRNA (miRNA) and long ncRNA (lncRNA) were identified both in PCOS and HT (283, 284). The ncRNAs were shown to be involved in regulating T cell production and differentiation (285). Furthermore, several ncRNAs were involved in regulation by the TGF-β signaling pathway. In HT, the expression of miR-141 was downregulated due to its involvement in TGF-β pathway and regulation through IL2 (286). In turn, lncRNA-IFNG-AS1 was upregulated in HT patients, and it was associated with IFN-γ expression in human CD4+ T cells and with the frequency of circulating Th1 cells (287). The lncRNA TGFB2-AS1 was shown to be transcriptionally regulated by TGF-β and to suppress TGF-β/BMP-mediated response of target genes (288). In granulosa cells from patients with PCOS, expression of miR-423 was downregulated, and both miR-33b and miR-142 were upregulated, compared to controls; miR-423 directly suppressed SMAD7, while miR-33b and miR-142 targeted TGF-β receptor 1 (TGFBR1) (289). Several lncRNA have been reported to positively regulate TGF-β/SMAD signaling (290). The lncRNAs can act directly or as miRNA sponges, thus reducing their regulatory effect on target mRNAs (291). In granulosa cells, lncRNA-NORFA directly interacts with miR-126 and prevents it from binding to TGFBR2 3'UTR (292). Another lncRNA, MALAT1, has been found to regulate TGF-β signaling through sponging miR-125b and miR-203a, TGF-β negative regulators by targeting TGFBR1 and TGFBR2 (293). Considering the above-mentioned examples of the role of ncRNA in modulating of TGF-β signaling, the involvement of ncRNA in the processes leading to the joint occurrence of PCOS and HT seems very likely; however, there are no strong data supporting this hypothesis and it requires confirmation in further studies.

CONCLUSIONS

PCOS and HT are two of the most common endocrine disorders among young women worldwide. Both syndromes are causally related to the risk of severe metabolic and reproductive disorders and are significant social problems. Thyroid dysfunction in PCOS appears to enhance the clinical symptoms of the disease and the severity of its consequences. The pathogenesis of the association of PCOS with HT is not clear, although the relationship between thyroid hormones and ovary function is indisputable. There is good evidence for a strong genetic impact on the development of both diseases; hence, a common genetic predisposition is possible. However, to date, it is not apparent. Furthermore, the question of whether PCOS predisposes to development HT, or whether HT is a forerunner of PCOS, remains open.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version. NZ-L wrote the original draft. EH wrote and critically revised the manuscript.

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