MECHANISMS IN ENDOCRINOLOGY

Thyroid and polycystic ovary syndrome

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Abstract

Thyroid disorders, especially Hashimoto's thyroiditis (HT), and polycystic ovary syndrome (PCOS) are closely associated, based on a number of studies showing a significantly higher prevalence of HT in women with PCOS than in controls. However, the mechanisms of this association are not as clear. Certainly, genetic susceptibility contributes an important part to the development of HT and PCOS. However, a common genetic background has not yet been established. Polymorphisms of the PCOS-related gene for fibrillin 3 (*FBN3*) could be involved in the pathogenesis of HT and PCOS. Fibrillins influence the activity of transforming growth factor beta (TGF β). Multifunctional TGF β is also a key regulator of immune tolerance by stimulating regulatory T cells (Tregs), which are known to inhibit excessive immune response. With lower TGF β and Treg levels, the autoimmune processes, well known in HT and assumed in PCOS, might develop. In fact, lower levels of TGF β 1 were found in HT as well as in PCOS women carrying allele 8 of D19S884 in the *FBN3* gene. Additionally, vitamin D deficiency was shown to decrease Tregs. Finally, high estrogen-to-progesterone ratio owing to anovulatory cycles in PCOS women could enhance the immune response. Harmful metabolic and reproductive effects were shown to be more pronounced in women with HT and PCOS when compared with women with HT alone or with controls. In conclusion, HT and PCOS are associated not only with respect to their prevalence, but also with regard to etiology and clinical consequences. However, a possible crosstalk of this association is yet to be elucidated.

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Introduction

Women of reproductive age are affected by polycystic ovary syndrome (PCOS) by ~5–7% depending on ethnicity and diagnostic criteria (1, 2). In a community sample, the prevalence of PCOS according to the Rotterdam criteria was shown to be ~12% and increased to ~18% when imputed values of the number of women with polycystic ovaries in the group that did not have ultrasound were included (3, 4).

PCOS is characterized by chronic oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and/or polycystic ovaries detected by ultrasound (3). Three main phenotypes of PCOS have been proposed: classic PCOS with hyperandrogenism and

anovulation, with or without polycystic ovaries; ovulatory PCOS with hyperandrogenism and polycystic ovaries; and anovulatory PCOS without hyperandrogenism and with polycystic ovaries (5). Several phenotypes are associated with a variable degree of metabolic and fertility irregularities. In PCOS, defects in insulin (INS) activity and INS secretion increase the risk of impaired glucose metabolism (6, 7). PCOS is the most frequent cause of anovulatory infertility in women (8) and is often associated with menstrual irregularities (9).

As thyroid dysfunction may also be associated with fertility problems, women with undiagnosed PCOS may often be referred to thyroid gland examination. Diseases

causing overt hyperthyroidism or hypothyroidism can also induce menstrual disorders and fertility consequences. In animal studies, all receptors in the signaling pathway of thyroid hormone function including thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), and thyroid hormones were shown to be present in monkey uterus and influenced by long-term steroid hormone treatment (10).

As extensively reviewed by Krassas *et al.* (11), in hyperthyroidism, higher levels of sex hormone-binding globulin (SHBG), estradiol (E_2), testosterone, androstenedione, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) compared with the euthyroid state were established (11). In addition, hyperthyroidism is associated with irregular menstrual cycles, while ovulation is usually preserved in otherwise healthy women (12).

In hypothyroidism, lower levels of SHBG, E_2 , testosterone, and androstenedione were described. Prolactin levels may be increased due to increased TRH secretion. Levels of LH and FSH were normal (11, 12). Hypothyroidism may be associated with ovarian cyst formation as shown in a case report (13). In gilts, hypothyroidism increased ovarian sensitivity to gonadotropin action and led to a marked hypertrophy of ovaries as well as to formation of multiple follicular cysts (14). Hypothyroidism may cause heavy, irregular menses, breakthrough bleeding, low endometrial thickness, ovulatory dysfunction, and sometimes non-proliferative endometrium due to anovulation (15).

The likelihood that a young woman with PCOS will have a disease causing overt or subclinical hyperthyroidism such as autoimmune Graves' disease with increased levels of TSH receptor-stimulating antibodies is very low as the prevalence of hyperthyroidism in the general Western population is low, as evident from the largest epidemiological National Health and Nutrition Examination Survey (NHANES) held in the USA, including more than 17 000 individuals (16). In the reproductive age, ~3% of the subjects had a TSH level below 0.4 mIU/l.

Slightly higher is the likelihood for a woman in the reproductive period to have a disease causing overt or subclinical hypothyroidism such as autoimmune Hashimoto's thyroiditis (HT) with increased levels of thyroid peroxidase (TPO) and/or thyroglobulin (Tg) antibodies. Clinical presentations of HT range from presence of thyroid antibodies with a normal thyroid function to subclinical and, finally, to overt hypothyroidism (17). Within the reproductive period, the prevalence of hypothyroidism with TSH levels above 4.5 mIU/l is ~4% as shown in the NHANES cohort (16). These numbers

increase with age as established in the Colorado thyroid disease prevalence study, which included more than 25 000 individuals (18). The highest is the likelihood that a young woman will have HT with a normal thyroid function and increased levels of TPO and/or Tg antibodies. In the age group between 20 and 29 years, the prevalence of TPO and Tg antibodies is 11.3 and 9.2% respectively and, in the age group between 30 and 39 years, the prevalence is 14.2 and 14.5% respectively (16).

Considering the high prevalence of HT and the high prevalence of PCOS in women in the reproductive period, the emphasis of our review will lie on the possible etiological and clinical connections between HT and PCOS. A systematic literature search in PubMed for PCOS- and thyroid-related English-language articles published until September 2013 was performed. A summary of our findings is given in Table 1.

Joint prevalence

Is the prevalence of autoimmune thyroid disease (AITD) in women with PCOS higher than that in women without PCOS? A first systematic prospective study addressing this question included 175 patients with PCOS and 168 healthy controls with a mean age of 28 years (19). Elevated levels of TPO or Tg antibodies distinctive for HT were found in 26.9% of PCOS patients and in 8.3% of controls. Young women with PCOS also had significantly higher levels of TPO and Tg antibodies and higher levels of TSH than controls. On thyroid ultrasound, 42.3% of patients with PCOS and only 6.5% of controls had a hypoechoic thyroid ultrasound pattern typical of HT (19). Increased thyroid antibodies and hypoechoic thyroid ultrasound pattern were found in 36 PCOS patients and 11 controls of this study (20.6 and 6.5% respectively). Taken together, the prevalence of HT in PCOS patients was found to be increased by threefold when compared with controls (19). Recently, the higher prevalence of HT in women with PCOS than in controls was confirmed in 113 Italian patients with the mean age of 24 years and in 100 controls (27 vs 8%) (20). Out of 168 young Brazilian PCOS women with the mean age of 24 years, 149 (88.7%) had normal thyroid function and 19 (11.3%) subclinical hypothyroidism with TSH levels between 4.5 and 10 mIU/l, representing a higher prevalence of subclinical hypothyroidism in PCOS than in the general population (21). Referring to the Colorado thyroid disease prevalence study, the prevalence of increased TSH levels in 24-year-old women should be ~4–5% (18). In Asia, in 80 patients with PCOS from Eastern India, aged between 13 and 45 years, a cross-sectional study

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Table 1 Shared characteristics of HT, PCOS, and HT in women with PCOS in the reproductive age with respect to prevalence, etiology, metabolic disorders, and fertility risks.

Parameter	HT	PCOS	PCOS with HT
Prevalence	11–14% (16, 18)	5–12% (1, 2, 4)	22.5–27% (19, 20, 22)
Genetic susceptibility to the disease	73% (29)	71% (31)	Not looked for
Role of TGFβ	Suspected (17, 52)	Suspected (44, 49)	Suspected
Levels of TGFβ	Lower than in healthy controls (51)	Lower in <i>FBN3</i> gene variants carrying allele 8 of D19S884 (50)	Not proven
Role of thymus	Immune tolerance (Tregs) (57)	Suspected (potential fetal origin of PCOS) (61, 64)	Not proven
Vitamin D receptor genetics	Associated with <i>VDR</i> gene variants (35)	Metabolic phenotypes associated with <i>VDR</i> gene variants (75)	Not proven
Vitamin D deficiency	Favors autoimmunity (70)	Favors metabolic syndrome (74)	Not proven
Metabolic disturbances	Features of metabolic syndrome (99, 100, 101)	Features of metabolic syndrome (6, 102, 103, 105, 106)	Worse features of metabolic syndrome (21, 27, 109, 110)
Reproductive disturbances	Infertility, miscarriage, and preterm delivery risk (112, 113, 114, 116)	Anovulatory infertility, gestational diabetes mellitus, pregnancy-induced hypertension, and preterm delivery risk (66, 122)	Clomiphene citrate resistance risk (123)

HT, Hashimoto's thyroiditis; PCOS, polycystic ovary syndrome; TGFβ, transforming growth factor beta; Tregs, regulatory T cells; *FBN3*, fibrillin 3; *VDR*, vitamin D receptor.

revealed a higher prevalence of TPO-positive autoimmune thyroiditis than in 80 controls (22.5 vs 1.25%). PCOS patients had higher mean TSH levels, a higher prevalence of goiter (27.5 vs 7.5%), and more frequently a hypoechoic thyroid ultrasound pattern (12.5 vs 2.5%) than controls (22). Recently, a case–control Iranian study has demonstrated that, in PCOS patients, the mean level of TPO antibodies and the prevalence of clinically proven goiter were higher than those in controls without PCOS (23). However, concentrations of TSH and Tg antibodies did not differ between PCOS and controls (23). Only one Turkish study was not able to establish higher levels of TPO and Tg antibodies in 84 women with PCOS than in 81 controls, although thyroid diseases were more prevalent in patients (24). Most of the above-mentioned studies were included into the recent meta-analysis, which confirmed a higher prevalence of AITD, positive TPO and Tg antibodies, and a higher level of TSH in PCOS patients than in controls (25).

The likelihood of having Graves' disease and PCOS together could be higher compared with subjects without PCOS. In this respect, no extensive epidemiological data were presented until now except for case reports (26). Considering the study by Janssen *et al.* (19), two patients with PCOS, but none of the controls, had been thyroidectomized because of Graves' disease. Additionally, two Indian patients with PCOS and no controls had Graves' disease (22).

Is the prevalence of PCOS in women with HT higher than that in women without HT? To the best of our

knowledge, only one study has addressed this question systematically. In 13- to 18-year-old girls with HT, the prevalence of PCOS was significantly higher than that in girls without HT who were negative for TPO antibodies (46.8 and 4.3% respectively) (27).

From the majority of studies, we can conclude that HT and PCOS frequently occur together. In the following section, possible causes for this phenomenon will be presented.

Joint etiology and pathogenesis

The etiology of HT is complex and includes predominantly genetic, as well as gender-associated and environmental factors such as iodine supply, drugs, infections, and chemicals (17). Similarly, the etiology of PCOS is supposed to involve genetic, ovarian-related as well as other hormonal and metabolic factors such as hyperinsulinemia (12).

For HT, a strong genetic susceptibility for the disease has been confirmed by family and twin studies (28, 29). For PCOS patients, genetic susceptibility and familial aggregation were also shown (30, 31). Several susceptibility genes have been proposed for HT and PCOS (16, 32). However, a common genetic background has not yet been established. Polymorphisms of susceptibility genes in the first disorder, HT, may influence the occurrence and characteristics of the second disorder, PCOS, and *vice versa*. Such possible connections will be

reviewed in more detail. Furthermore, HT is the most prevalent autoimmune disorder (16), and, in PCOS, a possible role of autoimmune phenomena in the etiology has been implied (27, 33). Therefore, putative genetic- and autoimmunity-related causal factors in both disorders will be elucidated, including the role of polymorphisms of susceptibility genes, as well as transforming growth factor beta (TGF β), regulatory T cells (Tregs), the thymus, vitamin D deficiency and sex hormone imbalances.

Susceptibility and candidate genes

In the following paragraph, susceptibility and candidate genes for HT and PCOS will be presented (see also Fig. 1).

In HT, family and twin studies enabled the recognition of a strong genetic predisposition. In children and siblings of patients with HT, the risk of developing HT is increased by 32- and 21-fold, respectively, with females being more frequently affected than males (28). As shown in a study of Danish twins, 73% of the susceptibility for the development of thyroid autoantibodies can be attributable to genetic factors (29). Several genes are known to be associated with the disease occurrence, progression, and severity. Among them are genes coding for human leukocyte antigen (HLA-DR), cytotoxic T-lymphocyte-associated protein 4 (CTLA4),

CD40, protein tyrosine phosphatase 22 (PTPN22), interleukin 2 receptor, alpha (IL2RA), and vitamin D receptor (VDR) (17, 34, 35). The only thyroid-specific gene associated with HT is the gene for Tg (17).

In PCOS, familial clustering is well established. In first-degree relatives of women with PCOS, an increased prevalence of components of PCOS has been documented (32, 36, 37). In Dutch twins, a tetrachoric correlation of 0.71 and 0.38 in monozygotic twin sisters and in dizygotic twins and other sisters, respectively, was established (31). Several candidate genes have been studied for PCOS, such as those coding for fibrillin 3 (FBN3), INS, INS receptor, insulin receptor substrate 1, transcription factor 7-like 2, calpain 10, the fat mass and obesity associated protein (38, 39), SHBG (32), and VDR (40). Results of a large number of candidate gene studies were mostly inconclusive (32). Lately, the *DENND1A* gene, which encodes a protein participating in the endosomal membrane transport, was recognized by genome-wide association studies (GWAS) as a true PCOS susceptibility gene in an Asian population (41) and later confirmed also in a European population (42).

The role of genetic polymorphisms

Possible connections between polymorphisms of susceptibility and candidate genes in HT and PCOS will be discussed in more detail.

Polymorphisms of the *FBN3* gene could be involved in the pathogenesis of PCOS as well as of HT by their influence on the activity of TGF β which is regulated by FBNs (see Fig. 2). Similar to FBN1 and FBN2, the *FBN3* gene is probably encoding FBNs, which are a component of microfibril networks in the extracellular matrix, providing binding possibilities for the sequestration of TGF β (43, 44). Until its release and activation, TGF β is bound to FBNs in an inactive complex. Therefore, FBNs regulate TGF β activity. Generally, TGF β cytokines, secreted by different cell types including macrophages, are known to be involved in cell proliferation, differentiation, recognition, and apoptosis (44, 45, 46, 47). Members of the TGF β superfamily such as activins, inhibins, and anti-Müllerian hormone are supposed to play a role in the pathogenesis of PCOS. However, no members of the TGF β signaling pathway have been shown by GWAS studies to be among the top signals for PCOS (44). Nevertheless, changes in TGF β have been implicated in the pathogenesis of PCOS with regard to fetal origins of PCOS, metabolic, as well as reproductive abnormalities (44). FBN3 is highly expressed in fetal tissues including fetal ovaries (48, 49). After the

Figure 1

Genetic associations in patients with HT and PCOS. HT, Hashimoto's thyroiditis; PCOS, polycystic ovary syndrome; HLA-DR, human leukocyte antigen; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PTPN22, protein tyrosine phosphatase 22; IL2RA, interleukin 2 receptor, alpha; VDR, vitamin D receptor; Tg, thyroglobulin; FBN3, fibrillin 3; INS, insulin; INSR, INS receptor; IRS1, insulin receptor substrate 1; TCF7L2, transcription factor 7-like 2; CAPN10, calpain 10; FTO, fat mass and obesity associated; SHBG, sex hormone-binding globulin; DENND1A, protein participating in the endosomal membrane transport; GnRHR, gonadotropin-releasing hormone receptor.

Figure 2

Associations of TGFβ with HT and PCOS. TGFβ, transforming growth factor beta; HT, Hashimoto's thyroiditis; PCOS, polycystic ovary syndrome; FBN3, fibrillin 3.

first trimester, FBN3 expression in the stromal compartments of fetal ovaries disappears. Therefore, FBN3 exerts its effects via the influence on the activity of TGFβ, which participates in the regulation of stromal formation and function during fetal development, thus supporting hypotheses on a potential fetal origin of PCOS (49). Polymorphisms of the *FBN3* gene have been shown to be associated with the levels of TGFβ. Women with PCOS carrying an allele 8 of D19S884 in the *FBN3* gene had lower levels of TGFβ1 and similar levels of TGFβ2 than PCOS women without allele 8. Both PCOS groups had lower levels of TGFβ2 than controls without PCOS, while allele 8-negative patients had higher levels of TGFβ1 compared with allele 8-negative controls. Moreover, allele 8-positive PCOS women had higher levels of inhibin B and aldosterone when compared with allele 8-negative PCOS women (50). Similarly, in hypothyroid HT, lower serum levels of TGFβ1 were found when compared with healthy controls. Moreover, levels of TGFβ1 did not increase after treatment with levothyroxine (L-T₄), indicating the connection between TGFβ1 and HT and not with TGFβ1 and hypothyroidism *per se* (51). In the development of immune tolerance, as will be discussed later, TGFβ induces the expression of transcription factor forkhead box P3 (FOXP3) and the generation of Tregs, and it acts as a key regulator of immune tolerance by stimulating suppressive Tregs and inhibiting T cell differentiation (17, 52). Therefore, TGFβ could also be involved in the occurrence of an autoimmune disorder such as HT. Given this background, we may hypothesize that PCOS women carrying allele 8 of D19S884 in the *FBN3* gene, and therefore having lower levels of TGFβ1, could be more prone to develop HT than PCOS women without allele 8, but this has not yet been investigated.

Recently, an association between the 3'-UTR variant rs1038426 of gonadotropin-releasing hormone receptor (GnRHR) and INS secretion in PCOS, as well as an association with serum TSH, serum INS levels, and INS sensitivity, has been shown. This may indicate an important role of GnRHR genetic variations in INS secretion and INS resistance in PCOS and their possible connection with thyroid function (53).

Finally, the *CYP11B1* locus is associated with PCOS and encodes an enzyme that oxidizes E₂ to 4-hydroxyestradiol. Polymorphism *CYP11B1* L432V (rs1056836) was associated with serum thyroxine (T₄), free triiodothyronine (fT₃), and free T₄ (fT₄) concentrations (54). This finding may represent a third link between thyroid function and PCOS with respect to genetics.

The role of the thymus

The role of the thymus in the regulation of the immune system and in the development of autoimmunity is well known. Its importance in the autoimmunity-related causal factors in HT and PCOS will be reviewed in this paragraph.

The maintenance of self-tolerance, and consequently the prevention of autoimmunity, is enabled by two mechanisms. The first is central immune tolerance with thymic deletion of autoreactive T cells during fetal life, and the second is peripheral immune tolerance where the leading role is played by Tregs (16, 55). These cells are derived from the thymus and from the naïve peripheral T cells. Tregs have a suppressive effect on the immune system and prevent an excessive immune response (56). In murine experimental autoimmune thyroiditis, a model for HT, the protection against autoimmunity was mediated by thymically derived CD4⁺CD25⁺FOXP3⁺ Tregs (57). Cytokine TGFβ induces the expression of FOXP3 and thereby the formation of Tregs (58). Lower serum levels of TGFβ1 were shown to be associated with HT, as mentioned previously (51). Therefore, if investigated, lower levels of Tregs would be expected in HT when compared with healthy subjects.

A role of estrogen-induced immune disruption in the development of PCOS has been shown in animal models. In female mice, estrogen injection before 10-days of age when thymus is in the final stage of development caused anovulation and follicular cysts (59). The influence of estrogen on the thymus was tested in estrogen-injected female mice with intact thymus, in which ovaries with follicular cysts were observed, whereas no cysts were noticed in mice thymectomized before estrogen

injections, and afterwards replaced with thymocytes from adult animals (60). When estrogen was unable to exert influence upon the thymus during its development, and adult thymic cells were provided later, the ovulation occurred and follicular cysts did not appear. Furthermore, estrogen-injected mice with intact thymus had significantly fewer number of thymocytes than controls (60). The absence of Tregs owing to estrogen-affected thymus was supposed to be a prerequisite for estrogen-induced cyst formation, thus supporting the autoimmune etiology of PCOS (60). Similarly, in women prenatally exposed to diethylstilbestrol (DES), a potent synthetic estrogen, which was prescribed in the USA from 1940 to 1971, the highest rate of infertility was found when they were exposed to DES from gestational weeks 9 to 12 (61). This is also the timeframe of the fastest development of the thymus (62). In DES-exposed women, an increased incidence of autoimmune diseases has been found (63). Modern pregnant women could in turn be exposed to higher levels of estrogen from phytoestrogens, present in flax seeds and soy bean products. Besides estrogens, adrenal steroids such as corticosterone were shown to diminish thymic weight and the number of thymocytes and to cause anovulation and the formation of ovarian cysts in mice (64).

To resume, various factors such as high levels of estrogen or severe stress with increased adrenal steroids could be responsible for the changes in fetal thymus and, thus, for the changes in the immune tolerance and for the joint appearance of HT and PCOS in the adult life in predisposed individuals.

The role of vitamin D deficiency

Vitamin D has been shown to have a beneficial effect on the immune system and seems to be protective with respect to autoimmune diseases (65). Inadequate vitamin D intake is linked to a higher incidence of autoimmune disorders (66, 67). Vitamin D exerts its action via VDR which is expressed in a wide variety of tissues, also on lymphocytes, monocytes, and dendritic cells (65, 68).

Polymorphisms of the *VDR* gene were related to HT, as mentioned previously (35). Moreover, polymorphisms of the *CYP27B1* hydroxylase gene catalyzing the conversion of 25 hydroxyvitamin D₃ (25(OH)D) to its active form were also associated with HT (69). Vitamin D levels were associated with VDR and vitamin D level-related genetic variants (70, 71). The latter finding was confirmed by Lin *et al.* (72). Genetic variants associated with lower vitamin D levels could play an important role in the development

of an autoimmune disorder such as HT. After all, vitamin D deficiency has been shown to be connected with the severity of thyroid autoimmunity and thyroid dysfunction (73). A possible explanation for this effect on the immune system is the influence of vitamin D on dendritic cells expressing VDRs. A stimulation of VDRs increases the tolerogenicity of dendritic cells, thus enabling them to promote the development of CD4⁺CD25⁺FOXP3⁺ Tregs with suppressive activity and to increase peripheral immune tolerance (74). Moreover, in healthy subjects, vitamin D supplementation significantly increased %Tregs compared with baseline levels (75, 76).

In women with PCOS, vitamin D deficiency also plays an important role. Associations between the low 25(OH)D levels and features of metabolic syndrome (77), and between VDR and vitamin D-related polymorphisms and metabolic and endocrine parameters, but not with PCOS susceptibility *per se*, have been shown (70). However, in PCOS women from Iran, an association of *VDR* ApaI polymorphisms with PCOS susceptibility was found (40). Vitamin D supplementation is supposed to improve menstrual frequency and metabolic disturbances in women with PCOS, as reviewed recently (78).

The role of sex hormones

The role of sex hormones in the pathogenesis of autoimmunity is presented in Fig. 3 and seems plausible as women are significantly more often affected by autoimmune diseases than men. Five percent of the world's population have an autoimmune disease and, of them, 78% are women (79). Even in Klinefelter's syndrome, a doubled chromosome X and a low androgen-to-estrogen ratio were supposed to play a role in the

Figure 3

The role of sex hormones in the pathogenesis of autoimmunity. ↑, increased; ↓, decreased; →, leads to; Tregs, regulatory T cells; IL6, interleukin 6.

pathogenesis of autoimmune diseases (80). In women, the onset of autoimmune diseases is earlier than that in men and often coincides with a rise in sex hormone levels (79). Accordingly, a female-to-male ratio in pre-pubertal children with chronic autoimmune thyroiditis has been shown to be significantly lower compared with pubertal adolescents or adults (1.6, 6.7, and 10.3 respectively) (81). In the course of the menstrual cycle, worsening of symptoms of multiple sclerosis was reported before or at the onset of menstruation, and the use of oral contraceptives proved to be protective in this regard (82). Similarly, estrogen usage negatively correlated with the presence of TPO antibodies (83). During the menstrual cycle, lower levels of estrogens during menstruation and luteal phase and higher levels of estrogens during the follicular phase lead to a shift from Th1- to Th2-mediated immunity respectively (84). Accordingly, in young women, levels of the Th2 cytokine interleukin 6 (IL6) negatively correlated with progesterone levels during the normal menstrual cycle. During the luteal phase, IL6 levels were the lowest, and in the follicular phase, the highest (85). IL6 was shown to inhibit the induction of FOXP3 and, consequently, the generation of Tregs (59). Conversely, estrogens were reported to enhance Treg formation (84). Accordingly, the number of Tregs was shown to decrease during the luteal phase and to increase during the late follicular phase (86). Pregnancy is associated with several adjustments in the immune system in order to tolerate the fetus, predominantly with a shift from a Th1 cytokine profile to a Th2 cytokine profile (87, 88). This is probably the consequence of the estrogen-induced expansion of Tregs, which suppress both Th1 and Th2 immune reactions, while the latter are less sensitive to Tregs and, therefore, prevail (89). After delivery, a decline in Tregs shifts the cytokine profile away from Th2 to Th1 causing an exacerbation or an aggravation of autoimmunity (89). Some large retrospective studies found a connection between the number of deliveries and the risk of AITD (90, 91).

Sex hormones have immune regulatory effects *in vitro* and *in vivo* (92). In animal models, estrogens related to a hyperactivity of B cells and a hypoactivity of T cells (93). Autoantibody production was higher in female mice than in male mice (94). Estrogens were described to decrease the activity of T suppressor cells, increase the activity of B cells, increase also the secretion of Th2 cytokine IL6, and to direct the immune response to Th2 and the formation of antibodies (33, 79). Women have a higher CD4⁺/CD8⁺ ratio with higher CD4⁺ levels and more antibodies than men (79). Androgens reduce most elements of the

immune system, enhance the activity of T suppressor cells, and enhance the Th1 response and activation of CD8⁺ (79, 95). Progesterone decreases the proliferation of macrophages, synthesis of IL6, and peripheral antibody production (95). Oscillations of progesterone concentrations during ovulatory cycle and in pregnancy are supposed to be connected with reversible changes in the immune system (96).

Women with PCOS usually have similar E₂, higher testosterone, and lower progesterone levels than women without PCOS (19). PCOS women with irregular menses and numerous anovulatory cycles can have no or very low progesterone and, therefore, an increased estrogen-to-progesterone ratio of long duration. Consequently, their susceptibility to autoimmune disorders will probably increase because of a stimulating action of estrogens on the immune system (19, 33). Otherwise, androgens could protect from autoimmune disease. However, their influence on the immune system and their level in PCOS is probably not high enough for the prevention of autoimmunity. Therefore, the imbalance among estrogen, progesterone, and androgens may promote the occurrence of HT. Considering this hypothesis and the three proposed phenotypes of PCOS (5), the highest prevalence of HT would be expected in PCOS women with chronic anovulation without hyperandrogenism, followed by classic PCOS with anovulation and hyperandrogenism, while the lowest incidence would be expected in ovulatory PCOS with hyperandrogenism. However, this hypothesis is yet to be confirmed.

Metabolic risks

Metabolic changes in HT and PCOS are heterogeneous and often similar in both disorders. Joint metabolic risks encompass higher BMI values and more expressed changes in glucose and lipid metabolism.

In HT, they are more pronounced in subclinically and overtly hypothyroid patients than in patients euthyroid by supplementation (97, 98). Thus, they are related to thyroid dysfunction and ameliorate after the restoration of normal thyroid status (97). However, even with increasing TSH levels within the normal range, an increase in total serum cholesterol, LDL cholesterol, non-HDL cholesterol, and triglycerides, a decrease in HDL cholesterol levels (99), and an association with LDL cholesterol and triglycerides levels have been observed (100). Moreover, euthyroid postmenopausal women with TSH levels within the upper quartile (2.48–4.00 mIU/l) had an adjusted odds ratio for the metabolic syndrome of 1.95

compared with women with TSH levels within the lower quartile (0.3–1.44 mIU/l) (100). Yet, no significant correlation between TSH levels within the normal range and parameters of glucose metabolism was found (100). Additionally, a cross-sectional population study revealed a positive association between BMI and serum TSH and a negative association between BMI and serum fT_4 . In addition, 5-year weight increases correlated with thyroid function, which accounted for 1% of the variation of BMI in this model. Thyroid function had a similar influence on BMI as tobacco smoking and physical activity (101).

Metabolic changes in women with PCOS, such as a higher incidence of INS resistance, type 2 diabetes, and hyperlipidemia, are generally recognized (6, 102). Postmenopausal women with PCOS also have central obesity, type 2 diabetes, and characteristic dyslipidemia of the metabolic syndrome more frequently than postmenopausal women without PCOS (103). Similar to HT, where the severity of metabolic changes correlates with the degree of thyroid dysfunction, the degree of INS resistance and the presence of markers of cardiovascular risk vary in different phenotypes of PCOS (5). Women with PCOS and regular menstrual cycle have significantly better metabolic parameters (BMI, fasting INS, and homeostasis model assessment-insulin resistance (HOMA-IR)) than women with PCOS and oligo/amenorrhea (104).

In PCOS, the prevalence of the metabolic syndrome has been shown to be ~33% in 29-year-old women with the highest INS levels and a mean BMI of 40.4 kg/m² (105). Recently, the prevalence of the metabolic syndrome in women with PCOS has been reported to be 11.9% (106). Furthermore, in 43-year-old women previously diagnosed with PCOS and with a mean BMI of 28.3 kg/m², the metabolic syndrome occurred after a mean follow-up of 13.9 years in only 23.8% (107). This was probably the consequence of lower BMI values in this population of Swedish women. A positive association between TSH levels and BMI has been established, as mentioned previously (104). Therefore, thyroid function seems to have an influence on clinical and biochemical parameters of PCOS. Accordingly, in women with PCOS as well as in healthy women, TSH levels correlated significantly with BMI, weight, waist circumference, diastolic blood pressure, and levels of LDL cholesterol, triglycerides, as well as HDL cholesterol (24). Even within the normal ranges of TSH concentrations, BMI and HOMA-IR were higher in women with PCOS showing TSH levels above 2 mIU/l compared with those with TSH below 2 mIU/l. In these two groups of women, HOMA-IR was not only different but also independent of BMI and age (108).

A combination of HT, based on clinical or subclinical hypothyroidism, or even normal thyroid function with the TSH level in the upper normal reference range, with PCOS might be associated with more pronounced metabolic changes than HT or PCOS alone. Indeed, girls with HT and PCOS had a higher BMI, higher fasting glucose and HOMA-IR, and higher cholesterol than girls with HT alone or controls. Components of PCOS significantly and inversely correlated with the quartiles of fT_4 (27). Similarly, women with PCOS and subclinical hypothyroidism had higher triglyceride levels, higher fasting INS levels, and higher HOMA-IR when compared with PCOS women with normal thyroid function. Groups did not differ with respect to total, HDL and LDL cholesterol (109). On the other hand, 62 subclinically hypothyroid young women with PCOS did not differ from 291 euthyroid women with PCOS with respect to fasting glucose and total cholesterol concentration. Only the levels of triglycerides were higher in the first group (110). In 19 young PCOS women with TSH levels between 4.5 and 10 mIU/l, only LDL cholesterol levels were significantly higher when compared with 149 PCOS women with TSH levels below 4.5 mIU/l (21). Discrepancies between these two studies in young PCOS women are probably related to the small number of cases and to the uneven number of cases vs controls in both studies.

Metformin is frequently prescribed to patients with PCOS. An interesting observation was reported in overweight patients with PCOS and hypothyroidism treated with either 1500 mg of metformin or placebo for 6 months (111). TSH levels decreased or even normalized 6 months after treatment with metformin when compared with healthy controls. However, levels of fT_4 and fT_3 did not change after treatment in any group indicating a central effect of metformin. Authors speculated that metformin could decrease the TSH level by increasing dopamine concentration in the hypothalamus (111).

Reproductive risks

Reproductive abnormalities are associated with HT as well as PCOS. As HT and PCOS frequently occur together, fertility problems could presumably appear more often and in a more pronounced way in patients with both disorders than in patients with only HT or PCOS.

The severity of HT varies and overt thyroid dysfunction is connected with serious fertility risks as mentioned previously; yet, they should gradually disappear during or after appropriate treatment. However, even patients with HT and normal thyroid function can have fertility

problems. Pooled results of several studies indicate that women with infertility more often present with AITD than controls, with an overall estimated relative risk for AITD of 2.1 (112). In some studies, AITD was shown to be associated with a three- to fivefold increase in overall miscarriage rate (113, 114). By contrast, in an observational cohort study of women with unexplained recurrent miscarriage, the prevalence of TPO antibodies was not higher than that in the general population. A TPO antibody-positive status did not have a prognostic value with respect to the outcome of the next pregnancy (115). However, in euthyroid TPO-positive pregnant women, treatment with L-T₄ decreased the rate of miscarriage from 13.8 to 3.5%, and the rate of premature delivery from 22.4 to 7% (116).

In 438 women undergoing assisted reproductive technology, the pregnancy success rate was similar in women with and without AITD, whereas the miscarriage rate was significantly higher in women with AITD compared with those without AITD (53 and 23% respectively) (117). On the other hand, in a population of 38-year-old or older women undergoing IVF procedure, other factors seemed to be more important in maintaining pregnancy, as the prevalence of AITD in the miscarriage group was not higher (118). Similarly, in a small group of women in an IVF program, no association was found between the presence of TPO antibodies before pregnancy and miscarriage. The presence of TPO antibodies before pregnancy did not diminish the chances to become pregnant (119). A systematic review and a meta-analysis confirmed the presence of maternal thyroid autoantibodies as a factor strongly associated with miscarriage and preterm delivery (120). Moreover, treatment with L-T₄ could attenuate the risks (120). Several hypotheses were proposed to explain the association between the presence of thyroid autoantibodies and pregnancy loss. Perhaps, women with higher levels of thyroid antibodies might have very mild hypothyroidism, or thyroid antibodies are only a secondary marker of a general autoimmune imbalance, or women with HT conceive later, whereas increased age represents an independent risk factor for pregnancy loss (112, 121).

Fertility problems are one of the main characteristics of PCOS, which is the most frequent cause of anovulatory infertility (66). Owing to its phenotypic heterogeneity, PCOS may present with or without clinically obvious ovary dysfunction. In pregnant women with PCOS, an increased risk of gestational diabetes mellitus, pregnancy-induced hypertension, and premature delivery has been established (122).

Although a very frequent combination, HT and PCOS have not often been studied with respect to fertility risks.

Only one case-control study revealed that patients with PCOS and increased levels of TPO antibodies are at a higher risk of clomiphene citrate resistance with an odds ratio of 7.7 (123).

Summary

Almost unanimously, prevalence studies report on a frequent joint appearance of PCOS and HT in women within the reproductive age. However, with respect to joint etiology, pathogenesis, and clinical consequences, data are very scarce. This is probably a result of a complex etiology of both disorders, and in case of PCOS, also a result of changing diagnostic criteria over time.

There is no doubt that genetic susceptibility contributes to the development of both disorders in more than 70% as shown by family and twin studies. Until now, a common genetic background has not yet been established. However, several gene polymorphisms associated with PCOS could also influence HT incidence. Among them, the *FBN3* gene variants seem to be the most plausible candidates due to their influence on TGF β activity, and consequently, on the level of Tregs. After all, the role of TGF β has already been implicated in the pathogenesis of PCOS and HT.

In line with this, several factors such as high levels of estrogen or cortisol could impair the development of fetal thymus and its role in the evolution of immune tolerance and, therefore, contribute to the occurrence of HT and PCOS.

Vitamin D deficiency, being involved in the etiology and manifestation of HT and PCOS, is associated with the severity of both disorders. In that respect, further studies in women with both disorders would surely be beneficial.

Women with PCOS could be more prone to develop HT as women without PCOS also because of their different balance between estrogen, progesterone, and androgen levels. Probably, estrogens are not sufficiently opposed by progesterone, which can be very low due to anovulation. Apparently, even relatively higher levels of androgens cannot prevent stimulating effects of estrogens on the immune system and the development of HT in predisposed individuals.

The mechanisms of more pronounced metabolic changes in thyroid dysfunction combined with PCOS when compared with thyroid dysfunction or PCOS alone are yet to be clarified.

With respect to reproductive risks, a combination of PCOS and HT has not yet been seriously investigated. Only a higher risk of clomiphene citrate resistance was reported in TPO-positive women with PCOS.

In conclusion, HT and PCOS are undoubtedly associated with each other, as observed from prevalence studies. However, with respect to etiology, pathogenesis, and clinical consequences, a much broader investigation is yet to be performed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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