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REVIEW

Genetic Predictors of the Development and Recurrence of Graves' Disease

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Summary

Graves' disease affects approximately 3 % of women and 0.5 % of men. The first-choice therapy is based on the administration of thyrostatic drugs. However, approximately half of patients relapse within two years of discontinuation. These patients must then decide whether to re-initiate thyrostatics, which may have serious side effects, or to undergo surgery or radioiodine treatment. Familial forms of Graves' disease indicate a significant genetic component, with twin studies demonstrating a contribution of genetic factors up to 70-80 %. The autoimmune nature of the disease involves the human leukocyte antigen (HLA) complex, which has a decisive impact on each individual's immune response. Within HLA, some variants of the *DRB1*, *DQA1* and *DQB1* genes appear to be possible predictors of the development and recurrence of Graves' disease. Outside the HLA region, many variants of immunocompetent genes have also been identified as potential Graves' disease predictors. Apart from the immune system, some thyroid-specific genes have been described in relation to the disease. Here, we present current knowledge regarding the genetic components involved in the development and recurrence of Graves' disease. Further, we present original pilot results from a cohort of Czech Graves' disease patients regarding the HLA variants.

Key words

Graves' disease • HLA variants • Autoimmunity • Treatment • Genetic predictors

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Introduction

Graves´ disease (GD) patients produce autoantibodies against the TSH receptor, resulting in the overproduction of thyroid hormones. The disproportion in incidence of GD between women (3 %) and men (0.5 %) can largely be attributed to genetic factors and will be discussed in more detail below. The first-choice therapy is based on the administration of thyrostatic drugs for many months or even years. However, approximately half of patients relapse within two years of discontinuation. It is then necessary to decide whether to re-initiate thyrostatic treatment, which may have serious side effects in some cases (liver disorder, agranulocytosis) or to choose a more radical approach (surgical removal of the thyroid gland or radioiodine administration). Clinically well-documented familial forms of GD indicate that the disease has a significant genetic component. Studies on twin pairs have demonstrated that the contribution of genetic factors can be up to 70-80 % (Brix *et al.* 2001). When individuals with a susceptible genetic background are exposed to specific environmental factors like stress, smoking, excessive iodine intake, the maternal postpartum period, microbiome-induced immunological changes (Astl and Šterzl 2015), and other undiscovered conditions, production of autoantigens against the TSH receptor is triggered. The autoimmune nature of the disease involves the human leukocyte antigen (HLA) complex, the most variable genetic system in the body, which has a decisive impact on each individual's immune response.

The HLA system

Over the past 50 years, HLA variants have been shown to significantly influence an individual´s susceptibility to many autoimmune features and complex autoimmune diseases such as type 1 diabetes mellitus (Rich 2017, Kantárová and Buc 2006), multiple sclerosis (Gianfrancesco *et al.* 2017), systemic lupus erythematosus (Langefeld *et al.* 2017), myositis (Miller *et al.* 2018), celiac disease (Verdu and Danska 2018, Jiskra *et al.* 2003), psoriasis (Hirata *et al.* 2018), autoimmune polyglandular syndrome (Hrdá *et al.* 2004) and many other autoimmune disorders including autoimmune thyroid disease (AITD) (Tomer 2010). Clearly, HLA is also essential for combating infectious diseases, and its variants are involved in an individual´s ability to cope with different infections (Matzaraki *et al.* 2017). The extreme variability of the HLA system is a consequence of long selection pressure as human populations moved around the world and faced different pathogens. The HLA complex consists of the three main regions, the classes I, II, and III. The class II molecule, which is important for a topic of this paper, is composed of α and β chains, which form receptors on antigen presenting cells. These cells include macrophages, dendritic cells, endothelial cells, B-lymphocytes and other organ-specific cells. Each of the α and β chains has a highly polymorphic region, together forming the antigen (more precisely epitope) binding groove. Of the HLA class II genes, some variants of the *DRB1*, *DQA1* and *DQB1* genes appear to be possible predictors of the development and recurrence of GD. All these genes are in linkage disequilibrium, which means that they are very close to each other on the chromosome and are therefore inherited en bloc as a haplotype.

GD was initially found to be associated with allelic group *DRB1**03 (Bech *et al.* 1977). As assessed in different Caucasian population groups around the world, the frequency of *DRB1**03 in GD patients was found to range between 40-55 %, but only approximately between 15-30 % in the general population, giving a relative risk (RR) for *DRB1**03 carriers of up to 4 (reviewed by Tomer and Davies 2003). Later, HLA allele *DQA1**05 was also reported to be associated with GD (Barlow *et al.* 1996, Yanagawa *et al.* 1993). Subsequently, the *DQB1**02 gene was included and many independent case-control and family studies identified haplotype *DRB1**03-*DQA1**05-*DQB1**02 as a predictor of the development of GD with an RR of 1.9-3.8 (Gough 2000).

A recent study by Vos *et al.* (2016) aimed to construct a predictive model to calculate the recurrence risk after a course of antithyroid drugs. The model was based on clinical parameters (age, goiter size, fT4, and TBII – thyrotropin binding inhibiting immunoglobulins) in combination with the presence of *DRB1**03, *DQA1**05, and *DQB1**02 and genotyping of the 1858C/T single nucleotide polymorphism (SNP) in the *PTPN22* gene (the role of which in GD will be discussed below) prior to the start of treatment. Antithyroid drugs were withdrawn after one year, and patients were followed for a further two years. Supplementing the clinical characterization of the patients with genotyping was found to be of great value in predicting the disease recurrence and therefore also in the choice of treatment strategy.

PTPN22

One of the strongest risk factors associated with GD outside of the HLA region is the *PTPN22* gene on chromosome 1p13.3-13.1 encoding for protein tyrosine phosphatase-22, a powerful inhibitor of T-cell activation. A single nucleotide polymorphism of *PTPN22*, rs2476601 also referred to as 1858C/T, leads to the substitution Arg620Trp, which disrupts an interaction motif in the protein (Stanford and Bottini 2014). This SNP has emerged as a major risk factor for the development of multiple autoimmune diseases including type 1 diabetes mellitus, rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, as well as autoimmune thyroid diseases such as Hashimoto´s thyroiditis and GD (Burn *et al.* 2011), suggesting that aberrant activity of this phosphatase can disrupt normal lymphocyte functions. The associations are highly reproducible in many different Caucasian populations; however, in Asian and African populations the minor allele is sporadic or absent (Nabi *et al.* 2016). The substitution at codon 620 was found to be associated with GD exhibiting odds ratio (OR) of 1.9 in British Caucasians (Velaga *et al.* 2004), 1.7 in a Polish population (Skórka *et al.* 2005), and even 4.2 in a Russian population (Zhebrun *et al.* 2011) and 4.3 in a Mexican population (López-Cano *et al.* 2017), suggesting a marked predisposing effect to GD considering that the OR is the ratio of the odds of the disease development occurring in the group of risk allele carriers to the odds of it occurring in the group of risk allele non-carriers.

Pilot results in Czech patients

The aim of our work in the Institute of Endocrinology in Prague was to verify the predictive potential of the model assessed on the Dutch population by Vos *et al.* (2016) in Czech GD patients who have undergone treatment and are continuously monitored in our Institute. We have classified the HLA-*DRB1*, HLA-*DQA1* and HLA-*DQB1* allelic groups as well as the *PTPN22* polymorphism rs2476601 in first 50 patients. The new generation sequencing performed on MiSeq (Illumina, San Diego, California, USA) was chosen as the most reliable haplotyping method. In our cohort, 40 % of the patients carried the *DRB1**03-*DQA1**05-*DQB1**02 haplotype, which confirms the findings of the above-mentioned meta- analysis (reviewed by Gough 2000) and complements that information with Czech data. For some HLA allelic groups, the Allele Frequencies website (http://www.allelefrequencies.net/) allowed a comparison of our results from GD patients with the Czech bone marrow donors (n=5,099): the *DRB1**03 allele was more frequent in the GD group (23 %) compared with bone marrow donors (12 %), which is in agreement with previous findings in other populations (Tomer and Davies 2003). On the contrary, the *DRB1**07 allele was less frequent in our GD group (4 %) in comparison with bone marrow donors (14 %). The *DRB1**07 allele has been reported to be protective for GD in UK Caucasians (Simmonds *et al.* 2005); however, a larger cohort of patients is necessary to assess its protective role in the Czech population. Concerning the patients whose disease had recurred (n=24), the eight hypothetically possible predisposing allelic variants (i.e. *DRB1**03, *DQA1**05, *DQB1**02, and PTPN22 rs2476601 variant, each from one or both parents) were distributed as follows: no risk allele in four individuals (16.7 %), one risk allele in six individuals (25 %), two risk alleles in two individuals (8.3 %), three risk alleles in six individuals (25 %), four risk alleles in five individuals (20.8 %), and five risk alleles in one individual (4.2 %). Unfortunately, there were only two patients who had not yet relapsed and whose remission had lasted for more than two years: one was carrying no risk allele and the other was carrying two risk alleles. Such a disproportion in the size of the groups to be compared limits us regarding statistical evaluations. Nevertheless, Fisher's exact test identified allelic group *DQA1**05 to be close to statistical significance in terms of the ability to predict the recurrence (p=0.06). In other words, there was no *DQA1**05 carrier among the successfully treated patients, while there were 19 out of 24 *DQA1**05 carriers (80 %) among the unsuccessfully treated patients. The remaining patients from the current cohort could not yet be included in the statistical analysis as they were still being treated or their remission period had not yet lasted two years (Vejrazkova *et al.* 2018).

Other immunocompetent genes outside the HLA region

CTLA-4

The cytotoxic T lymphocyte antigen 4 gene (*CTLA-4*) encodes a vital regulatory molecule of the immune system that participates in the presentation of antigenic peptides to T-cells. Several polymorphisms in this gene may decrease its expression or function (Ueda *et al.* 2003), thereby promoting the development of autoimmunity, including autoimmune thyroid diseases. Numerous case-control studies have shown an association between GD susceptibility and different *CTLA-4* SNPs or microsatellite markers with an OR of approximately 2-3 (Tomer and Davies 2003, Fouad *et al.* 2017). Major findings resulted from the study conducted by Kim *et al.* (2007), which suggested that genetic markers associated with the development of the disease cannot always be used to predict the disease relapse. Although strongly associated with the development of GD, *CTLA-4* is not associated with the occurrence of relapse after antithyroid withdrawal. This was also true of the *CD40* gene (see below).

CD40

The cluster of differentiation 40 (*CD40*) plays a fundamental role in the cross-talk between antigen presenting cells and T-cells and is essential for B cell activation, providing a crucial signal for their proliferation, differentiation, and immunoglobulin production. As a strong humoral component the gene has been shown to be relevant in autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis (van der Linden *et al.* 2009, Gaffney *et al.* 2006, Australia and New Zealand Multiple Sclerosis Genetics Consortium 2009). Already in 1996, *CD40* was postulated to be linked with susceptibility to GD in mice (Resetkova *et al.* 1996). These findings have been replicated in humans, regardless of ethnicity (Jacobson *et al.* 2005, reviewed by Jacobson and Tomer 2007). However, as said above, variants in the *CD40* gene cannot predict the recurrence of GD (Kim *et al.* 2007).

FOXP3

FOXP3, located on the X chromosome, encodes a forkhead/winged helix transcription factor that is a critical determinant of T regulatory cell development and function. Mutations in this gene result in severe immunodysregulation, polyendocrinopathy, and enteropathy – a serious X-linked disability known as IPEX syndrome. Polymorphisms in *FOXP3* and its promoter have been associated with the risk of developing GD with an OR>5 in some populations (Ban *et al.* 2007, Bossowski *et al.* 2014, Shehjar *et al.* 2018). One study found that the association of *FOXP3* with GD was mostly in the subset of patients with the juvenile form of the disease (Tomer *et al.* 2007).

ARID5B

The *ARID5B* gene encodes for AT-rich interaction domain 5B, which is a DNA binding protein that regulates the transcription of target genes involved in adipogenesis and liver development. This gene also plays a role in the differentiation of B-lymphocyte progenitors, and it is therefore believed to be a general autoimmunity gene. A meta-analysis of GWAS reported a significant association of the gene with rheumatoid arthritis and AITD, including both Hashimoto's thyroiditis and Graves´ disease (Okada *et al.* 2012, Tomer *et al.* 2013).

NRXN3

NRXN3, or neurexin 3, is a neuronal cell surface protein that functions as a cell adhesion molecule and is thought to be involved in synaptic plasticity. Polymorphisms in *NRXN3* have been linked to genetic predispositions to a number of conditions such as alcohol and drug addictions, obesity, and thyroid autoimmunity with an OR=2.3 for GD (Tomer *et al.* 2013).

IKZF3

The *IKZF3* gene encodes a zinc-finger protein that plays an important role in the proliferation and differentiation of B-lymphocytes. Recently, an association between five SNPs in this gene and GD was reported, with an OR in the range of 1.3-2.0 (Li *et al.* 2018). Furthermore, the study suggested that *IKZF3* is probably not the susceptible gene for Hashimoto's thyroiditis, since no locus resulted in a statistical difference between patients and controls. It seems clear that even though Graves´ disease and Hashimoto's thyroiditis share a similar pathogenetic mechanism in which autoreactive T-cells escape tolerance and infiltrate the thyroid gland, genetic factors playing roles in their etiology may be different.

Thyroid-specific genes

Polymorphisms in the thyroglobulin gene and variants in TSHR gene have also been found to be associated with GD pathogenesis (Tomer *et al.* 2002, Hsiao *et al.* 2008, Fujii *et al.* 2017).

Thyroglobulin

A whole-genome linkage study identified the thyroglobulin gene as a major AITD susceptible gene (Tomer *et al.* 2003), and was replicated in various ethnic groups. Further, a possible genetic interaction has been described between one of the thyroglobulin variants and the HLA *DRB1* gene conferring a high susceptibility to GD (Hodge *et al.* 2006).

TSHR

As TSHR is the main target of the immune response in GD, the gene for the receptor was, along with the HLA genes, among the first to be tested for an association with GD. Associations were found in several intronic regions, with the most consistent variants in Caucasians being found in intron 1, altering the transcription and splicing of the gene (Stefan and Faustino 2017). The variant rs2268458 was associated with GD (OR 1.7-1.8), but not with autoimmune hypothyroidism, indicating that this TSHR region represents a GD-specific locus (Dechairo *et al.* 2005, Yin *et al.* 2008).

The gender issue – the influence of two X chromosomes and parity

GD is 5-10 times more frequent in women. This may be to some extent explained by estrogenic sex steroids in promoting autoimmunity (Grossman *et al.* 1991), but this does not explain the observed female preponderance of GD in early childhood. Genetic factors were already mentioned in connection with the X-linked gene *FOXP3*. However, there are additional immune regulatory genetic factors such as the *CD40* ligand gene and others located on the X chromosome: therefore, females with two X chromosomes are twice as likely to inherit susceptible X-linked regulatory elements. Furthermore, X chromosome inactivation during early embryonic development is a major regulatory feature in

which one X chromosome of each female embryo cell is transcriptionally silenced. Inactivation is a random process, so sometimes skewed inactivation occurs when one X chromosome is silenced proportionately more often than the other one. This imbalance has been proposed as a potential mechanism explaining the female preponderance of GD. In the case of significantly skewed inactivation, the immune repertoire will not entirely tolerate the minor version of the two self-antigens encoded by the two X chromosomes. Lymphocytes could then be autoreactive to those minor X-linked antigens and induce an autoimmune process (Simmonds *et al.* 2014).

Another exclusively female issue that can induce the development of autoimmune processes is the postpartum period. During pregnancy, fetal cells are known to reach maternal circulation and infiltrate a variety of tissues including the thyroid gland (Ando *et al.* 2002). This condition, known as fetal microchimerism, can persist for more than 20 years after the childbirth. Fetal cells, representing semi-alloantigens for the mother, may provoke the immune system and trigger autoreactivity. To make things more complicated, there is another circumstance that may pose a threat during the postpartum period. In pregnant women, the immune system is suppressed to protect the fetus, but in the first postpartum months its activation occurs and, in predisposed women, thyroid autoantibody production may rise (Draca 2002).

Conclusion

Studies in the past two decades have demonstrated that multiple factors are involved in the development and recurrence of GD. The interaction of environmental factors with susceptible genes may have synergistic effects in breaking the immunological tolerance and trigger the disease. This interaction is mediated through dynamic epigenetic modulation (Wang *et al.* 2017, Imgenberg-Kreuz *et al.* 2018). Epigenetic modifications caused by an inconstant environment may drive genetically predisposed individuals to develop the disease. In addition to HLA variants, many non-HLA genes have been confirmed as being susceptibility genes contributing to the etiology of GD, some are unique to the recurrence of GD, while others are common to both the disease development and recurrence. Others pose a risk for the development of AITD in general or even a wider range of autoimmune diseases. The current unprecedented progress in genetics is increasingly identifying genetic variants for which the mechanisms of influence on the development of autoimmune diseases are better understood (Tomer 2014). Support for research is the only way to advance the mechanism-based therapy of autoimmune diseases, personalized choice of treatment approaches and more accurate predictions of treatment outcomes.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

AITD – autoimmune thyroid disease, ARID5B – AT-rich interaction domain 5B, CD40 – cluster of differentiation 40, CTLA-4 – cytotoxic T lymphocyte antigen 4 gene, FOXP3 – forkhead box P3, GD – Graves´ disease, HLA – human leukocyte antigen, IKZF3 – Ikaros family zinc finger protein 3, NRXN3 – neurexin 3, OR – odds ratio, PTPN22 – protein tyrosine phosphatase-22, RR – relative risk, SNP – single nucleotide polymorphism, TBII – thyrotropin binding inhibiting immunoglobulins, TSH – thyroid-stimulating hormone, TSHR – receptor for thyroid-stimulating hormone.

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