Genes and environment in Graves’ hyperthyroidism: A prospective cohort study

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Citation for published version (APA):
Chapter 1

Introduction
INTRODUCTION

Graves’ hyperthyroidism is an autoimmune disease characterized by stimulating thyrotropin receptor (TSHR) autoantibodies which bind to and activate the TSHR on thyroid epithelial cells, resulting in overproduction of thyroid hormones.

PATHOGENESIS

Autoantibodies to the TSHR are made by thyroid-infiltrating lymphocytes. These intrathyroidal inflammatory cells also produce cytokines (interleukin-1, tumor necrosis factor α, and interferon-γ) that induce the expression of adhesion molecules (i.e. CD54), regulatory molecules (i.e. CD40), and HLA class II molecules on thyroid epithelial cells, which in turn activate local inflammatory cells. These cytokines also induce thyrocytes to synthesize cytokines that may help sustain the intrathyroidal autoimmune process¹ (Figure 1). Graves’ hyperthyroidism is a multifactorial disease with many predisposing

Figure 1. Pathogenesis of Graves’ hyperthyroidism (adopted from Weetman AP. N Engl J Med 2000;343:1236-1248)
genetic and environmental factors. Good quantitative evidence exists that genetic factors play an important role in the etiology of Graves’ hyperthyroidism. Twin studies show significantly higher probandwise concordance rates in monozygotic twins than in dizygotic twins2-4. Using structural equation modeling, the heritability of Graves’ disease has been reported to be 79% (95% CI, 38–90%)5. It means the relative impact of genetic factors is around 75%; consequently, the relative contribution of environmental factors would be around 25%.

ENVIRONMENTAL FACTORS

Smoking
Smoking has been associated with increased risk of developing Graves’ disease6,7 and especially Graves’ orbitopathy (GO)8. GO manifests more severely in smokers6. Mechanisms whereby smoking may negatively affect Graves’ disease, in particular GO, are unclear. They may include superoxide radicals generated by smoking (inducing orbital fibroblasts to proliferate9), hypoxia (stimulating orbital fibroblasts to proliferate and produce glycosaminoglycans10), and nicotine and tar (inducing class II HLA molecule expression by orbital fibroblasts in the presence of interferon gamma11).

Stress
Several reports showed an association between stress and Graves’ disease. Since the first description by Parry12 of an association between a stressful life event and the occurrence of hyperthyroidism in 1825, many studies have reported a greater number of stressful life events in the year preceding the diagnosis of Graves’ hyperthyroidism when compared with controls13-16.

Iodine
Iodine is necessary for thyroid hormone production. Hyperthyroidism due to multinodular goiter (but not Graves’ disease) is more common in iodine-deficient than in iodine-deplete areas17. Increasing dietary iodine intake is associated with a (transient) increase in the prevalence of Graves’ hyperthyroidism18,19.

Drugs
Several drugs may provoke Graves’ hyperthyroidism. Iodine excess due to iodine-containing drugs (i.e. amiodarone) may precipitate Graves’ hyperthyroidism in susceptible individuals with latent Graves’ disease20. Lithium therapy is associated with hypothyroidism and goiter but also with Graves’ hyperthyroidism, possibly through the immunologic effects of the drug21. High active antiretroviral therapy (HAART) has been associated with
Graves’ hyperthyroidism, likely related to the induced increase in the number or change in the function of CD4+ T cells\textsuperscript{22}. Also interferon alpha treatment for patients with hepatitis C infection has been associated with Graves’ hyperthyroidism\textsuperscript{23}.

**GENETIC FACTORS**

Genes shown to confer susceptibility to Graves’ disease can be divided into thyroid-specific genes (\textit{TSHR}, \textit{Tg}) and immune regulatory genes (\textit{HLA}, \textit{CTLA4}, \textit{PTPN22}, \textit{CD40}, \textit{IL2RA}, \textit{FCRL3}). The strongest associated genes will be described below.

Since anti-TSHR antibodies in serum are the hallmark of Graves’ hyperthyroidism, \textit{TSHR} gene was the most obvious candidate for genetic studies. TSHR gene polymorphisms are indeed associated with Graves’ hyperthyroidism. But the strongest associated single nuclear polymorphism (SNP) in the TSHR gene differs between Caucasians (intron 1)\textsuperscript{24-26} and Asians (intron 7)\textsuperscript{27}. Thyroglobulin (Tg) accounts for approximately 75 - 80% of total thyroidal protein and serves as a precursor and veritable storehouse for the thyroid hormones T3 and T4. Association of \textit{Tg} polymorphisms with Graves’ hyperthyroidism have been found in some studies\textsuperscript{28,29} but could not be uniformly replicated\textsuperscript{30}. HLA Class II haplotypes \textit{DRB1-03, DQA1-05, and DQB1-02} are well documented as being associated with an increased risk of developing Graves’ hyperthyroidism in Caucasian people\textsuperscript{31}. The HLA class II region is involved in the encoding of many membrane bound proteins expressed on the cell surface of B-lymphocytes, macrophages, dendritic cells and activated T-cells, which are involved in the presentation of antigens to CD4+ Th cells. The \textit{DRB1, DQA1} and \textit{DQB1} genes, aberrantly expressed on thyroid follicular cells, play a major role in maintaining tolerance to self-thyroid antigens. CTLA-4 protein acts as a potent negative regulator of T-cell response and both \textit{CTLA4-49}\textsuperscript{32,33} and \textit{CTLA4-60}\textsuperscript{34,35} SNP’s are associated with developing Graves’ hyperthyroidism. The \textit{PTPN22 C1858T} SNP is strongly associated with Graves’ hyperthyroidism and is involved in several signalling pathways associated with the immune response\textsuperscript{36,37}. CD40, expressed on B-cells and other antigen presenting cells (APC), plays an elementary role both in B-cell activation and antibody secretion. CD40 expression has been documented on thyroid follicular cells and thyroid fibroblasts and is up-regulated in thyroid tissues from Graves’ disease patients\textsuperscript{38}. Recent meta-analysis has shown that an association between Graves’ hyperthyroidism and \textit{CD40} polymorphisms exists, especially in Asians\textsuperscript{39}. \textit{CD25} which plays a major role for the IL-2-receptor pathway in the development and function of T cells in the control of autoimmunity is associated with Graves’ disease\textsuperscript{40}. \textit{FCRL3} gene encodes a member of the immunoglobulin receptor superfamily. The encoded protein may play a role in regulation of the immune system. Available data suggest that \textit{FCRL3} polymorphisms are associated with susceptibility for Graves’ hyperthyroidism. However, the primarily associated variant(s) remain(s) to be found\textsuperscript{41}. In conclusion, there is much
evidence for the association of particular polymorphisms in relation to susceptibility to Graves’ disease. However, studies show sometimes discrepant results, possibly because they are performed in different ethnic groups.

**CLINICAL PRESENTATION**

The clinical presentation of Graves’ disease is diverse. Biochemical severity and the age of the patient determine to some extent the manifestations of hyperthyroidism. The most common symptoms are nervousness, sweating, tremor, weakness, rapid heartbeat or palpitations, diarrhea, heat intolerance, and weight loss. With increasing age, weight loss and decreased appetite become more common, whereas irritability and heat intolerance are less common. Cardiac manifestations like atrial fibrillation are rare in patients who are younger than 50 years old but occur more often in older patients. Graves’ orbitopathy (mostly mild forms) is clinically apparent in 30 – 50% of patients with Graves’ hyperthyroidism (Figure 2), but it is detected in 80% of patients who undergo assessment by means of orbital imaging. Pretibial myxedema is another manifestation of Graves’ disease occurring in about 5% of patients.

**Figure 2.** Left panel Graves’ orbitopathy. Right panel large goiter size caused by Graves’ disease.
The annual incidence of Graves’ hyperthyroidism is around 0.5 per 1000 subjects per year with the highest risk of onset at middle age, although the disease can occur at any age. The female-to-male ratio is between 5:1 and 10:1 and the median age at diagnosis is lower in females than in males\textsuperscript{1,44}. The prevalence of Graves’ hyperthyroidism is similar among whites and Asians, but lower among blacks\textsuperscript{1}.

The diagnosis of Graves’ hyperthyroidism is based on clinical and laboratory findings. Decreased serum TSH and increased serum free thyroxine (fT4) and/or triiodothyronine confirm the diagnosis of thyrotoxicosis. Presence of diffuse goiter (Figure 2), orbitopathy or pretibial myxedema indicates Graves’ disease as the cause of thyrotoxicosis. When such clinical manifestations are absent, a diffuse homogeneous uptake on thyroid scintigraphy and the presence of TSHR autoantibodies may confirm the diagnosis Graves’ hyperthyroidism.

Treatment options of Graves’ hyperthyroidism are antithyroid drugs, radioiodine therapy, or surgery. All three modalities have advantages and disadvantages (Table 1). The therapeutic goal of radioiodine therapy and surgery is usually to make the patient hypothyroid, necessitating lifelong thyroid hormone replacement. Antithyroid drugs

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<th>Treatment</th>
<th>Advantages and preferences</th>
<th>Disadvantages</th>
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<tr>
<td>Radioactive iodine ablation</td>
<td>- Definitive treatment</td>
<td>- Requires lifelong thyroid hormone replacement.</td>
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<td></td>
<td>- May be preferred for patients with increased surgical risk.</td>
<td>- Risk of worsening Graves’ orbitopathy in patients with active disease.</td>
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<tr>
<td>Thyroidectomy</td>
<td>- Definitive treatment and rapid resolution of hyperthyroidism.</td>
<td>- Inherent surgical risks</td>
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<td>- Preferred in patients with documented or suspected thyroid malignancy or coexisting hyperparathyroidism requiring surgery.</td>
<td>- Requires lifelong thyroid hormone replacement (total thyroidectomy).</td>
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<td>- Simplifies management of future pregnancy.</td>
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<td></td>
<td>- Preferred in patients with moderate to severe active Graves’ orbitopathy.</td>
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<tr>
<td>Antithyroid drugs</td>
<td>- May induce remission</td>
<td>- Potential serious side effects of hepatic dysfunction and agranulocytosis.</td>
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<td>- May be preferred for patients with increased surgical risk, necks previously operated upon, or limited life expectancy.</td>
<td>- Need for continued monitoring of thyroid hormone levels.</td>
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<td></td>
<td>- Preferred in patients with moderate to severe active Graves’ orbitopathy.</td>
<td>- Possibility of disease recurrence.</td>
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can be given either in a titration regimen, or as a block and replace regimen (BRT) in which a stable dose of methimazole blocks thyroid hormone production and thyroxine is added to prevent hypothyroidism. Antithyroid drug regimens are generally given for a limited period of time (1 – 2 years) because of potentially serious side effects including agranulocytosis and hepatotoxicity. About 30 – 50% of patients experience recurrent hyperthyroidism after withdrawal of antithyroid drugs, finally requiring definitive treatment with either thyroidectomy or radioiodine therapy.

AIM OF THE THESIS

Although much is known about Graves’ disease, several issues regarding pathogenesis, diagnosis and best therapeutic approach are still unclarified. The evidence that environmental stimuli like exposure to stress, cigarette smoke, iodine excess, and several drugs may provoke Graves’ hyperthyroidism in genetically susceptible subjects is rather good, but the quantitative relation between the exposure to environmental stressors and the severity of the provoked thyrotoxicosis has scarcely been studied. An increasing number of gene polymorphisms are found to be associated with Graves’ hyperthyroidism. Nevertheless, little is known whether specific genotypes are associated with differences in clinical presentation of Graves’ hyperthyroidism. With regard to the diagnosis of Graves’ hyperthyroidism in clinical practice, the assay of serum TSH-R autoantibodies is routinely performed by commercially available kits measuring thyrotropin-binding inhibitor immunoglobulins (TBII). Companies producing these assays claim to reach a sensitivity of nearly 100%, but available studies lack a ‘golden standard’ (not involving TBII) against which the diagnosis accuracy of TBII can be evaluated.

Finally, with regard to treatment there is a great geographical variability in the preference for each of the three types of treatment for a first episode of Graves’ hyperthyroidism. Uncertainty about the chance of remission after a course of antithyroid drugs might play a role in this respect. To clarify some of these issues we collected a cohort of 263 consecutive untreated patients (69 males and 194 females) with a first episode of Graves’ hyperthyroidism, who were all treated for one year with antithyroid drugs (block and replace regimen), and followed up for two years after withdrawal of antithyroid drugs.

In chapter 2 the sensitivity of a 2nd generation TBII assay for the diagnosis of Graves’ hyperthyroidism was determined in our population of patients with Graves’ hyperthyroidism, diagnosed according to a ‘golden standard’ composed of suppressed serum TSH, elevated fT4 and/or T3, and a diffuse homogeneous uptake on thyroid scintigraphy (\(^{99m}\)Tc-pertechnetate). TSH-R mutation analysis was performed in TBII-seronegative
patients to exclude familiar non-autoimmune hyperthyroidism. Differences in clinical characteristics and in exposure to environmental factors between TBII-seronegative and TBII-seropositive patients were assessed to investigate if TBII-seronegative patients belong to a particular subset of Graves’ hyperthyroidism.

**Chapter 3:** Age has consistently been observed as a significant modulating factor, advancing age being associated with less severe Graves’ hyperthyroidism. Nevertheless, the mechanism behind less severe Graves’ hyperthyroidism in the older age groups is incompletely understood. Advancing age is associated with a decrease in self-reported stress (recent life events and daily hassles). It has been shown that psychological stress has a differential effect on immune responses, suppressing cellular and potentiating humoral immunity. In chapter 3 we tested our hypothesis that advancing age is associated with less exposure to stress, resulting in lower production of TSH receptor antibodies and thereby less severe Graves’ hyperthyroidism.

**Chapter 4:** Possibly as a consequence of the multifactorial etiology of Graves’ hyperthyroidism, there is marked variation in phenotypic appearance (as judged from differences in severity of hyperthyroidism, presence of Graves’ orbitopathy or pretibial myxedema, and goiter size). Sometimes patients present themselves after a very short time of thyrotoxic complaints. In our experience these patients have frequently severe Graves’ hyperthyroidism and respond remarkably fast to antithyroid drugs. We hypothesized that a short duration of thyrotoxic symptoms until diagnosis is related to more severe Graves’ hyperthyroidism. It is well known that autoimmune thyroid disease (AITD) clusters in families. In view of genetic anticipation [a phenomenon whereby the symptoms of a genetic disorder become apparent at an earlier age as it is passed on to the next generation], we hypothesized that Graves’ hyperthyroidism develops at a younger age in patients with a positive family history for AITD compared to patients with a negative family history. Both hypotheses were tested.

Elaborating on the multifactorial etiology of Graves’ hyperthyroidism, genetic polymorphisms and environmental factors are both involved in the pathogenesis of Graves’ hyperthyroidism. However, their interaction and effect on Graves’ phenotypes have scarcely been investigated. In chapter 5 we tested our hypothesis that subjects with susceptibility genotypes develop more severe Graves’ hyperthyroidism at a younger age and after lower exposure to environmental factors. Special attention was given to possible differences in gender: in view of the strong female preponderance in AITD, the development of Graves’ disease in males might require a much heavier load of either susceptibility genes or exposure to environmental factors.

Treatment options of Graves’ hyperthyroidism are antithyroid drugs, radioiodine therapy, or surgery. Treatment is best tailored to the needs of individual patients, taking into account co-morbidities and personal preferences. A rather accurate estimate of the likelihood of remission after a course of antithyroid drugs would be very relevant for selecting a particular treatment. In chapter 6 we determined clinical and genetic markers...
Chapter 1

that are independently associated with recurrence after antithyroid drug therapy, and constructed a prediction model to calculate risk of recurrence after a course of antithyroid drugs, based on clinical and genetic parameters prior to the start of treatment.
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Chapter 1


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