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

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Chapter 7

General discussion
GENERAL DISCUSSION

All studies presented in this thesis are performed in the same cohort of 263 consecutive, untreated patients with a first episode of Graves’ hyperthyroidism. The main strength of the cohort studies is their prospective nature, allowing simultaneous assessment of clinical, environmental, and genetic parameters in each patient at baseline. Furthermore, all patients were treated in the same manner with a one-year course of standardized antithyroid drug therapy and follow-up until two years after withdrawal of therapy. The cohort might well be representative for the whole Dutch adult population of patients with Graves’ hyperthyroidism, because we included consecutive patients originating from one academic and eight local hospitals throughout The Netherlands; almost all hyperthyroid patients in The Netherlands are treated by hospital-based specialists. Baseline characteristics of our study population indeed correspond well with literature data on clinical1-4 and genetic5-12 characteristics of patients with newly diagnosed Graves’ hyperthyroidism.

It is sometimes claimed that second generation thyrotropin binding inhibiting immunoglobulin (TBII) assays have a 100% sensitivity for the diagnosis of Graves’ hyperthyroidism13. To our opinion this claim is unlikely to be true because a sensitivity of 100% for any diagnostic test is almost never reached, and some false positive and false negative test results are almost inevitable. In chapter 2 we determined the sensitivity of a 2nd generation assay of TBII for the diagnosis of Graves’ hyperthyroidism in our cohort. Hallmark of the study design was that the diagnosis of Graves’ hyperthyroidism was established independent from TBII results, and that we envisaged to exclude an alternative diagnosis in TBII seronegative patients by TSH receptor mutation analysis. In the presence of biochemical hyperthyroidism and a diffuse homogeneous thyroidal uptake in 99mTc-pertechnetate scan the only alternative diagnosis is familial non-autoimmune hyperthyroidism, caused by gain-of-function mutations in the TSH receptor gene. We observed a prevalence of 5.4% of TBII seronegativity in our cohort. TSH receptor mutation analysis did not detect mutations in the TSH receptor gene in the seronegative patients. Consequently, the TBII seronegative patients can still be considered ‘bona fide’ cases of Graves’ hyperthyroidism. The sensitivity of the TBII assay in our cohort is thus 94.6%.

Graves’ hyperthyroidism is caused by TSH receptor stimulating autoantibodies (TSI, thyroid stimulating immunoglobulins) which bind to the TSH receptor on thyroid follicular cells, followed by increased thyroid hormone synthesis and release by the thyroid gland. The question that arises how the absence of TSH receptor antibodies in serum can be reconciled with the observed hyperthyroid state. We offer three explanations. The first explanation is that the sensitivity of the employed 2nd generation TBII assay is too low. Recently, a meta-analysis has shown that the sensitivity of 2nd generation TBII assays is 97%14. In none of studies included in this meta-analysis was the diagnosis of Graves’ hyperthyroidism established independently of serum TBII. Furthermore, studies
were retrospectively designed and used different serum TBII cut-off levels. Since 2004 3\textsuperscript{rd} generation TBII assays are available in which the autoantibodies in patients sera inhibit binding of a human monoclonal thyroid stimulating antibody to ELISA plate wells coated with TSH-receptors\textsuperscript{15}. Sensitivities of these assays are higher, up to 98.3\%\textsuperscript{14}. A second explanation is that TBII assays are not necessarily measuring TSH receptor stimulating antibodies, which bind to but also activate TSH receptors inducing (via cAMP signaling pathways) increased thyroid hormone synthesis and release. In recent years it has been recognized that the nature of TSH receptor autoantibodies in Graves’ disease can be stimulating, blocking, or neutral with respect to activation of TSH receptor\textsuperscript{16}; they may occur simultaneously. The TBII assays cannot discriminate between these three types of antibodies as all three will interfere with the binding of labeled TSH (or monoclonal antibodies against TSH receptor) to TSH receptors. Bioassays measuring functional activity of TSH-receptor antibodies by detecting stimulating signal level (cyclic adenosine monophosphate) can discriminate between stimulating and binding activity (like a Mc4 assay)\textsuperscript{17}. The distinction may be of clinical value. For example in Graves’ orbitopathy, high persistent TSI levels are associated with active and more severe manifestations with poor responses to therapy\textsuperscript{18,19}. Although functional assays have the advantage of measuring responses that can be correlated with disease activity, these bioassays are complex and time consuming. Recently, some new highly sensitive and specific bioassays are developed\textsuperscript{17,20,21}. A retrospectively designed study in 106 untreated Graves’ disease patients found a sensitivity of 94.3\% in a Mc4 assay\textsuperscript{22}. However, diagnosis of Graves’ hyperthyroidism was not established independent of TSH receptor antibodies. Another study by Giuliani et al. found 100\% sensitivity and 98.5\% specificity for a new Mc4 bioassay\textsuperscript{17} but also this study lacks diagnostic criteria independent of serum TBII. The third and most attractive explanation is that the production of TSH receptor antibodies in our TBII seronegative patients might still be confined to the thyroid gland and adjacent lymph nodes, without spill-over of the antibodies into the circulation. Indeed, the thyroid gland is the primary site of autoantibody secretion in autoimmune thyroid disease\textsuperscript{23}, autoantibodies being produced by lymphocytes infiltrating the thyroid gland. In support of this explanation is our observation that TBII seronegative patients were characterized by biochemically mild hyperthyroidism, likely indicating early stages of disease. In line with this proposed explanation is a case report of Hashimoto’s goiter. Baker et al. have shown that lymphocytes isolated from the goiter (surgical pathology clear Hashimoto’s disease) of a euthyroid patient without thyroperoxidase (TPO) or thyroglobulin (Tg) autoantibodies in serum, did produce TPO and Tg antibodies, whereas peripheral blood lymphocytes did not produce these antibodies\textsuperscript{24}. Nowadays in clinical practice indications to measure TSH receptor antibodies are limited to support the diagnosis of Graves’ hyperthyroidism when clinical assessment and thyroid scintigraphy do not give clues for the right diagnosis, to support the diagnosis of
Graves’ orbitopathy in euthyroid patients; to estimate prognosis in Graves’ orbitopathy; to estimate the risk of neonatal hyperthyroidism in pregnant patients; and to estimate the risk of recurrent Graves’ hyperthyroidism after antithyroid drug therapy, normal levels indicating greater chance for remission. Indeed guidelines of the American Thyroid Association recommend to measure TSH-receptor antibodies not routinely but only when a thyroid scan and uptake are unavailable or contraindicated (e.g. during pregnancy and nursing). However, recently it has been shown that incorporating TSH-receptor antibody measurement in routine diagnostic algorithms might lead to 46% faster time to diagnosis and 47% overall cost savings. These effects are in large part due to reductions in costly procedures and less follow-up visits. In 2011, a worldwide survey under 730 physicians showed that 58% of physicians use TSH-receptor antibody measurements in routine clinical practice.

In chapter 3 we tested our hypothesis that less severe Graves’ hyperthyroidism with advancing age is causally associated with less exposure to stress. The first finding was that advancing age was indeed associated with less severe Graves’ hyperthyroidism as evident from lower serum thyroid hormone levels, lower serum autoantibodies concentrations (TBII and TPO-antibodies), and smaller goiter size. These findings are in agreement with previous published studies. The second finding was that advancing age was also associated with less exposure to stressful life events (pleasant and unpleasant) and daily hassles. These interesting observations on stress are in line with previous studies in which older adults reported fewer undesirable daily events. When stressors do occur, older age is related to reductions in perceived severity and affective distress. No association could, however, be found in our study between the amount of stress exposure and serum thyroid hormone and autoantibody levels. Therefore we had to reject our hypothesis that less stress exposure in old age is causally related to less severe Graves’ hyperthyroidism in elderly people.

What mechanism then underlies the effect of less severe Graves’ hyperthyroidism with advancing age? There is good evidence that the immune system deteriorates with age. For example, the repertoire of naive and memory T cells is less diverse, and a gradual decline in thymic generation of new naive T cells occurs during aging. Animal models on experimental autoimmune thyroiditis have shown that lymphocytes from old mice are less effective in transferring thyroiditis than lymphocytes from young donors. In addition, antibodies produced by aged animals are generally of lower affinity than those produced by younger animals. These age-associated changes that cause alterations of the immune response are termed immunosenescence. This phenomenon might lead to a lower TSH-receptor autoantibody production in elderly Graves’ hyperthyroid patients. On the other hand, prevalence of autoantibodies associated with systemic autoimmune diseases increases with age but probably with lower affinity and effectiveness. Moreover, aging is associated with increased serum TSH concentrations, with no change in fT4 concentrations. This suggests that the TSH increase arises from reduced TSH...
bioactivity with a decreased thyroidal response leading to lower thyroid hormone production, and subsequently an alteration in the set point of the hypothalamus-pituitary-thyroid axis (towards higher TSH in order to maintain fT4 levels). As with TSH, the same decreased thyroidal response to TSH-receptor stimulating antibodies might be the case in elderly subjects with Graves’ hyperthyroidism.

Chapter 4 describes the effect of a positive familial history for autoimmune thyroid disease (AITD) on phenotypic appearance of Graves’ hyperthyroidism. A positive family history for AITD was present in 43% of our patients. This is in good agreement with a frequency of around 50% reported in literature\textsuperscript{1,2}. Manje et al. recorded a positive family history on the basis of patient’s recall as having a relative positive for AITD\textsuperscript{2}. In our study the autoimmune nature of thyroid disease in affected relatives was verified via written confirmation by the relatives themselves.

Why is the frequency of around 50% not higher, because twin studies by Brix et al. have shown that around 80% of the susceptibility to develop Graves’ disease can be attributed to genetic factors\textsuperscript{46}? Does is mean that single nuclear polymorphisms (SNP’s) in susceptible genes are sometimes inherited, but can also arise \textit{de novo}? If so, this will count just for a very small part of patients. Does it mean that genetic susceptibility for Graves’ disease is lower in patients with a negative family history and would they have been exposed more environmental factors? No differences were found in exposure to more environmental factors between patients with a positive and patients with a negative family history for AITD in our study. Presumably, gene-environmental interactions in Graves’ hyperthyroidism are more complex than nowadays presumed. Consequently, the estimation of the susceptibility of genetic and environmental factors as calculated by Brix et al.\textsuperscript{46} might be skewed. Calculations were for 1/3 based on a cohort of patients born between 1870 and 1920. During the follow-up time iodine intake was insufficient in Denmark leading to endemic goiter. Furthermore, the model used by Brix et al. was based on “nonshared unique environmental effect” while most twins grow up together and are exposed to the same environmental factors. American twin studies indicate that the estimated pairwise concordance rates for Graves’ disease was slightly lower in monozygotic and dizygotic twins compared to the Danish study (0.29 and 0.04 vs. 0.35 and 0.03 respectively)\textsuperscript{47}. All together the study by Brix et al. may overestimate the influence of genetic factors in the etiology of Graves’ disease and the contribution of environmental factors might be higher.

Genetic anticipation is a phenomenon whereby the symptoms of a genetic disorder become apparent at an earlier age as it is passed on to the next generation. In view of genetic anticipation we hypothesized that Graves’ hyperthyroidism develops at a younger age in patients with a positive family history for AITD. In our study we found that age at diagnosis was much lower in the group with the highest number of affected relatives and degree of kinship compared to patients with a lower familial predisposition; the difference varied between 8 – 13 years. Patients with the highest familial predisposition
were more often male. No differences were found in exposure to environmental factors or in biochemical and clinical severity of hyperthyroidism. Brix et al. investigated age at diagnosis in 33 same-gender parent-offspring pairs with Graves’ disease from multiply affected families and found that mean age at diagnosis in children was 12.5 years lower than in their parents. Manji et al. found that reporting of a relative with thyroid dysfunction was associated with a lower median age at diagnosis of Graves’ disease (in women, 38 vs. 43 years in patients with no family history, and in men 42 vs. 47 years respectively). They also observed that the greater the number of affected relatives, the greater the effect was. In view of these findings a lower median age in view of genetic anticipation in Graves’ disease seems to be real.

19th century psychiatrists already described that within certain families there was a tendency for various diseases to manifest earlier in succeeding generations, as opposed to the commonly perceived pattern of inheritance where the disease manifested at the same time generation after generation. Later on, anticipation was invoked as a way of explaining findings of lower age of onset and increasing severity of disease in dementia praecox, Huntington disease, myotonic dystrophy, Leber’s hereditary optic neuropathy, Fragile X syndrome, and diabetes. From the early 1990’s sequencing of the myotonic dystrophy, Fragile X, X-linked spinal and bulbar muscular atrophy, and Huntington disease genes found a region of unstable DNA where changes occurred in the copy number of a trinucleotide repeat. The longer and more unstable the repeat areas become, the earlier and more severely the disease manifests. Yaturu et al. described in type II diabetes that patients in the second affected generation seemed to acquire the disease at an earlier time in life, suggesting evidence for genetic anticipation. Like Diabetes Mellitus, Graves’ hyperthyroidism is a non-Mendelian disorder with most cases occurring sporadically, differing from unstable DNA diseases like Fragile X, Huntington disease, and myotonic dystrophy. However, the absence of trinucleotide repeats in non-Mendelian diseases does not exclude genetic anticipation. Earlier age at onset has been described in several non-Mendelian disorders i.e. familial breast cancer, colon cancer, Alzheimer disease, and insulin-dependent diabetes. In conclusion, our study supports our hypothesis that genetic anticipation in Graves’ hyperthyroidism occurs, possibly via other molecular mechanisms than via trinucleotide repeats.

We investigated in chapter 5 the effect of specific genotypes in relation to phenotypic appearance and exposure to environmental factors in Graves’ hyperthyroidism. We hypothesized that subjects with genetic susceptibility to Graves’ disease are younger at the time of diagnosis and present with more severe disease. The investigated genetic variants in HLA, CTLA4, and PTPN22 all carry an independent risk for Graves’ disease. Frequencies of observed genetic variants in our study are in good agreement with frequencies found in other studies in adult Caucasian patients with Graves’ disease. The well-known linkage disequilibrium between Graves’ disease and genes in the HLA class II region, also observed in our study, is due to the extended haplotype DRB1*304-
DQB1*02-DQA1*501. Interestingly, no differences were found between the frequencies of HLA class II polymorphisms and susceptibility alleles of CTLA4 49A/G, CTLA4 CT60, and PTPN22 SNPs in the familial and sporadic cases of Graves’ hyperthyroidism in our study. 

CTLA4 49A/G and CTLA4 CT60 SNP’s were both dose dependently associated with younger age at the time of diagnosis: G/G homozygotes were respectively 7 and 8 years younger compared to wild types (A/A genotype). No associations between genetic variants in HLA class II and PTPN22 genes and age at diagnosis of Graves’ hyperthyroidism were observed. Our data are at variance with a Polish study in adult Caucasian patients with Graves’ hyperthyroidism, which observed no relation between age at onset and HLA variants or CTLA4 49A/G but patients with G-alleles of the PTPN22 SNP were 10 years younger. Ban at al. found higher frequencies of the HLA DRB1-03 genotype in patients under the age of 20 years compared to older patients. However the literature on this topic is scarce, other studies did not find an association between specific genotypes and age at onset of Graves’ hyperthyroidism. In view of the polygenic etiology of Graves’ hyperthyroidism one may presume that the effect of a single polymorphism is too small to ensure such a great effect. CTLA4 is one of the strongest associated genes in Graves’ hyperthyroidism, and consequently its relationship with Caucasian Graves’ hyperthyroid patients is more readily detected.

In the second part of this study we hypothesized that subjects with genetic susceptibility to Graves’ disease require less exposure to environmental factors for contracting Graves’ hyperthyroidism. Polymorphism CTLA4 49A/G (but none of the other genotypes) was quantitatively related to stress exposure. The total number of daily hassles, the intensity per hassle, and the total intensity of daily hassles were all lower in carriers of the G-allele of this polymorphism in a dose-dependent manner. The CTLA4 49A/G SNP was not related to mood (PANAS scales) or to recent life events. According to psychological studies, everyday exposure to daily hassles in general constitutes a greater burden of stressors than incidental exposure to major life events. The observation of less daily hassles is not biased by mood changes in our study (current mood state may influence the perception and thereby the scores of daily hassles and recent life events). Less daily hassles in carriers of G-alleles of CTLA4 49A/G can also not be explained by their lower age, as recent studies have shown a progressive decrease in daily hassles with advancing age in healthy subjects with highest scores occurring in the youngest age groups of <40 year. The finding that SNP CTLA4 49A/G is associated with younger age at diagnosis and with less exposure to stress, supports our hypothesis that subjects with genetic susceptibility for Graves’ disease might require less exposure to environmental factors for contracting Graves’ hyperthyroidism. No other studies related genetic variants in HLA, CTLA4 and PTPN22 to environmental factors, apart from the notion that the frequency of tobacco smoking was not related to the SNP in PTPN22.
A major risk factor in Graves’ hyperthyroidism is female sex. Graves’ disease is 5 – 10 times more common in females than in males and median age at diagnosis is lower in women than in men\(^2\). In view of the 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. We found that males (but not females) in HLA linkage disequilibrium had more severe (biochemical and immunological) hyperthyroidism and a tendency to younger age at diagnosis, compared with those not in linkage disequilibrium. Furthermore, the amount of current smokers in men with HLA linkage disequilibrium was lower compared to males with no linkage disequilibrium albeit failing statistical significance (35% vs. 57%, p = 0.12). With regard to CTLA4 49A/G, male carriers of a G-allele were 9 years younger at time of diagnosis compared to wild-type males. This association was much weaker in female carriers of G-alleles but the trend was in the same direction. For the PTPN22 and CTLA4 C760 genes this association was not found. These data support our hypothesis that the impact of genotypes in males seems to be greater than in females although a much larger sample size would be required for definitive proof. In female Graves’ hyperthyroid patients modulation of the immune response by estrogen is presumed. There is a large body of evidence that estrogen enhances immunologic reactivity\(^65;67\). On the other hand, the enhanced susceptibility of Graves’ disease in women can also be related to the X-chromosome. X-chromosome inactivation has been associated with autoimmune thyroid disease\(^68;69\). Furthermore, pregnancy is associated with Graves’ disease\(^70;71\). It has been suggested that fetal microchimerism (the presence of fetal cells in maternal tissue) might play a role in the development of autoimmune thyroid disease\(^72\). Treatment options of Graves’ hyperthyroidism are antithyroid drugs, radioiodine therapy, and surgery. Recently a worldwide survey under 730 physicians showed great geographical variation in preferred treatment for a first episode of Graves’ hyperthyroidism\(^27\). In North America radioiodine therapy is the first choice in 58.6%, whereas in Europe antithyroid drugs are favored (85.7%) above radioiodine therapy (13.3%). Efficacy, recurrence, complications, and costs differ among the three treatment options. The likelihood of remission after a course of antithyroid drugs would be very relevant information for selecting a particular treatment. In chapter 6 we developed a predictive score to estimate the risk of recurrence after a course of antithyroid drugs, based on clinical and genetic parameters prior to the start of treatment. In our cohort of 178 Dutch Caucasian patients with a first episode of Graves’ hyperthyroidism 37% of patients had recurrent Graves’ hyperthyroidism within two years after antithyroid drugs withdrawal. These findings are in good agreement with 30-50% recurrence rates in the literature\(^29;73;74\). At time of diagnosis and before the start of therapy, we found that younger age (< 40 years), more severe biochemical hyperthyroidism by means of higher fT4 (≥ 40 pmol/l) and higher TBII (≥ 6 IU/l), and large goiter size (grade II or higher) were all independently associated with recurrence after antithyroid drugs. We did not find differences in sex, but Allahabadia et
al. found higher recurrence rate in males than in females in a UK population (80% vs. 60%)\textsuperscript{29}; the discrepancy might be explained by the much larger sample size in the UK study. Young age is observed in some but not all previous studies\textsuperscript{29,75-80}. Like in our study, agreement exists on more severe biochemical hyperthyroidism and larger goiter size as independent risk factor for recurrence before start of therapy\textsuperscript{29,76,77,79,81}. The contribution of high serum TSH receptor antibody levels before start of treatment varies between studies, one reporting positive but other report negative results\textsuperscript{76,79,82-87}. It should be noted that most of these studies are performed using a first generation TSH receptor antibody assays. Nowadays with the availability of much higher sensitive 2\textsuperscript{nd} and 3\textsuperscript{rd} generation TBI assays together with bioassays measuring TSH receptor blocking, neutral and/or stimulating antibodies, prediction of recurrence might be improved. Recently, Giuliani \textit{et al.} showed that risk of recurrence could be predicted at the end of antithyroid drug treatment with a new Mc4-bioassay\textsuperscript{88}. However, to stratify for the best treatment modality measurement of TSH receptor antibodies should be performed at time of first presentation.

HLA Class II haplotypes DRB1-03, DQA1-05, and DQB1-02 are well documented as being associated with an increased risk of developing Graves’ hyperthyroidism in Caucasians\textsuperscript{5,8}. We found that HLA DRB1-03, DQA1-05, and DQB1-02 polymorphisms are strong predictors for recurrence after antithyroid drug therapy. Only one study published about recurrence risk and HLA DQA1-05 and found no relationship\textsuperscript{89}. In our study also the PTPN22 C/T SNP was associated with risk of recurrence in Graves’ hyperthyroidism, not reported so far. We did not find an association between recurrence rate and the CTLA4-49 and CTLA4-60 SNP’s. Both SNP’s are associated with developing Graves’s hyperthyroidism\textsuperscript{5,7,9,11,90}. Japanese, Turkish and Chinese studies have shown that the CTLA4-49 polymorphism is associated with recurrence\textsuperscript{60,91,92} but German and Korean studies could not find such an association\textsuperscript{89,93}. It has recently been shown that Graves disease associated alleles identified in Chinese Hans, and those identified in other Asian studies are totally distinct from the known associated alleles in Caucasians\textsuperscript{94}. The most prominent susceptibility allele in Caucasians, DRB1*03:01, has a much lower frequency in Asians, ranging from less than 3% in Japanese and Koreans to 4–9% in Chinese\textsuperscript{95,96}. According to the ethnic differences in the prevalence of genetic polymorphisms, discrepant results are comprehensible. Furthermore, multiple gene polymorphisms are found to be associated with Graves’ disease. Genome-wide association studies have a dramatic impact on susceptibility locus discoveries. Recently seven newly identified loci for autoimmune thyroid disease have been found by the ImmunoChip project in which twelve groups world-wide are collaborating\textsuperscript{97}. However, for each associated gene polymorphisms the absolute risk in the pathogenesis of Graves’ disease is low. Based on the Hazard ratios of the individual risk markers two predictive scores of recurrence were constructed. In the first model called the GREAT (Graves’ Recurrent Events After Therapy) score, patients were stratified into different classes of recurrence.
risk according to clinical markers (age < 40 years, serum fT4 $\geq$ 40, serum TBII $\geq$ 6, and goiter size $\geq$ II). For the second model patients were also stratified into different risk classed but based on clinical parameters supplemented with genetic parameters (HLA and PTPN22 polymorphisms). The GREAT score consists of simple clinical parameters that can be used in everyday practice. When patients fall into GREAT Class I we would advise to start antithyroid drug therapy because of the low recurrence risk after withdrawal (16%). Patients who fall in Class II of the GREAT score have a change of 44% for recurrence after antithyroid drug therapy. In these patients additional measurement of HLA polymorphisms and PTPN22 SNP can give supporting information for the best therapeutic approach. After genotyping, about a third of patients will be reclassified into the lowest risk groups (GREAT+ class I+ - II+: recurrence risk 4 – 21%) and antithyroid drug therapy would be advised. A small part of GREAT score Class II patients will be re-classified as very high risk (GREAT+ class IV+) with a recurrence rate of 84%. The remaining patients can be treated according to preferences of patient or physician and co-morbidity. For the GREAT score highest risk class III (recurrence rate 68%) we would recommend radiiodine therapy or surgery. Use of our predictive scores model has a number of advantages. First, patients can be better informed about the treatment strategy and recurrence risk. This leads to better understanding by the patient, likely to better treatment compliance, and in the end lower recurrence rates. Secondly, looking at the healthcare costs, as a result of the lower recurrence rates fewer patients will need adjuvant therapy, resulting in less follow-up visits and finally lower treatment costs. Patel et al. have shown in a UK cohort of Graves’ hyperthyroid patients that treatment with radiiodine is the most cost effective modality after two years follow up for cured patients compared to antithyroid drug therapy and surgery\textsuperscript{98}. However, no long-term cost effectiveness data is known but in the end 54% will develop post-radiiodine hypothyroidism necessitating lifelong thyroxin treatment\textsuperscript{99}. In conclusion, in the present era of personalized medicine, our prediction model based on simple clinical assessment can be of great value in individualized treatment of newly diagnosed patients with Graves’ hyperthyroidism in routine clinical practice. Nowadays there is convincing evidence for a limited number of genes that have consequently been reported as Graves’ disease susceptibility loci. Future studies using sophisticated gene-scanning techniques will definitely find more associated genes. In our opinion new affordable DNA techniques in the nearby future will make it possible to screen patient in daily practice on multiple Graves’ disease susceptible loci at time of presentation which in combination with clinical markers will make it possible to refine the prediction for the risk of recurrence. Our prediction score can serve as a basis for future refinement.
FUTURE STUDY TOPICS

Although the sensitivity of newly developed TSH receptor antibody (bio)assays for the diagnosis of Graves’ hyperthyroidism is very high, 100% sensitivity will probably never be reached. To substantiate our hypothesis that in early Graves’ hyperthyroidism the TSH receptor antibodies are confined to the thyroid gland, without spill-over into the circulation, it will be of great interest to evaluate TSH receptor antibodies production by isolated interthyroidal lymphocytes of TBII-seronegative patients with a diagnosis of bona fide Graves’ hyperthyroidism.

With regard to immunosenescence it will be of great interest to test if the affinity or bioactivity of TSH receptor autoantibodies is decreased in elderly Graves’ hyperthyroid patients. This can be tested by comparing the potencies of IgGs in cAMP bioassays between young and old Graves’ hyperthyroid patients. Secondly, we are wondering if the effect of TSH receptor stimulation is diminished in elderly Graves’ hyperthyroid patients. This can be tested by assessing the response to either recombinant TSH or a monoclonal antibody against the TSH receptor (like M22) in vivo or in vitro, looking for age differences.

Decades of research have shown that genes and environment both are involved in the pathogenesis of Graves’ hyperthyroidism. Another fruitful area of future research would be gene-environment interactions. A notable example is a Brazilian study on genetic polymorphisms associated with cigarette smoking and the risk of Graves’ disease\textsuperscript{100}. The effect of alcohol consumption has scarcely been studied. A Danish paper reported that alcohol could be a confounding factor in the relationship between stress and Graves’ disease\textsuperscript{101}. As controls consumed alcohol more often than cases, alcohol consumption appeared to be protective against Graves’ disease.

Despite the absence of trinucleotide repeats in non-Mendelian diseases like Graves’ disease, genetic anticipation seems to occur. The molecular mechanism of the effect should be clarified in familial studies of successive generations with Graves’ disease.

Our prediction model for the recurrence of Graves’ hyperthyroidism should prospectively be tested in a group of newly diagnosed, untreated Graves’ hyperthyroidism patients. It will be interesting to investigate if the newest generation of TSH receptor assays improve prediction of recurrence after antithyroid drug therapy. Furthermore, more Graves’ disease associated genes will undoubtedly be found and it may become affordable to test for multiple genes in each patient to further refine our prediction model of risk of recurrence.
REFERENCES


17. Kamijo K, Murayama H, Uzu T, Cong K, Kahaly GJ. A novel bioreporter assay for thyrotropin receptor antibodies using a chimeric thyrotropin receptor (mc4) is more useful in differentiation of Graves’ disease from painless thyroiditis than conventional thyrotropin-stimulating antibody assay using porcine thyroid cells. Thyroid 2010;20:851-856.

Chapter 7


General discussion


55. Tomer Y. Genetic susceptibility to autoimmune thyroid disease: present, past, and future. *Thyroid* 2010;20:715-725.


Chapter 7


