Early stages of thyroid autoimmunity: follow-up studies in the Amsterdam AITD cohort
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Citation for published version (APA):
Effraimidis, G. (2012). Early stages of thyroid autoimmunity: follow-up studies in the Amsterdam AITD cohort

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Chapter 1

Introduction
A SHORT HISTORICAL OVERVIEW OF AUTOIMMUNE THYROID DISEASE

The first presentation of the classic symptoms of hypothyroidism was in 1883 at the meeting of the Clinical Society of London [1], although there had been already a few other early observations on myxedema in 1850 [2]. It was at the same time that Theodor Kocher observed that patients who had undergone total thyroidectomy developed a clinical picture similar to that of patients with cretinism [3]. In 1888 the Clinical Society of London published a detailed report which linked cretinism and myxedema with the destruction of the thyroid gland [4]. It became clear that the lack of one or more substances secreted by the thyroid was responsible for the disease. At the end of the 19th century hypothyroidism was not curable but very soon thyroid extracts from sheep proved to be effective [5]. In 1912 the Japanese physician Hakaru Hashimoto described a new pathologic entity of the thyroid gland in four women who had goiters with diffuse lymphocytic infiltration, fibrosis and parenchymal atrophy (struma lymphomatosoma) [6].

In 1956, it was reported for the first time by Roitt et al. [7] that serum of patients with lymphadenoid goiter contains antibodies against thyroid tissue (thyroglobulin antibodies) which are associated with Hashimoto’s disease. In the same year, Rose and Witebsky provided evidence that immunization of animals with homologous thyroid extract developed thyroiditis lesions with similar features as those seen in the human disease [8]. The production of experimental autoimmune thyroiditis and the detection of antithyroglobulin antibodies laid the foundation stone for the causal relationship between autoimmunization and human disease [9,10]. In 1958, Belyavin and Trotter reported on another type of thyroid autoantibodies which were found to react with crude preparations of thyroid cell membranes, the anti-microsomal autoantibodies [11]. The microsomal antigen remained unidentified until 1985 when it was recognized as the thyroid peroxidase (TPO) [12,13].

The cardinal diagnostic test of hypothyroidism caused by destruction of thyroid gland is an elevated serum TSH. However until the mid 1960s serum TSH assays were not available. Until that time a diagnosis of primary hypothyroidism was confirmed by measuring basal metabolic rate, later replaced subsequently by protein bound iodine, hormonal iodine and T4 assays. By the early 1980s the methods for assay of serum TSH had been improved: TSH assays had become sufficiently sensitive to discriminate between normal and suppressed TSH values. The wide employment of TSH assays in clinical practice led to the realization that besides patients with overt hypothyroidism (elevated TSH in combination with low T4 levels) there were patients with an elevated TSH in the presence of normal T4 serum concentrations, a condition called subclinical hypothyroidism.

The first cases of exophthalmos and goiter were published in 1825. Ten years later Robert Graves described four women with goiter and palpitations while one of them women had also exophthalmos [14]. The same constellation of symptoms was independently reported by Karl von Basedow in 1840, the historical “Merseburger Triad” as it is called in textbooks [15]. Paul Julius Moebius first brought up the idea that the disorder is caused by hypersecretion of a ductless gland in 1886 [16]. By the end of 19th century the thyroid was recognized as the causative factor because patients who underwent thyroidectomy were cured from
thyroid hormone excess. Therefore, thyroidectomy remained the core for the treatment of hyperthyroidism in the first half of the 20th century.

In 1956 Adams and Purves while setting up an assay for serum TSH in guinea pigs found a stimulator in the serum of Graves’ disease patients distinct from TSH [17]. Their findings were confirmed in 1958 by McKenzie who used mice as assay model [18]. The stimulator was initially called long-acting thyroid stimulator (LATS). Kriss et al. in 1964 proved that LATS was an immunoglobulin G, and subsequently the autoimmune nature of the disease became established [19]. During the next 20 years it became obvious that the thyroid stimulating activity is caused by antibodies against the TSH receptor. At present very sensitive assays for the TSH-receptor antibodies are available [20].

**Autoimmune Thyroid Disease:**
A Complex Multifactorial Entity

Hashimoto’s thyroiditis (hypothyroidism) and Graves’ disease (hyperthyroidism) can be considered as two separate diseases. Although clinically the opposite, both conditions share immunological features like a variable degree of lymphocytic infiltration and the presence of autoantibodies to TPO and Tg in serum. Moreover, it is well known that sometimes (although rarely) patients with hypothyroid Hashimoto goiter may convert into Graves’ hyperthyroid cases; also a number of Graves’ hyperthyroid patients may spontaneously convert to hypothyroidism in the long term. Furthermore, in the same family there can be patients with Graves’ hyperthyroidism and Hashimoto’s hypothyroidism. Consequently, one may consider the two conditions as representing the opposites sides of the same coin. In this view Graves’ disease and Hashimoto’s thyroiditis belong to one and the same disease entity, namely autoimmune thyroid disease (AITD).

AITD is nowadays generally considered to be a complex multifactorial entity in which the interplay between genetic and environmental factors result in the expression of the disease. There is a good evidence that the genetic predisposition plays a major role in the pathogenesis of AITD as siblings and other family members of AITD patients are at increased risk for AITD [21,22]. The sibling recurrence risk ratio (λs) is 11.6 for Graves’ and 28.0 for Hashimoto’s disease [23,24]. In women with at least one first degree relative with AITD, the incidence of Graves’ hyperthyroidism is 4.1-4.3 times higher and of Hashimoto’s hypothyroidism 1.9-2.7 times higher than the general female population [25]. Twin studies suggest that the concordance rate for Graves’ disease and for autoimmune hypothyroidism is significantly higher in monozygotic twins compared with dizygotic twins (0.35 vs 0.03, p=0.001 and 0.55 vs. 0.0, p=0.01 respectively) [26,27]. A model fitting analysis estimated that 79% and 73% of the likelihood of developing Graves’ disease and thyroid antibodies respectively is due to genetic factors [27-29] and it can be assumed that the relative impact of genetic factors in the phenotypes of AITD is most likely around 75% [29]. The nature of these genetics factors is gradually being discovered over the last decades. The importance of the HLA gene complex in AITD was early identified. Graves’ disease has been
associated with HLA-DR3 in Caucasians [30] (primary susceptibility allele HLA-DRB1*03 [24]) while Hashimoto’s thyroiditis has been reported to be associated with DR3 and DR4 in Caucasians [30] and with a pocket HLA-DR amino acid signature [31]. Later, it has been reported that polymorphisms in the gene that encodes cytotoxic T lymphocyte antigen 4 (CTLA-4) [32] and protein tyrosine phosphatase, non-receptor type 22 (PTPN22) [30,33] are associated with AITD. Polymorphisms in CD40, thyroglobulin and thyrotropin receptor genes have also been identified as contributing to thyroid autoimmunity [22,30]. Two comments can be made in these studies. First, the observed genetic associations are not always specific for AITD as the same genetic factors (HLA, CD40, CTLA-4, PTPN22) are associated with a number of other autoimmune diseases (like type 1 diabetes, rheumatoid arthritis, Addison disease). Second, the contribution of each of these genetic factors to the risk of contracting AITD is very small indeed. It has been estimated that combining the presently known polymorphisms that carry a risk for AITD, ends up to only 5-10% of the genetic susceptibility. Consequently there must be many more genes involved in the pathogenesis of AITD than currently known.

Whereas multiple genes contribute to AITD, environmental factors are also important in the pathogenesis of the disease. According to twins studies environmental factors will contribute for about 30% to the development of AITD. Exposure to a multitude environmental factors have been studied and found to be associated with AITD like iodine, smoking, stress, pregnancy, exogenous estrogens and certain infections (e.g. with Yersinia enterocolitica) [34,35].

**AIM OF THE PRESENT THESIS**

So far, the importance of environmental factors in the pathogenesis of AITD has been studied mostly in a retrospective manner in cross-sectional case-control or population-based studies (exceptions are ambient iodine intake and exposure to particular drugs which have been studied in a prospective manner). For instance the well established association between stress and Graves’ hyperthyroidism has been studied just in retrospective studies, which are liable to recall bias as Graves’ disease patients were asked to report on stressful life events either at the time they were still hyperthyroid or even later when euthyroidism was restored. Prospective studies in general provide more solid evidence in establishing associations between environmental factors and autoimmune thyroid disease.

Since the first descriptions of hypothyroid and hyperthyroid patients in the 19th century, a large body of literature has provided many pieces of information that help to solve the puzzle of the pathogenesis of AITD. The overall picture that emerges is as follows. AITD starts with a particular genetic background. Next thyroid antibodies (TPO-Ab, Tg-Ab) occur in serum, which can be taken as an early sign of thyroid autoimmunity; thyroid function tests (TSH, fT4) at this stage are still within the normal reference range. Thereafter serum TSH values may become abnormal while serum fT4 concentrations are still normal: the stage of subclinical hypothyroidism or subclinical hyperthyroidism. AITD ends in many cases with
overt hypo- or hyperthyroidism characterized by both abnormal TSH and fT4 concentrations. However there remain still many unanswered questions. With respect to environmental factors, these have been studied mostly in a retrospective manner and in relation to the occurrence of the final stages of AITD (overt hypo- or hyperthyroidism) and not to the earlier stages of AITD (occurrence of thyroid antibodies). In order to get better insight into environmental factors involved in the pathogenesis of AITD and especially in the early stages of AITD, we evaluated exposure to a number of environmental insults in a prospective manner. This was done by nested case-control studies in the Amsterdam AITD cohort. The Amsterdam AITD cohort consists of healthy euthyroid women at risk for AITD because they were relatives of patients with AITD; subjects were followed prospectively with annual assessments for 5 years. The cohort also allows evaluation of the relative importance of susceptibility genes and assessment of early aberrations in immunological surveillance in AITD, but such studies are outside the scope of the present thesis which is devoted to environmental factors.

Amsterdam AITD cohort

The cohort has been described previously in detail [36,37]. Subjects willing to participate in our study, were recruited from all over The Netherlands through patients visiting our department, through advertisements in local newspapers, and through thyroid patient self-support associations. The Medical Ethics Committee of the Academic Medical Center in Amsterdam approved the study. All subjects were seen at our Institution and gave their informed written consent. We screened 1003 female subjects with at least one 1st or 2nd degree relative with documented autoimmune hyper- or hypothyroidism, who were in self-proclaimed good health, without a history of thyroid disease, and between 18 and 65 years of age. We checked the medical history of the affected relative(s) regarding the autoimmune nature of their thyroid disease after obtaining their informed consent. Autoimmune thyroid disease for this purpose was defined as documented hyper- or hypothyroidism in the presence of autoantibodies against thyroid peroxidase (TPO), TSH-receptor autoantibodies, histology compatible with autoimmune thyroiditis, or thyroid eye disease. In 200 of the 1003 screened subjects proof of the autoimmune nature of the thyroid disease in at least one family member could not be ascertained, leaving 803 participants to be included in the cohort. After excluding 13 females with overt hyper- or hypothyroidism at baseline, 790 first or second degree female relatives of AITD patients were eligible for follow-up. Endpoints of follow-up were the development of overt hyperthyroidism or hypothyroidism (events) defined by abnormal TSH values in combination with abnormal fT4 concentrations in plasma. Endpoints were assessed every year for 5 years. At each visit blood was sampled for assay of TSH, fT4, TPO-Ab, Tg-Ab, TBII (TSH binding inhibitory immunoglobulins) and Yersinia enterocolitica serology. In addition, annual questionnaires assessed smoking habits, alcohol consumption, use of oral contraceptives or other estrogens, pregnancies, exposure to iodine excess, and stress exposure.
At the end of the 5-yr follow-up of the Amsterdam AITD cohort, 38 subjects had developed autoimmune hypothyroidism and 13 hyperthyroidism [25]. The cumulative event rate was 7.5% over 5 years, the mean annual event rate was 1.5%.

**OUTLINE OF THE THESIS**

In *chapter 2* we describe the time course of the transition from euthyroidism to either overt hyperthyroidism or overt hypothyroidism during the natural history of AITD. We also evaluated whether exposure to some environmental factors change in the years preceding the development of overt AITD in two nested case-control studies.

In *chapter 3* we evaluate environmental factors (especially smoking) in relation to de novo occurrence of thyroid antibodies (TPO-Ab, Tg-Ab). Smoking is a well known risk factor for Graves’ hyperthyroidism, and recent literature data indicate that in contrast smoking seems to protect against overt autoimmune hypothyroidism.

In *chapter 4* the hypothesis is tested that alcohol consumption reduces the risk of developing AITD. We examined the effect of alcohol intake on both early (development of TPO-Ab) and late stages (overt hypothyroidism) of AITD. Previous studies suggest a protective effect of alcohol on the development of other autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus.

In *chapter 5* the hypothesis is tested that early stages of thyroid autoimmunity (i.e. genetic susceptibility and development of TPO-Ab) are associated with low vitamin D levels. Vitamin D deficiency has been identified as a risk factor for a number of autoimmune diseases including type 1 diabetes and multiple sclerosis.

In *chapter 6* the hypothesis is tested that recent life events and daily hassles are associated with the development of TPO-Ab and overt hyper- or hypothyroidism. Exposure to stress has been related to the onset of Graves’ hyperthyroidism in retrospective studies, but the role of stress in the development of thyroid antibodies and Hashimoto’s hypothyroidism has scarcely been studied.

In *chapter 7* we report age-dependent changes in recent life events and daily hassles in healthy women who remained euthyroid without any serological sign of thyroid autoimmunity during the 5-yr follow-up.

In *chapter 8* we evaluate the relationship between Yersinia Enterocolitica infection and de novo occurrence of thyroid antibodies and the development of overt autoimmune hypo- or hyperthyroidism. So far, conflicting results are reported in the literature on the putative relationship between Yersinia enterocolitica infection and AITD.

In the general discussion of *chapter 9*, an overview of the above mentioned studies is presented, and the scientific and clinical implications of the obtained results are commented.
Figure 1. Timeline of the history of autoimmune thyroid disease.
References