The Amsterdam autoimmune thyroid disease cohort
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General Discussion

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A. Family background

Our Amsterdam AITD cohort was composed of 803 adult women in self declared good health, with documented proof of one or more 1st or 2nd degree relatives with AITD. At study entrance, the prevalence of autoimmune hypothyroidism was 3.6% (overt hypothyroidism 1.3%, subclinical hypothyroidism 2.3%) and of autoimmune hyperthyroidism 1.9% (overt hyperthyroidism 0.4%, subclinical hyperthyroidism 1.5%) (§3). Our prevalence figures can be compared with those obtained in the general population. For a proper comparison, confounding factors like age, sex and ambient iodine intake should be taken into account. Our cohort was assembled in The Netherlands, an iodine-replete country (1). Because prevalence figures are substantially influenced by ambient iodine intake (2) we focussed on available surveys done in iodine-sufficient countries like the U.K. (3) and the U.S.A. (4,5), excluding studies done in iodine-deficient regions (6,7). The prevalence of unsuspected overt hypothyroidism in the Whickham survey among adult women in the U.K. was 0.3% and of subclinical hypothyroidism 7.5% (3). In the Colorado study (mean age 56 yr, 56% females and 44% males) the prevalence figures in subjects not taking thyroid hormone medication are: overt hypothyroidism 0.4%, subclinical hypothyroidism 8.5%, overt hyperthyroidism 0.1%, subclinical hyperthyroidism 0.9% (4). The NHANES III study in the U.S.A. reports the following prevalences in the disease-free population (that is the total investigated population except those who were pregnant or taking estrogens and those who reported to have thyroid disease, goiter, or thyroid medication): overt hypothyroidism 0.2%, subclinical hypothyroidism in 3.9%, overt hyperthyroidism 0.2%, subclinical hyperthyroidism
0.2% (not specified for sex) (5). The prevalence figures in our Amsterdam AITD cohort are three times higher than those in the general population, except the prevalence of subclinical hypothyroidism which was about three times lower. The latter can be explained by the difference in age: the mean age of our cohort is 36 yr, 20 years younger than e.g. the median age of 56 yr in the Colorado study; it is well known that the occurrence of subclinical hypothyroidism increases with advancing age (3,4,5). It thus seems reasonable to conclude that the prevalence of autoimmune thyroid dysfunction in the Amsterdam AITD cohort is higher than in the general female population. The finding was to be expected in view of the familial clustering of AITD (8). It must be bear in mind that the prevalence in our study may have been influenced by unintentional selection in the recruitment of participants.

In the Amsterdam AITD cohort study we followed 790 women for 5 years (leaving out the 13 subjects with clearly overt thyroid dysfunction at study entrance). The cumulative incidence of overt hypothyroidism and overt hyperthyroidism in the cohort was 7.5% (§7). The calculated hypothyroidism incidence rate was 9.6 /1000 women years in our population, and the hyperthyroidism incidence rate was 3.3 /1000 women years. These incidence figures can be compared to those in two studies from the U.K., the prospective Whickham study (9) and the retrospective Tayside study (10); we did not consider the Danthyr study performed in iodine-deficient Denmark (2). The incidence rates of hypothyroidism in the two English studies were 3.5 and 4.98 /1000 women years, and of hyperthyroidism 0.80 and 0.77 /1000 women years, respectively. The incidence of overt hypothyroidism in our Amsterdam AITD cohort is thus 1.9-2.7 times higher than that in the general female population, and that of overt hyperthyroidism is 4.1-4.3 times higher. The figures once again demonstrate that subjects with a family history of AITD are at increased risk for thyroid disease. The risk in siblings of parents with AITD (with reported $\lambda_s$-values as high as 11.6 for Graves’ disease and 28.0 for Hashimoto’s disease) (8) is much higher than observed in the Amsterdam AITD cohort. This is understandable because relatives with AITD in our study could be children, brothers, sisters, parents, grandparents, uncles or aunts of the participants. Our figures thus fit nicely between the lower risk in the general population and the higher risk in siblings.

The autoimmune nature of thyroid disease in family members of the Amsterdam AITD cohort participants was ascertained by letters from physicians who had treated those family members. This proved to be a slow and tedious process. Definitive evidence of having at least one relative with AITD was obtained in all participants, but definitive evidence of having two or more relatives with AITD was obtained in 55% of participants at study entrance (§3) and in
77% of participants in the 5-yr follow-up (§7). The difference is explained by the ongoing efforts of retrieving information about family members, treated often decades ago. The collected data suggest there are AITD families in whom only Graves’ disease or only Hashimoto’s disease occurs. However, we cannot be absolutely sure about this because we had not always the opportunity to investigate all possibly affected family members. Nevertheless, it is certain from our data that Graves’ and Hashimoto’s disease coexist in some AITD families. This suggests a common genetic background. Indeed particular HLA and CTLA-4 polymorphisms have been identified as susceptibility factors for both diseases (11,12). Slow but steady progress is being made in the recognition of other susceptibility genes which are linked specifically to either Graves’ or Hashimoto’s disease (13). Twin studies indicate that genetic factors contribute for about 70% to the risk of developing AITD (14,15). In subjects with a certain genetic make-up environmental factors may determine whether they will develop Graves’ hyperthyroidism or Hashimoto hypothyroidism.

The specific nature of the familial background was an independent risk factor for the occurrence of autoimmune hypo- or hyperthyroidism in the 5-yr follow-up, and therefore included in the predictive THEA-score (§7). Hypothyroid events occurred more often in subjects having one or two relatives with Hashimoto disease than in subjects having one or two relatives with Graves’ disease (7.3% vs 3.2%). Hyperthyroid events however, did not occur more often in subjects having one or two relatives with Graves’ disease than in subjects having one or two relatives with Hashimoto disease (1.7% vs 1.6%). Our data are in partial agreement with those of a large U.K. study (16): in Hashimoto hypothyroidism, a family history of hypothyroidism was more common than hyperthyroidism (42.1% vs 22.8% in affected females), but in Graves’ hyperthyroidism a family history of hyperthyroidism in any relative was more frequent than hypothyroidism (30.1% vs 24.4% in affected females). The discrepancy with regard to Graves’ disease between our cohort and the U.K. study could be due to the much larger sample size of the U.K. study; on the other hand, The U.K. study is liable to bias because a family history of thyroid dysfunction was recorded on the basis of subject recall and not checked objectively as in the Amsterdam AITD cohort.

B. Age

The mean age of the Amsterdam AITD cohort was 36 yr at study entrance, but participants who at that time already had an abnormal TSH were older (43 yr in the hypothyroid group and 44 yr in the hyperthyroid group) (§3). Age was an independent risk factor for hypothyroidism
but not for hyperthyroidism in our cohort. This is in accordance with surveys in the general population, all reporting a higher prevalence of hypothyroidism with advancing age (3,4,5); the relation was not observed for hyperthyroidism (9). The mean age at diagnosis of hypothyroidism and hyperthyroidism (e.g. 57 yr and 48 yr respectively in the Whickham survey) in the general population is higher than in our cohort. However, these population-based studies include all causes of thyroid dysfunction. In this respect a more proper comparison is with the U.K. study also dealing exclusively with autoimmune hypo- and hyperthyroidism (16): the median age at diagnosis of both Hashimoto hypothyroidism and Graves’ hyperthyroidism was 41 yr (for both sexes), a figure close to our observed ages of 43 and 44 yr. The peak ages for diagnosis of both Graves’ and Hashimoto disease in the U.K. study were the fourth to sixth decades.

Higher age was an independent determinant of the presence of TPO antibodies in the euthyroid participants of the Amsterdam AITD cohort at baseline. This finding is in complete agreement with the higher prevalence of TPO antibodies with advancing age in the disease-free population of the NHANES III study (5,17).

In the 5-yr follow-up of the Amsterdam AITD cohort, age was not an independent risk factor for the occurrence of autoimmune hypo- or hyperthyroidism according to multiple logistic regression analysis, and consequently was not included in the predictive THEA-score (§7). The trend to a higher event rate in older age was nonsignificant, probably due to the small number of subjects in the age group 60 to 65 yr and an overriding effect of higher TPO antibodies concentration with advancing age (9). The mean age at which participants in our cohort developed overt hypothyroidism or hyperthyroidism during follow-up, was 41 yr and 44 yr respectively (§8); again, very close to the median age of 41 yr in the U.K. study (16). It is interesting to mention that in the U.K. study the median age in women who had Graves’ hyperthyroidism, was lower in those with a family history of AITD than in those without (38 yr vs 43 yr), and the same was true for women with Hashimoto hypothyroidism (38 yr vs 48 yr). The U.K. study also reports an inverse relationship between the number of relatives with thyroid dysfunction and age at diagnosis, but this has yet not been assessed in our cohort.

C. Biochemical tests

C.1. Thyroid function
The prevalence of an abnormal TSH in the Amsterdam AITD cohort at baseline was 5.5% (§3). In the disease-free population of NHANES III the prevalence of an abnormal TSH was
7.1% for all ages, falling to 4.9% when excluding subjects with thyroid antibodies (17). Among our participants with a normal TSH at baseline, TSH was higher in subjects with TPO antibodies than without TPO antibodies (2.3 vs 1.6 mU/l), although FT4 was not different between both groups (§3). This interesting finding has later been confirmed in other studies (17). The presence of TPO antibodies indicate the existence of chronic lymphocytic thyroiditis, which may slightly compromise thyroid hormone synthesis and release; it results in a slight change of TSH towards higher values but still within the normal reference range. FT4 values were similar irrespective of the presence or absence of TPO antibodies; this can be explained by the exquisite sensitivity of pituitary thyrotrophs to respond to minute changes in circulating FT4, as illustrated by the log-linear relationship between TSH and FT4.

In the 5-yr follow-up of the Amsterdam AITD cohort TSH (but not FT4) levels at baseline were an independent risk factor for the occurrence of overt hypo- or hyperthyroidism, and therefore included in the predictive THEA-score (§7). The risk was dose-dependent, and not restricted to TSH levels outside the reference range (two of the 13 hyperthyroid cases (15.4%) had a baseline TSH <0.4mU/l, and seven of the 38 hypothyroid cases (18.4%) had a baseline TSH >5.7mU/l. The risk clearly increased at TSH above 2.0 mU/l, that is at values within the customary reference range. The cut-off of 2.0 mU/l is exactly the same as observed in the twenty-year follow-up of the Whickham survey, in which the probability of developing hypothyroidism increased sharply at baseline TSH values >2.0 mU/l irrespective of the presence of thyroid antibodies (9). The finding has two important implications. First, optimal thyroid health might be associated with TSH levels between 0.4 and 2.0mU/l. Secondly, the upper normal limit of TSH could or should be lowered to 2.0mU/l. The latter notion has been strengthened by the observation that in reference populations the distribution of TSH values is not normal but skewed to higher values. Proponents and opponents of a narrower TSH reference range hold each other in balance (18,19). We ourselves, are of the opinion that the upper normal limit of TSH should not be lowered to 2.0 mU/l: it will label a sizable proportion of the population as being abnormal, thereby creating a huge burden to health services, without any proof that medical intervention in subjects with TSH values between 2.0 and 4.0 mU/l improves long-term health outcomes (20).

The nested case-control study in the 5-yr follow-up of the Amsterdam AITD cohort demonstrated higher TSH and lower FT4 values in the hypothyroid cases than in controls, both at baseline and one year before the event (§8). The absolute change in TSH values in this time period was greater in cases than in controls (ΔTSH 1.1 [0.5-2.1] vs 0.4 [0.1-0.9] mU/l, p <0.000). In contrast, TSH and FT4 values were not different between hyperthyroid cases and
their controls, neither at baseline nor at one year before the event. The absolute change in TSH in this time period also did not differ between cases and controls (ΔTSH 0.2 [0.0-0.5] vs 0.3 [0.0-0.6] mU/l, p=0.6). No differences were found in absolute changes in FT4 between hypothyroid/hyperthyroid cases and their respective controls. The findings suggest that progression towards overt autoimmune hypothyroidism is a gradual process taking several years, but that in contrast overt autoimmune hyperthyroidism develops faster in terms of months rather than years.

C.2. Thyroid antibodies

The prevalence of TPO-Ab was 27% and of Tg-Ab 8% in the Amsterdam AITD cohort at study entrance. The prevalence was higher in subjects with an elevated TSH (TPO-Ab 86%, Tg-Ab 24%) than in subjects with a suppressed TSH (TPO-Ab 53%, Tg-Ab 20%; in subjects with a normal TSH the prevalence of TPO-Ab was 24% and of Tg-Ab 7% (§3). Our observed overall prevalence of 27% of TPO-Ab among 1st and 2nd degree relatives of AITD patients lies nicely in between the lower prevalence of TPO-Ab in the general population (10.3% in the Whickham survey, 13.0% in NHANES III) (3,5) and the higher prevalence of TPO-Ab in first-degree relatives of AITD patients (43-48%) (21,22). The findings once again underline the notion that subjects with a family history of AITD are at increased risk for thyroid antibodies. Twin studies (23) and linkage analysis (24) confirm the strong genetic contribution to the development of thyroid antibodies. The lower prevalence of Tg-Ab in comparison with TPO-Ab is in agreement with population studies. Also, the lower prevalence of TPO-Ab and Tg-Ab in hyperthyroid subjects in comparison with hypothyroid subjects is well known. Of interest is the dose-dependent effect of TPO-Ab on TSH values in the participants with a normal TSH, as discussed above in section C.1. TPO-Ab > 100 kU/l (but not Tg-Ab) constitute an independent dose-dependent risk factor for the occurrence of overt thyroid dysfunction in the 5-yr follow-up of the Amsterdam AITD cohort, and are consequently included in the productive THEA-score (§7). This was true for both autoimmune hypothyroid and hyperthyroid events. Baseline TPO-Ab >100 kU/l were observed in 30 of the 38 hypothyroid cases (79%) and in 9 of the 13 hyperthyroid cases (69%). The data are in good agreement with the 20-yr follow-up of the Whickham survey (9): in women, the odds ratios with 95% confidence intervals were 8 (3-20) if serum TSH alone was raised, 8 (5-15) if thyroid antibodies alone were positive, and 38 (22-65) if serum TSH was raised and thyroid antibodies were positive. The fact that Tg-Ab in our cohort did not contribute to the risk, is likely related to the unknown biologic relevance (if any at all) of Tg-
Ab; in contrast, TPO-Ab contribute in the immunopathogenesis of AITD by complement-fixation and antibody-dependent cell-mediated cytotoxicity.

The nested case-control study in the 5-yr follow-up of the Amsterdam AITD cohort demonstrated higher TPO-Ab and Tg-Ab levels in hypothyroid cases than in controls, both at study entrance and at one year before the event (§8). The same was true for the hyperthyroid cases and their controls, although less pronounced for Tg-Ab. The absolute change in TPO-Ab over this time period was greater in hypothyroid cases than in their controls (\(\Delta TPO-Ab 385 [85-853] \text{ vs } <30 [<30-<30], \ p<0.000\)), but not different between hyperthyroid cases and their controls (\(\Delta TPO-Ab <30 [<30-242] \text{ vs } [<30-48], \ p=0.8\)). Like mentioned in section C.1 for TSH, the data suggest a gradual slow development of autoimmune hypothyroidism, whereas overt autoimmune hyperthyroidism develops much faster. The latter notion is further substantiated by the finding that one year before the occurrence of autoimmune hyperthyroidism causative antibodies against the TSH receptor (TBII) were not demonstrable. In line is the very low prevalence of TBII in the general population as well as in our cohort.

**D. Environmental factors**

**D.1. Smoking**

The prevalence of ever smoking was 58% and of current smoking 35% in the Amsterdam AITD cohort at study entrance. The figures for ever smoking among participants with an elevated, normal or suppressed TSH are 69%, 57% and 47%, and for current smoking 42%, 35%, and 13% respectively; differences between those with an abnormal and a normal TSH were not significant. The prevalence of current smoking (but not of ever smoking) among the euthyroid subjects with a normal TSH was lower in those with TPO-Ab than in those without TPO-Ab (25% vs 38%, \(p<0.001\)) (§3). Smoking behavior at study entrance did not contribute to the risk of developing overt hypo- or hyperthyroidism in the 5-yr follow-up of the Amsterdam AITD cohort (§7). However, smoking behavior did change in some subjects during follow-up, and in the nested case-control study there were less current smokers in the hypothyroid cases than in their controls (13% vs 28%, \(p=0.08\)) at the time of the event; this was not observed in the hyperthyroid cases and their controls (§8).

Of particular interest is the finding that current smoking appears to be protective against the development of TPO-Ab. Subsequent studies have confirmed this original observation. Data from the NHANES III population published in 2004 indicated that fewer smokers (11%, 95% CI 10-13%) had TPO-Ab and/or Tg-Ab compared to nonsmokers (18%, 95 CI 17-19%) (25).
The relationship persisted when analyzing the presence of one antibody independently of the status of the other antibody. The odds of having thyroid antibodies in serum was lower by 1.1% for every 10 ng/ml increase in serum cotinine after adjustment for confounders. A recent Danish population study published in 2008 also reports a negative association between smoking and the presence of TPO-Ab and Tg-Ab (26). Odds ratio’s were in the order of 0.5-0.6 (comparable to the OR of 0.688 in our cohort), with no difference in the risk between moderate and heavy smokers neither between never and ex-smokers.

One may thus ask the question if smoking decreases the risk on hypothyroidism. Studies in the past have shown conflicting results, and a meta-analysis in 2002 could not prove a negative association between smoking and hypothyroidism although it detected a trend (27). A Danish study from 2002 found a lower prevalence of mild hypothyroidism among smokers compared to nonsmokers (2.6% vs 5.3%) with an adjusted odds ratio of 0.47 (95% CI 0.33-0.67) (28). In the NHANES III smokers had less frequently an elevated TSH value (>4.5mU/l) compared with nonsmokers (2.6%, 95% CI 2.0-3.2% vs 5.5%, 95% CI 4.7-6.3%) (25). A recent Norwegian population study from 2007 reported a lower prevalence of overt and subclinical hypothyroidism among current smokers compared to never smokers (odds ratio’s are 0.60, 95% CI 0.38-0.95 for overt hypothyroidism and 0.54, 95% CI 0.45-0.66 for subclinical hypothyroidism, both in women) (29). The studies do not distinguish between the various causes of hypothyroidism, but most likely the majority will be due to chronic lymphocytic thyroiditis. In line with these recent studies is our nested case-control study showing a protective effect of smoking on the development of overt autoimmune hypothyroidism, although the p value of 0.08 was just not significant most likely due to a limited sample size. The available data strongly suggest that the preventive effect of smoking on hypothyroidism is – at least partly – explained by the preventive effect of smoking on the development of TPO-Ab.

In sharp contrast to the findings in hypothyroidism are the many studies showing that smoking is a risk factor for Graves’hyperthyroidism (with an odds ratio of 1.9, 95% CI 1.1-3.2 in a particular case-control study) (30). We did not observe this association in our cohort studies, most likely due to the small number of subjects with a suppressed TSH. Thus the peculiar situation arises that refraining from smoking increases the risk on Graves’ disease. Nevertheless the recommendation should be to stop smoking in view of the many other adverse health outcomes associated with smoking.

D.2. Exogenous estrogens
Exogenous estrogens in our studies comprise both oral contraceptive pills and hormone replacement therapy. The prevalence of ever estrogen use in the Amsterdam AITD cohort at baseline was lower in participants with a suppressed TSH than with a normal TSH (67% vs 81%), and this was also true for current estrogen use (13% vs 46%) (§3). No differences existed between those with an elevated and a normal TSH. Ever estrogen use (but not current estrogen use) was an independent (protective) risk factor for hyperthyroidism according to multiple logistic regression analysis (OR 0.169, 95% CI 0.055-0.523). In the subjects with a normal TSH, the prevalence of ever and current estrogen use was lower in the absence than in the presence of TPO-Ab, but only ever estrogen use was an independent (protective) risk factor for TPO-Ab (OR 0.578, 95% CI 0.345-0.967) (§3). In the 5-yr follow-up of the Amsterdam AITD cohort estrogen medication at baseline had no predictive value for the occurrence of overt hypo- or hyperthyroidism (§7). Also in the nested case-control study evidence for a modulating effect of estrogen use on the occurrence of an event was not found (§8).

Oral contraceptives are used by over 100 million women worldwide, but there are remarkably few studies on their use and the development of AITD. An early large study among 46000 women found that the incidence of hypo- and hyperthyroidism together was lower in oral contraceptive users than in controls (RR 0.68, 95% CI 0.52-0.85) (31). Although we did not find a protective effect of estrogens in the 5-yr follow-up of the Amsterdam AITD cohort (which has still a limited sample size), the prevalence of Graves’ hyperthyroidism at baseline was lower in estrogen users independent of the number of previous pregnancies. This is in agreement with the observation that the use of contraceptives had a protective effect for the development of Graves’ disease (odds ratio 0.68, 95% CI 0.49-0.93), but not for Hashimoto disease (32).

With regard to the effect of estrogens on TPO-Ab, our finding has not been confirmed in a recent population study reporting no association between thyroid antibodies and the use of oral contraceptives, although women aged 60-65 years receiving hormone replacement therapy now or previously had a lower prevalence of Tg-Ab, but not of TPO-Ab (33).

D.3. Pregnancy

The prevalence of having been pregnant in the Amsterdam AITD cohort at study entrance was higher in participants with a suppressed TSH than with a normal TSH (87% vs 51%), and pregnancies were an independent risk factor for hyperthyroidism according to multiple logistic regression analyses (odds ratio 6.88, 95% CI 1.50-30.96) (§3). The prevalence of
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pregnancies was not associated with an elevated TSH, nor was pregnancy an independent risk factor for the presence of TPO-Ab in subjects with a normal TSH at baseline (§3). A history of pregnancy at baseline did not contribute to the risk of developing overt autoimmune hypothyroidism in the 5-yr follow-up of the Amsterdam AITD cohort (§7). In the nested case-control study parity did not differ between cases and controls at study entrance (§8). However, at the time of the event there were more hypothyroid cases in the postpartum period than controls (21% vs 4%), whereas the proportion of women who had never been pregnant was lower in hyperthyroid cases than in their controls (8% vs 38%), both at one year before the event and at the times of the event.

Our data suggest that the postpartum period carries a risk for autoimmune hypothyroidism, and that never been pregnant protects against autoimmune hyperthyroidism. The year after delivery puts the woman at risk for AITD, as illustrated by the high incidence of postpartum thyroiditis (5.2% in a Dutch population study) (34). Late development (after five years or more) of permanent hypothyroidism occurs in 25% of patients with postpartum thyroiditis (35). The prevalence of postpartum Graves’ disease (both persistent and transient) is estimated at 11% of those with postpartum thyroid dysfunction and 0.54% of the general population (36). Recent large population-based studies report conflicting results on the association between parity and AITD. An Australian study found (after adjustment for age) that women who had previously been pregnant did not have a significantly increased risk of positive thyroid antibodies or a raised or reduced TSH compared with women who had never been pregnant (37). Likewise, a Danish study failed to demonstrate an association between previous pregnancy, parity and thyroid antibodies (33). In contrast, a German study did find an association between parity and AITD (38). Women with at least one pregnancy had increased odds for AITD (OR 4.6, 95% CI 1.4-15.1) compared to women who had never been pregnant. Similar results were obtained using positive TPO-Ab (OR 1.8, 95% CI 1.0-3.3) as separate dependent variable or using number of birth as alternate independent variable.

D.4. *Yersinia enterocolitica*

The prevalence of *Yersinia enterocolitica* (YE) infection in the Amsterdam AITD cohort at study entrance is higher than in controls, both for YOP IgG-Ab (40% vs 20%) and for YOP IgA-Ab (22% vs 13%) (§4). The prevalence of these antibodies did not differ between subjects with a normal or abnormal TSH, nor was it associated with the presence of TPO-Ab. YOP antibodies at study entrance did not predict the occurrence of overt thyroid dysfunction in the 5-yr follow-up of the Amsterdam AITD cohort (§7). In the nested case-control study
the proportion of subjects positive for YOP-IgG and YOP-IgA did not differ between hypothyroid or hyperthyroid cases and their respective controls, neither at baseline nor at one year before the event (§8).

Infection with YE has been shown to produce a lipoprotein that can cross-react with the TSH receptor and thus can act as a potential trigger of thyroid autoimmunity, in particular Graves’ disease (39). Our Amsterdam AITD cohort studies, both at baseline and during follow-up, did not provide any evidence that YE infection is causally related to AITD. The observed higher prevalence of YOP antibodies in our cohort relative to a control group, suggesting a higher rate of persistent YE infection in AITD relatives, could be explained by assuming that susceptibility genes for AITD may also confer a risk for YE infection.

Our results are in agreement with a more recent Danish twin study, reporting that YE infection does not confer an increased risk of thyroid antibodies (40). The same group of authors, however, concluded in their latest paper that it is too early to dismiss YE infection in the etiology of Graves’ disease (41). In a classical case-control study the prevalence of YOP-IgG and YOP-IgA in Graves’ disease was higher than in controls (51% vs 35% and 49 vs 34% respectively). Similar results were found in twin pairs discordant for Graves’ disease. Odds ratio’s were significantly increased. Clearly, the issue has not been settled yet, and longitudinal studies examining temporal relationships are required.

**D.5. Iodine excess**

Iodine excess in the Amsterdam AITD cohort was assessed according to the participant’s history of taking iodine-containing nutritional supplements, kelp tablets, seaweed, iodine containing drugs like amiodarone, or iodine-containing contrast agents. The prevalence of selfreported iodine excess over the preceding year excess was 7% at baseline, with no differences between participants with an abnormal or normal TSH, nor in the normal TSH group between those with or without TPO-Ab (§3). Iodine excess at baseline was not a predictive factor for the occurrence of an event in the 5-yr follow-up of the Amsterdam AITD cohort (§7). The occurrence of an event was also not related to exposure to iodine excess in the preceding years as evident from the nested case-control study (§8).

This is not to deny that cases of iodine-induced hypothyroidism and Jod-Basedow do occur. Their incidence in the general population is very low, and iodine excess accounts for only a small proportion of all hypothyroid and hyperthyroid patients. Amiodarone carries a rather high risk for induction of thyroid dysfunction, but none of the cohort participants used this drug.
D.6. Stress

Stress exposure in the Amsterdam AITD cohort was assessed by three validated questionnaires: the Recently Experienced Stressful Life Events (counting the number and impact of major life events experienced in the past 12 months), the Dutch Everyday Problem Checklist (counting the number and intensity of daily hassles experienced in the last 2 months), and the positive and negative affect schedule (the PANAS, measuring the current mood in terms of positive and negative effect). Among the euthyroid subjects with a normal TSH of the Amsterdam AITD cohort at study entrance, stress scores were not different between those with or without TPO-Ab (§5). Stress exposure in the 5-yr follow-up of the Amsterdam AITD cohort was not associated with the occurrence of autoimmune hypo- or hyperthyroidism as evident from the nested case-control study (§8).

We rejected our hypothesis that stress might be associated with the presence of TPO-Ab, an early marker of AITD. Likewise we did not find evidence for a higher stress exposure preceding the onset of overt autoimmune hypothyroidism. There are only two other studies on a possible association between stress and Hashimoto disease. A cross-sectional French study concluded that major stressors did not have any triggering role in Hashimoto disease (42). An English study in pregnant women did not report an excess of life events in women who developed postpartum thyroid dysfunction, and there was no difference in the number of life events between antibody positive and antibody negative women (43). Stress was evaluated just once in both studies: when the disease was clearly diagnosed (42) and at 8 weeks postpartum (43). In this respect our longitudinal study is more reliable, although equally negative in its conclusions.

The absence of an effect of stress exposure on the occurrence of autoimmune hyperthyroidism in our nested case-control study is most likely due to the small number (n=13) of hyperthyroid cases. Indeed, most of the recent case-control studies have supported stress as a factor that affects the onset and clinical course of Graves’ disease (44). However, these studies have been retrospective in nature and liable to recall bias.

There is another interesting issue related to the PANAS scores in our cohort, although highly speculative. The PANAS scores of cohort participants at study entrance are higher than those observed in a non-clinical sample broadly representative of the general adult female UK population (n=537, mean age 42.9 ± 15.7 yr) (45): median negative affect scores in our study vs the UK study are 21 and 15 respectively, and median positive affect scores are 39 and 31 respectively. In most clinical contexts, the concern will be whether negative affect scores (reference range 10-42) are unusually high, and whether positive affect scores (range 10-50)
are unusually low. A high positive affect score points to neuroticism, a low negative affect score is typical for a depressed affect. The high positive and negative affect scores in our AITD cohort can be interpreted as a reactive affect balance style (46).

E. Immunopathogenesis

The Amsterdam AITD cohort study was primarily designed to assess which environmental factors contribute to the development of AITD, not to investigate the mechanism by which identified environmental factors modulate thyroid autoimmunity. Nevertheless, the cohort allowed doing some studies providing further insight in the immunopathogenesis of AITD as described in §6.

Key element in the immune response is the presentation of antigen (bound to MHC class II molecules on antigen-presenting cells) to T cell receptors on CD4+ T-cells. Formation of the complex is followed by activation of the CD4+ T-cell, a process that involves expression of interleukin-2 (IL-2) receptor and autocrine stimulation by IL-2 release and leads to T-cell proliferation, secretion of other cytokines, and thus the development of effector function. Activated CD4+ T-cells can, depending on their pattern of cytokine secretion, develop into type 1 helper (Th1) cells (leading to a destructive process as seen in organ-specific endocrinopathies) or type 2 helper (Th2) cells promoting antibody production. Other T-cell subsets exist. In the past suppressor functions have been assigned to the typically cytotoxic CD8+ T-cells, but the most promising regulatory T-cell subset (specifically with regard to autoimmune diseases) is CD4+CD25+ (IL-2 receptor). A defect in CD4+CD25+ T regulatory cells breaks the immunological tolerance (47). A mutation in the FOXP3 gene encoding for a transcription factor expressed on CD4+CD25+ T-cells, is associated with a decreased number of T regulatory cells (48,49).

Against this background we investigated whether activated CD4+ T-cells could be an early marker of AITD. To this end we investigated participants of the Amsterdam AITD cohort who were euthyroid at study entrance, contrasting those with and without TPO-Ab (§6). We observed a reduced expression of CD25 on CD4+ T-cells and lower levels of soluble IL-2 receptors in serum in our female relatives of AITD patients as compared to controls; there were no differences between TPO-Ab positive and negative women. Our study suggests that women at risk for AITD exhibit various signs of a reduced T-cell activation, in particular signs of a reduced expansion of the T-cell pool, also already in those without thyroid antibodies.
In this study we made some further observations on the effect of smoking and estrogen use on the immune response. Smoking activates the IL-2/IL-2R system, and smokers in our study (in contrast to nonsmokers) had near normal sIL-2R serum levels and a slightly higher percentage of CD4+CD25+ T-cells. Hence smoking may have corrected the reduced T-cell functions in our AITD relatives, in line with the protective effect of smoking on the presence of TPO-Ab (see D.1). Estrogens suppress IL-2 and the IL-2R, and are immunosuppressive in many autoimmune diseases (see D.2). In our AITD relatives current estrogen use was associated with lower percentages of CD4+CD25+ T-cells compared to nonusers, but this was not reflected by extra reduced sIL-2R levels. These findings are in contrast with a study reporting that estrogens drive expansion of the CD4+CD25+ regulatory T-cell compartment (50). The weak point of our study, however, is the small size of subgroups.

F. Clinical applicability

The clinical applicability of our findings in the Amsterdam AITD cohort is described in §7. An accurate simple predictive score (called the Thyroid Events Amsterdam or THEA score) was developed to estimate the 5-yr risk of overt autoimmune hypothyroidism or hyperthyroidism in female relatives of AITD patients. The numerical THEA score predicts events by weighting the three independent risk factors: TSH, TPO-Ab, and family background. The higher the THEA score, the higher the risk on developing overt thyroid dysfunction within 5 years.

The THEA score can be useful in day-to-day clinical practice. For, many patients with AITD ask their treating physician about the risk that their daughters or sisters will also get this disease. Assessment of the THEA score in family members allows answering this question in an evidence-based quantitative manner. At low THEA scores (0-7) subjects can be reassured of a very high likelihood of maintaining a normal thyroid function in the next 5 years. If THEA scores are higher, reassessment after 1 yr might be warranted. The THEA score might be very useful for young women of AITD families who want to become pregnant in the next few years. Screening of thyroid function and thyroid antibodies seems to be indicated in these women in view of adverse pregnancy outcomes for mother and child when thyroid function is abnormal (51), but also when thyroid function is normal but TPO-Ab are present (52). Thyroxine treatment should be considered under these circumstances. The high abortion rate among euthyroid pregnant women with TPO-Ab is reduced to the abortion rate in TPO-Ab...
negative women by levothyroxine treatment, as reported in a recent randomized clinical trial (53).

It is unknown whether the THEA-score is also applicable in male AITD relatives. Very likely this will be the case, although the weights attached to TSH and TPO-Ab concentrations in the THEA score might be different in view of their higher odds for developing hypothyroidism in males than in females in the 20-yr follow-up of the Whickham study (9): in males, the odds ratio’s are 44 (95% CI 19-104) for raised serum TSH alone, 25 (95% CI 10-63) for positive thyroid antibodies alone, and 173 (95% CI 81-370) for both raised serum TSH and positive thyroid antibodies (for comparison with the odds in females, see section C.2). However, the overall risk to develop AITD is much lower in males. Therefore, one might speculate that at a given THEA-score the risk to develop overt hypothyroidism in the next 5 years will be greater in men than in women.

**G. Future research**

The Amsterdam AITD cohort provides many opportunities for future research studies. First, many more cases of overt hypo- and hyperthyroidism will occur among the participants who were still clinically euthyroid at the end of the study. An extended follow-up would be very valuable. Second, better characterization of T-cell subsets (especially of regulatory T-cells) might provide deeper insight in the immunopathogenesis of AITD at its early stages. Third, assessment of polymorphisms in susceptibility genes for AITD and incorporating these genetic risk factors in the THEA-score, may enhance the predictive value of the THEA-score. Fourth, the cohort allows studying gene-environment interactions. Such studies have scarcely been done in the field of thyroid autoimmunity. A notable exception is a recent study on genetic polymorphisms associated with cigarette smoking and the risk of Graves’ disease (54). Last but not least, the cohort opens up the possibility to do preventive intervention studies. Prevention of disease is better than treatment of disease, but has so far hardly been given any attention in AITD. The ideal population for preventive intervention would be subjects at risk for AITD but in whom thyroid function is still normal. The Amsterdam AITD cohort has identified such subjects: female relatives of AITD patients, in whom TSH is normal but TPO-Ab are already present. The question then is, which kind of preventive measure should be tested in a prospective controlled trial apart from advice promoting healthy lifestyles. Intervention with selenium in these early stages of thyroid autoimmunity might be a very attractive option. Selenium plays a role in the immune system, and acts as an anti-
oxidant (55). Selenium deficiency results in increased H2O2 levels in the thyroid gland, presumably increasing the activity and immunogenecity of TPO. Indeed in subjects on thyroxine replacement TPO-Ab concentrations fall upon selenium supplementation, as shown in several randomized clinical trials (56, 57, 58). Selenium supplementation has also a favourable effect on postpartum thyroid status in pregnant women with thyroid antibodies (59). It remains to be seen, however, if selenium supplementation in euthyroid subjects with TPO-Ab can prevent the development towards subclinical and overt autoimmune hypothyroidism.
References