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Serological Evidence of Thyroid Autoimmunity Among Schoolchildren in Two Different Socio-Economic Environments

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Structured Abstract

Context: The mechanisms leading to thyroid autoimmunity are largely unknown.

Objective: To assess the role of environment in the development of thyroid autoimmunity.

Design: Prevalence of thyroid autoantibodies in two neighboring populations living in completely different socio-economic circumstances (Russian Karelia and Finland).

Setting: Two population-based cohorts sharing partly the same ancestry.

Patients or Other Participants: 532 schoolchildren from Russian Karelia and 532 schoolchildren in Finland matched for the age, gender and season of the blood sampling.

Interventions: None.

Main Outcome Measures: Prevalence of thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb) and HLA-DQ alleles.

Results: The prevalence of TPOAb was significantly lower in Russian Karelian than in Finnish children (0.4 % vs. 2.6 %, $P=0.006$). A similar difference was observed for TGAb (0.6 % vs. 3.4 %, $P=0.002$). Finnish girls tested more frequently positive for both TPOAb (4.3 % vs. 0.4 %, $P=0.01$) and TGAb (5.3 % vs. 0.9 %, $P=0.01$) than boys. Seven of the 23 tested subjects with signs of thyroid autoimmunity (30%) had increased serum TSH concentrations as a sign of subclinical hypothyroidism. The frequency of HLA genotypes did not differ between the two countries or between autoantibody-positive and negative subjects.

Conclusions: The prevalence of thyroid autoimmunity is lower in Russian Karelia than in Finland. This difference was not related to ethnic background or HLA-DQ alleles. The results support the idea that the Russian Karelian environment which is characterized by inferior prosperity and standard of hygiene may provide protection against thyroid autoimmunity.

Introduction

Epidemiological and clinical data indicate that autoimmune thyroid disease (AITD) is quite common, affecting about 2 % of females and 0.2% of males in most populations characterized by a Western life style (1-5). AITD is characterized by infiltration of T lymphocytes in the thyroid gland, proliferative responses of T cells against thyroid autoantigens and production of autoantibodies against human thyroglobulin (TG), thyroid peroxidase (TPO) and thyroid-stimulating hormone receptor (TSHR) (6, 7). Thyroid autoantibodies are widely used in the diagnosis of AITD, and they often precede the development of clinical disease (8-10).

The pathogenesis of AITD is a multi-step process leading to the breakdown of the immune tolerance to thyroid autoantigens and accumulation of autoreactive T lymphocytes in the thyroid gland (11, 12). Both the Th1 and Th2 type helper T cells seem to be involved in all types of AITD (6,13). Usually the process is slow, and characterized by gradual spreading and diversification of the autoimmune response.

In spite of the quite well-characterized autoimmune component in AITD, relatively little is known about the mechanisms underlying the breakdown of tolerance leading to the autoimmune process. Like other organ-specific diseases AITD seem to have a multifactorial etiology where interactions between the effects of multiple genes and environmental factors are important, and the right combination of genetic, environmental and endogenous factors is required for the initiation of the disease process (14). It appears that multiple environmental risk factors may be involved including low birth weight, fetal microchimerism, viral and bacterial infections, iodine intake, selenium deficiency, radiation and stress (15-18). In addition, female sex and other genetic factors, such as the major histocompatibility complex (MHC) region, the gene encoding the cytotoxic T-lymphocyte antigen-4 (CTLA4) on the long arm of chromosome 2, and a locus on the long arm of chromosome 5, have been shown to contribute to the pathogenesis of AITD (19-23).

The genetic disease susceptibility maps particularly to the HLA-DRB1 locus (24). The CTLA-4 locus has been confirmed as an additional susceptibility marker associated with increased risk of Grave's disease and autoimmune hypothyroidism (25, 26). A variety of studies have also reported associations between polymorphisms of genes encoding the TSHR, TPO, TG and risk of AITD (27-29).

Additional chromosomal locations, such as 14q31, 18q21, 20q11, 6p, 13q32, 12q22 have also been implicated as potential susceptibility factors for AITD (21, 30).

The role of non-genetic factors is supported by the fact that the concordance rate for AITD among monozygotic twins is in the range of 35-55 % compared with 3% in dizygotic twins (31, 32). The causative role of certain infections has been implicated, and AITD has been linked to a series of infectious agents (e.g. *Yersinia enterocolitica*, retroviruses). Infectious agents may induce autoimmunity by different mechanisms, such as molecular mimicry, polyclonal T-cell activation and enhanced expression of MHC molecules on the thyroid epithelial cells (16). Interferon-gamma is an example of such cytokines which can induce MHC class II expression on thyroid cells during microbial infections (33). In addition, TPO antibodies (TPOAb) are frequent in individuals who have experienced congenital rubella infection *in utero* (34).

The aim of the present study is to assess the frequency of signs of thyroid autoimmunity in the background population in two adjacent regions which differ conspicuously in their socio-economic circumstances, i.e. Russian Karelia and Finland. Due to population mixing during earlier centuries these populations share partly the same kind of ancestry, which makes this epidemiological setting optimal for the detection of possible gene-environment interactions underlying the development of thyroid autoimmunity. We have previously observed that the frequency of microbial infections differs markedly between these populations (35, 36), while the frequency of type 1 diabetes, celiac disease and allergy show an opposite trend (36-38). This led us to generate the hypothesis that the environment in Russian Karelia includes factors which may protect against several immune-mediated diseases, and that this protection might be linked to a strong microbial exposure.

Subjects and Methods

Subjects

A total of 1064 randomly selected schoolchildren (532 schoolchildren from Russian Karelia and 532 from Finland) were included in the present study. These children were recruited in schools as a part of a larger cohort of children as described in detail elsewhere (39). Children from both countries were matched pair-wise for age, gender and season of the blood sampling. Mean age was

11.4 (± 2.0 ; SD) years (range 7.1-15.0) and 57% ($n=304$) were girls in both countries. The children from Russian Karelia were enriched for Finnish/Karelian ancestry. In Russian Karelia the ethnic background of the child was recorded according to the ancestry of both the mother and father and was classified as follows: 302 (56.8%) children with both parents having Finnish or Karelian background, 57 (10.7%) children with a Russian background and 173 (32.5%) with a mixed or other ethnic background. In Finland all children were of Finnish ancestry. Serum samples and EDTA whole blood samples were taken from all children and stored at -20°C until analyzed.

The children and their parents gave informed consent. The study was approved by the local Ethical Committee of the Faculty of Medicine, University of Oulu, Finland and by Ministry of Health in the Karelian Republic of Russia.

Laboratory analyses

IgG class serum TPOAb and TG antibodies (TGAb) were measured using UniCAP assay (Thyroglobulin ImmunoCAP and Thyroid Peroxidase ImmunoCAP, Phadia, Freiburg, Germany) according to the manufacturer's instructions in the Centre of Laboratory Medicine, Tampere University Hospital, Tampere, Finland. The cut-off limit was 60 IU/ml for TPOAb positivity and 220 IU/ml for TGAb positivity. Thyrotropin (TSH) and free thyroxine (FT4) levels were analyzed using a chemiluminescence assay (Abbott, Architect TSH and free T4, Abbot Park, IL). The reference range for TSH in healthy children was 0.35-5.0 mU/l and for FT4 9.0-19.0 pmol/l. HLA class II alleles were typed using polymerase chain reaction and microtiter plate-based hybridization with lanthanide-labeled oligonucleotide probes as previously described (40). Samples that were positive for the HLA-DQB1*02 allele were further analyzed for the presence of the associated HLA alleles DQA1*0201 and DQA1*05 to define the HLA DR3-DQ2 haplotype. EDTA samples were available for genotyping from all Finnish schoolchildren and from 99.6% (530) of the schoolchildren from Russian Karelia. All HLA-analyses were performed in the Immunogenetics Laboratory, University of Turku, Turku, Finland.

Statistical analyses

χ^2 test and Fisher's exact test were used to compare the frequency of autoantibodies.

For the comparison of paired data, McNemar's test was used. Autoantibody levels were compared between the groups with the Mann-Whitney U-test. P values of less than 0.05 were considered significant. The SPSS statistical package (version 12.1, SPSS Inc., Chicago, IL) was used for the data analyses.

Results

TPOAb were detected in 14 [2.6%; 95% confidence interval (CI) 1.5-4.4%] of the 532 Finnish children compared to two (0.4%; CI 0.05-1.4%) of the 532 Karelian children ($P=0.006$, Fig. 1). A similar difference was observed in the frequency of TGAb between the Finnish and Karelian children [3.4 % (CI 2.0-5.3%) vs. 0.6% (CI 0.1-1.6%); $P=0.002$, Fig. 1). There were no significant differences in the autoantibody levels between autoantibody-positive Finnish children and Karelian children; median TPOAb levels were 225.5 IU/ml in Finnish children and 148 IU/ml in Russian Karelian children ($P=0.63$) and TGAb levels 545.5 IU/ml vs. 359 IU/ml ($P=0.55$), respectively. Serum TSH and FT4 concentrations were measured from 24 (92.3%) children who were positive for either TPOAb or TGAb. Seven (29.2%) of these children had elevated TSH as a marker of subclinical hypothyroidism and they were all Finnish children. None of the children had abnormal FT4 concentrations (Table 1).

Thirteen Finnish girls (4.3%, CI 2.5-7.6%) and only one boy (0.4%; CI 0.01-2.4%; $P=0.01$) tested positive for TPOAb. A similar gender difference was seen in terms of positivity for TGAb (Finnish girls 5.3%, CI 3.0-8.4% vs. boys 0.9%, CI 0.1-3.1%; $P=0.01$). In Russian Karelia all three autoantibody-positive subjects were girls; two girls (0.6%, CI 0.08-2.4%) tested positive for TPOAb and three (1.0%, CI 0.2-2.9%) for TGAb. Accordingly altogether 88.5 % of those children who were either TPOAb or TGAb positive in these two populations were girls ($P<0.001$). In addition, the three boys who tested positive for thyroid autoantibodies had very low antibody levels (Table 1). Autoantibody-positive children were slightly older than autoantibody-negative ones, the mean age of autoantibody-positive subjects being 12.7 years, while the mean age of autoantibody-negative children was 11.4 years ($P=0.003$).

Among the 26 autoantibody-positive children two Finnish girls had clinical hypothyroidism (Table 1, cases 11 and 13). One of them (case 13) had been diagnosed almost 8 years before the sampling at the age of 5.2

years, while the other (case 11) developed hypothyroidism one month after the sampling at the age of 12.2 years.

There was no significant difference in the distribution of HLA genotypes between the two countries, when the HLA genotypes were categorized into three groups [DR3-DQ2/x (x=any allele), DR4-DQ8/y (y=any allele) and other genotypes (Table 2). There was no clear-cut association between HLA genotypes and the frequency of autoantibodies. The proportion of autoantibody-positive children was higher in Finland when compared to Russian Karelia especially among those with the genotype including DR4-DQ8 [6.1% (CI 2.6-11.6%) vs. 0.0% (CI 0.0-2.6%); $P=0.007$]. One of the autoantibody-positive children in Russian Karelia was of Russian ancestry, one had Finnish/Karelian ancestry and one had other ethnic background. Thus, the prevalence of autoantibodies in these ethnic groups was 1.7%, 0.3% and 0.6%, respectively.

Discussion

Thyroid antibodies are reliable markers of thyroid autoimmunity and surrogates of subsequent thyroid dysfunction (7, 9). However, there are no previous studies evaluating the prevalence of thyroid autoantibodies in children in a large cohort representing the background population. The study confirms that these antibodies are less frequent in this age group compared to older subjects. The autoimmune process detected by the presence of these autoantibodies was in most cases in its early subclinical phase and had not progressed close to the presentation of clinical disease, as only seven (29.2%) of the 24 autoantibody-positive children had increased TSH levels and two had been diagnosed with hypothyreosis.

The effect of gender on thyroid autoimmunity was obvious already in these young children. Thyroid antibodies were almost eight times more frequent in girls compared to boys. In addition, the few antibody-positive boys had very low antibody levels. This suggests that gender has a strong influence on thyroid autoimmunity not only in the clinical phase of AITD but also in the early subclinical phase. The fact that the gender bias is obvious already in such young children (mean age 11 years) indicates that factors other than the hormonal changes during puberty may contribute to this phenomenon. However, most of the autoantibody-positive children were older than 11 years and therefore the effect of pubertal hormonal changes can not be excluded.

Thyroid autoantibodies were more than five times more frequent in Finland than in Russian Karelia. Such a marked difference clearly indicates that the predisposition to thyroid autoimmunity differs in these populations. Genetic factors could be one possible explanation. In previous studies it has been shown that AITD is associated with the HLA-DR3 and HLA-DR4 alleles (40-44), and it may be possible that the frequency of these alleles should differ in the two populations. However, this was not the case because we observed that the frequency of susceptible HLA alleles was quite similar in Russian Karelia and Finland. This is also supported by the fact that thyroid antibodies showed no clear association with HLA alleles. Thus, the risk effect of HLA, which has been described previously in Grave's disease or Hashimoto's thyroiditis, seems to be related to the progression of the autoimmune process to clinical disease rather than to the early stage of thyroid autoimmunity *per se*. Thyroid autoimmunity was also rare in Russian Karelian children who had Finnish ancestry further supporting the contribution of non-genetic (environmental) factors.

Although these populations live close to each other and have partly the same ancestry, their socioeconomic environment differs markedly. This is due to the inferior hygienic circumstances and lower economic status in Russian Karelia (in 2004 the gross national product in Russian Karelia was USD 3,410 per person compared to 32,790 in Finland). This is clearly reflected by a conspicuously higher frequency of a variety of microbial infections in Russian Karelia. We have previously reported that 73% of these schoolchildren were seropositive for *Helicobacter pylori* in Russian Karelia compared to 5% in Finland (36). A same kind of difference was observed in hepatitis A virus, *Toxoplasma gondii* and enteroviruses. Accordingly, the lower risk of thyroid autoimmunity in Russian Karelia could well be explained by the hygiene hypothesis. Frequent microbial exposure in Russian Karelia could protect the children from thyroid autoimmunity by stimulating the regulatory elements of the immune system. If this is the case then one would expect that also other autoimmune diseases and allergies should be less frequent in Russian Karelia than in Finland. In fact, this is exactly what we have observed in our previous studies indicating that type 1 diabetes, celiac disease and IgE-mediated allergic sensitization are all considerably less common in Russian Karelia (36-38). Genetic risk for type 1 diabetes

and celiac disease, defined according to susceptible and protective HLA-allele combinations, was quite similar in both populations. Our results support the hypothesis that lack of microbial exposures may play a role in the pathogenesis of a wide range of immune-mediated diseases (45, 46). Accordingly, this influence could be mediated by a general effect of microbes on immune regulation rather than by microbe-specific mechanisms.

In conclusion, the increased risk for thyroid autoimmunity in Finland could indicate that there are some driving environmental factors or a lack of protective elements which are present in Russian Karelia. These factors are probably related to the different socioeconomic environment and microbial exposure in these countries. The fact that a similar gradient exists also in other immune-mediated diseases implies that this effect is mediated by general immunoregulatory pathways. Altogether, the findings fit well with the hygiene hypothesis, and further studies are needed to identify microbe-host interactions which could mediate protection against thyroid autoimmunity. Such interactions may be common for several immune-mediated diseases.

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Legend to Figure 1.

Frequency of TPOAb and TGAb in schoolchildren in Russian Karelia (black bars) and in Finland (white bars). The error bars represent the 95% confidence intervals.

TABLE 1. Characteristics of the 23 Finnish children and three Russian Karelian children who tested positive for thyroid autoantibodies. Elevated values given in **bold**. ND=not done.**Finland**

Number	Age, years	Sex	TPOAb, IU/ml	TGAb, IU/ml	TSH, mU/l	FT4, pmol/l	HLA-type		Clinical symptoms
		M-male F-female					DQB1	DQA1	
1	11.7	M	<26	270	4.2	17.1	0602		No
2	13.6	M	<26	238	4.4	13.2	X		No
3	14.6	M	118	<220	2.4	15.2	X		No
4	7.8	F	104	6327	2.9	15.5	0201	05,0201	No
5	8.6	F	256	3417	6.6	13.7	02,0302	03,05	No
6	9.8	F	425	2287	14.1	13.0	0301,0302		No
7	11.0	F	67.5	582	3.8	14.9	0201,0302	01,05	No
8	11.4	F	560	482	6.6	12.2	0301		No
9	11.5	F	<26	444	8.0	13.3	X		No
10	11.7	F	311	<220	3.6	14.8	0301,0603		No
11	12.1	F	195	<220	24.5	9.0	0302		Hypothyreosis diagnosed 1 month after the sampling

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12	12.5	F	163	<220	5.1	14.9	0302		No
13	12.9	F	402	586	3.7	12.6	02	05	Hypothyreosis diagnosed at the age of 5.2 years
14	13.2	F	1338	295	2.9	13.4	0301,0603		No
15	13.3	F	83.1	<220	6.7	9.7	0302		No
16	13.6	F	<26	482	1.3	12.4	0301,0603		No
17	14.0	F	121	458	1.6	14.2	0302		No
18	14.0	F	411	2269	4.1	10.9	0301,0603		No
19	14.1	F	39.3	588	3.7	14.9	0301		No
20	14.4	F	<26	299	ND	13.9	0301		No
21	14.5	F	<26	3191	3.8	16.4	02	03,0201	No
22	14.6	F	<26	509	2.0	13.4	X		No
23	14.7	F	35.2	373	1.5	12.7	0302		No

Russian Karelia

MS # 07-1644

Number	Age, years	Sex M-male F- female	TPOab, IU/ml	TGab, mU/ml	TSH, mU/l	FT4, pmol/l	HLA-type		Clinical symptoms
							DQB1	DQA1	
1	11.3	F	172	986	3.2	14.6	02,0602	05	No
2	12.7	F	35.4	359	0.8	11.9	02,0301	05	No
3	14.0	F	124	326	ND	12.0	0301		No

TABLE 2. Proportion of children with thyroid autoantibodies in three HLA genotype groups in Finland (n=532) and Russian Karelia (n=530).

	Finland		Russian Kareliaa		
HLA genotype	Number (%) of carries	Number (%) of antibody-positive children	Number (%) of carries	Number (%) of antibody-positive children	P value^a
DR3-DQ2/X (DQA1*05-DQB1*02)	87 (16.4%) ^b	4 (4.6%) ^c	80 (15.1%) ^b	2 (2.6%)	0.381
DR4-DQ8/Y (DQA1*03-DQB1*0302)	132 (24.8%) ^b	8 (6.1%) ^c	112 (21.1%) ^b	0 (0%)	0.007
Other genotypes	326 (61.3%)	13 (4.0%)	343 (64.7%)	1 (0.3%)	0.0006

^aDifferences between countries in the proportion of children with thyroid autoantibodies in the various genotype groups.

^bThirteen Finnish children and five Karelian subjects were heterozygotes (DR3-DQ2/ DR4-DQ8) and they are included in both groups

^cTwo autoantibody-positive Finnish children were heterozygotes (DR3-DQ2/ DR4-DQ8) and they are included in both groups

