

# A Genome-Wide Scan for Autoimmune Thyroiditis in the Old Order Amish: Replication of Genetic Linkage on Chromosome 5q11.2-q14.3

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**Autoimmune thyroiditis (AITD) is a common disorder characterized by circulating antibodies to epitopes of thyroid tissue and hypothyroidism (Hashimoto's thyroiditis or AITD-hypothyroidism), although many subjects with AITD are euthyroid. Current evidence suggests that AITD is familial and polygenic. We studied AITD in a homogeneous founder Caucasian population, the Old Order Amish of Lancaster County, Pennsylvania. We found autoimmune thyroiditis, defined by the presence of circulating antimicrosomal antibodies, to be relatively common in the Amish, with a prevalence of 22.7%. The prevalence of AITD-hypothyroidism was 9.2%. We performed a genome-wide linkage analysis with 373 short**

**tandem repeat markers in 445 subjects from 29 families. We observed suggestive evidence of linkage of AITD to a locus on chromosome 5q11.2-q14.3 (LOD, 2.30;  $P = 0.0006$  at 94 cM; closest marker, D5S428), a region that was previously reported to be linked to AITD-hypothyroidism in a Japanese study. AITD-hypothyroidism showed a more modest linkage peak to the same region (LOD, 1.46;  $P = 0.005$ ). Possible linkage (nominal  $P < 0.01$ ) to autoimmune thyroiditis and/or AITD-hypothyroidism was also detected in five other regions. We conclude that a gene on chromosome 5q11.2-q14.3 is likely to contribute to susceptibility to AITD in the Amish. (*J Clin Endocrinol Metab* 88: 1292–1296, 2003)**

**A**UTOIMMUNE THYROIDITIS (AITD) is a common disorder characterized by the presence of autoreactive T lymphocytes, thyroid-specific antibodies, and cell-mediated immunity directed against a variety of antigens, including thyroid peroxidase (TPO), thyroglobulin, thyroid hormone, colloid, and sodium-iodide symporter (1, 2). Phenotypically, patients with AITD may have hypothyroidism [Hashimoto's thyroiditis (HT) or AITD-hypothyroidism] or normal thyroid function. Like many other autoimmune disorders, AITD has a strong female predominance, clusters in families, and frequently occurs in conjunction with other autoimmune disorders with which it may share common susceptibility genes (3–6). Several small studies have shown a high concordance for AITD-hypothyroidism in monozygotic twins compared with dizygotic twins (7). Relatives of patients with AITD have increased prevalence of antithyroid antibodies (5, 8–10). In addition, epitope recognition by antithyroid peroxidase antibodies may be familial (11). Evidence suggests that susceptibility to typical AITD is likely to be influenced by the combined effects of several genes and their interactions with environmental provocations (2).

The cytotoxic T cell lymphocyte antigen 4 (chromosome 2q33) and major histocompatibility complex class 2 (chromosome 6p21) loci have been linked to AITD-hypothyroidism. However, not all studies concur (12, 13). Tomer *et al.* (14)

performed a genome-wide linkage analysis in multiplex families from the United States, Italy, and the United Kingdom. Autoimmune thyroid disease was linked with regions on chromosomes 6 (AITD-1, 80 cM; MLS, 2.9), 12 (HT-1, 96 cM; MLS, 2.1) and 13 (HT-2, 97 cM; MLS, 3.8). In an analysis of Japanese affected sibling pairs, evidence for linkage to AITD-hypothyroidism was observed on chromosome 5 (MLS, 3.14; closest marker, D5S436) and chromosome 8 (MLS, 3.77; closest marker, D8S272) (15). Recently, Villanueva *et al.* (16) reported a genome scan in a multiplex Chinese-American family that provided evidence for linkage of AITD to regions on chromosomes 11 and 9.

The Old Order Amish of Lancaster County, Pennsylvania, are a genetically homogeneous founder population with large sibships and a relatively homogeneous lifestyle. Consequently, the Amish are excellent subjects for genetic studies (17). The present study was carried out to examine the epidemiology of AITD in the Amish and to perform genome-wide linkage analysis to identify chromosome regions that may harbor AITD susceptibility genes. Our findings show that AITD is relatively common in the Amish. Furthermore, we provide independent confirmation of a locus linked to AITD on chromosome 5q11.2-q14.3 as well as modest evidence for linkage to five other chromosomal regions.

## Subjects and Methods

### Subjects

The Old Order Amish emigrated to the United States from Europe in the early 18th century. Approximately 30,000 descendants from some

Abbreviations: AFDS, Amish Family Diabetes Study; AITD, autoimmune thyroiditis; AMA, antimicrosomal antibodies; HT, Hashimoto's thyroiditis; MLS, maximum LOD score; TPO, thyroid peroxidase.

200 founding families are currently living in Lancaster County, Pennsylvania, and surrounding counties. Most Amish can trace their lineage back to a few common ancestors 12–14 generations ago. The Amish are a rural-living population and are characterized by their strict religious beliefs, eschewal of technological innovation, and strong interest in their ancestry and genealogical relationships. Moreover, there is considerable homogeneity in the Amish lifestyle (17).

Recruitment for the Amish Family Diabetes Study (AFDS) began in 1995 with the goal of identifying susceptibility genes for type 2 diabetes and related traits (17). Subjects were identified by door to door interviews and word of mouth. Proband was defined as individuals with previously diagnosed type 2 diabetes (subsequently confirmed by blood testing). All first and second degree family members over the age of 18 yr were recruited around diabetic probands. If another diabetic subject was identified in the family, the family was expanded to include the individual's first and second degree relatives. Based on this ascertainment scheme, a total of 953 individuals were recruited. Through the use of the Amish Geneologic Database (18, 19), these families can be combined into a single 11-generation pedigree. The study protocol was approved by the institutional review board at University of Maryland School of Medicine, and informed consent was obtained from each study participant.

### Phenotypes

Phenotypic characterization relevant to AITD included self-reported history of thyroid disease and thyroid hormone replacement therapy. Serum levels of TSH (TSH immunoradiometric assay, Nichols Institute Diagnostics, San Juan Capistrano, CA) and antimicrosomal antibodies (AMA; Kronus, Boise, ID) were determined in the first 445 AFDS participants. Anti-TPO (Quest Diagnostics, Inc., Baltimore, MD) were measured in a subset of 243 samples that were also assayed for AMA; the concordance rate between these 2 markers of thyroid autoimmunity was 95%. Of the subjects who had both AMA and anti-TPO antibodies measured, the sensitivity of AMA to detect hypothyroidism was 82.4%, whereas the sensitivity of anti-TPO to detect hypothyroidism was 88.2%.

For the purposes of this study we used two case definitions. The more inclusive case definition was AITD, and this was defined as having serum AMA levels of 1 IU/ml or greater regardless of thyroid function. This case definition defines all individuals with evidence of thyroid autoimmunity, although an indeterminate number of these affected individuals will never develop hypothyroidism. The more restrictive case definition was AITD with hypothyroidism (AITD-hypothyroidism) and was defined as having a serum AMA level of 1 IU/ml or greater and a serum TSH level of 5 mU/liter or greater. This case definition resulted in a smaller number of individuals, all clinically affected, but excluded individuals at increased risk for the future development of hypothyroidism. Due to the small number of patients with confirmed hyperthyroidism ( $n = 5$ ), these subjects were excluded from further analysis.

As high iodine intake is believed to be a risk factor for AITD (20, 21), urinary iodine excretion, a marker for dietary iodine intake, was measured by Dr. Lewis Braverman in 100 randomly selected AFDS participants. The median urinary iodine excretion was 14.4  $\mu\text{g}/\text{dl}$  (range, 1.5–261.8  $\mu\text{g}/\text{dl}$ ), a level similar to that observed in the general U.S. population (22). The frequency of cigarette smoking, another potential risk factor for AITD, is relatively low in the Amish, with fewer than 3% of all Amish reporting that they currently smoke on a regular basis (17).

### Genotype analysis

DNA was extracted from leukocytes and a screening set of 373 highly polymorphic microsatellite short tandem repeat markers was genotyped from the ABI Prism Linkage Mapping Set (PE Applied Biosystems/PerkinElmer, Foster City, CA) on 22 autosomes and the X chromosome. The mean marker heterozygosity was 0.75 (range, 0.33–0.91). The average intermarker interval was 9.7 cM. The largest gap between markers was 25.4 cM, occurring on chromosome 7. The average genotyping error rate based on blind replicates was 0.16%.

### Statistical analysis

Although all 445 subjects can be related by tracing their ancestors back multiple generations, we divided the sample into 29 discrete families to

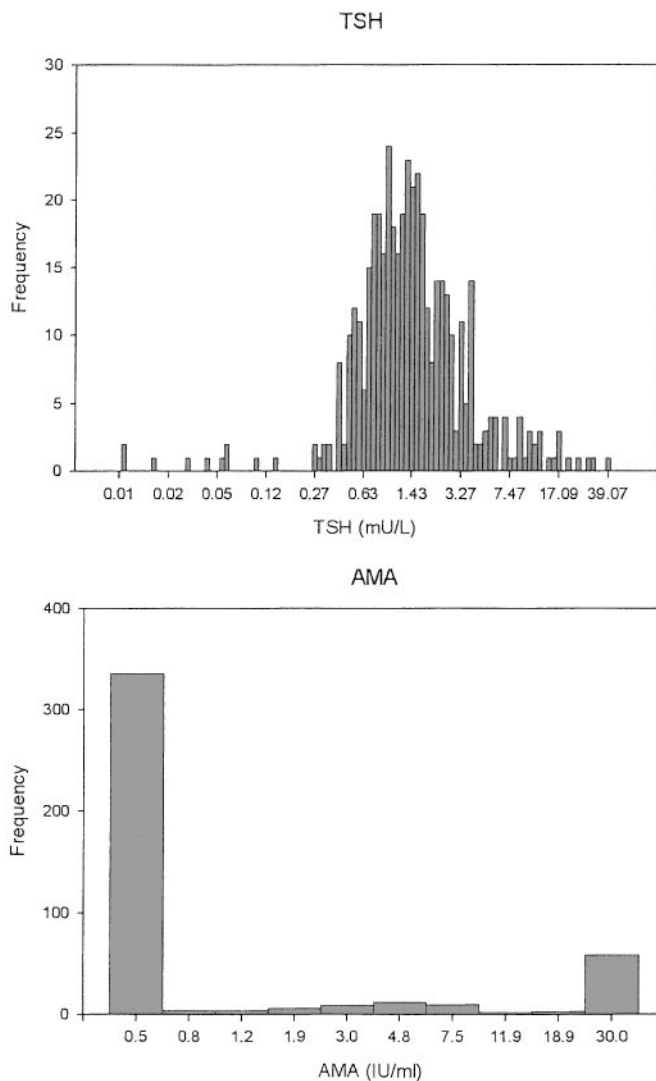


FIG. 1. Distribution of serum TSH and AMA levels in the Old Order Amish.

reduce computational burden. These 29 families ranged in size from 2–57 subjects and provided a large number of relative pairs, including 352 parent-offspring pairs, 813 sibling pairs, 738 avuncular (aunt/uncle-niece/nephew) pairs, and 757 first cousin pairs.

The heritabilities of AITD-hypothyroidism and AITD were computed using an extension of quantitative genetic theory, which is based on specifying the phenotypic similarities among relatives as a function of their expected genetic covariances (23). The dichotomous trait extension to this approach is made by assuming an underlying risk (liability) for each individual that follows a multivariate normal distribution (24). The individual's affection status is then determined by whether his or her underlying liability exceeds a certain threshold (e.g. the population prevalence of the disease). Using this approach, the heritability of the dichotomous trait can be estimated as a component of the total trait variance. In addition to the variance components, we included as covariates the effects of age, age<sup>2</sup>, and sex in the model. Parameter estimates were obtained using maximum likelihood procedures, as implemented in the SOLAR software package (25).

Genome-wide qualitative trait linkage analysis of the two case definitions (AITD and AITD-hypothyroidism) was carried out using an extension of the variance components methodology implemented in the SOLAR program (25). The method is based on a threshold model that assigns an individual a specific affection status if his/her underlying genetically determined risk or liability exceeds a specific threshold on

**TABLE 1.** Prevalence of AITD in the Old Order Amish

Age (yr)	AITD <sup>a</sup>			AITD-hypothyroidism <sup>b</sup>		
	Total	Male	Female	Total	Male	Female
<30	11/58 (19.0)	3/20 (15.0)	8/38 (21.1)	2/48 (4.2)	0/17 (0.0)	2/31 (6.5)
30–40	20/101 (19.8)	6/48 (12.5)	14/51 (27.5)	4/85 (4.7)	1/43 (2.3)	3/42 (7.1)
40–50	32/127 (25.2)	7/50 (14.0)	27/77 (32.5)	11/105 (10.5)	0/53 (0.0)	11/62 (7.2)
50–60	22/89 (24.7)	4/40 (10.0)	18/49 (36.7)	7/73 (9.6)	3/39 (7.7)	4/34 (1.8)
>60	16/70 (22.9)	6/32 (18.8)	10/38 (26.3)	10/64 (15.6)	2/28 (7.1)	8/36 (22.2)
Total	101/445 (22.7)	26/190 (13.7)	75/255 (29.4)	34/375 (9.1)	6/170 (3.5)	28/205 (13.7)

Data represent no. of subjects/total no. of subjects (%).

<sup>a</sup> From a total of 445 Amish individuals who had AMA levels measured.

<sup>b</sup> From a total of 375 Amish individuals who had both AMA and TSH levels measured.

a normally distributed liability curve after the adjustment for covariate effects. The latent liability was assumed to have an underlying multivariate normal distribution with equal unit variances of liability in both the general population and in relatives of affected individuals. We modeled variability with liability as a function of individual-specific covariates (*e.g.* age and sex), the relationship between individuals (*i.e.* the residual arising from sharing one or two alleles identical by descent), heritability, and a locus-specific effect. The hypothesis of linkage was evaluated by the likelihood ratio test, which tests whether the locus-specific effect is significantly greater than zero (*i.e.*  $H_0: \sigma^2_{QTL} = 0$  vs.  $H_A: \sigma^2_{QTL} > 0$ ). The statistical significance of the linkage was shown in logarithm of the odds (LOD) score, computed as:  $LOD = \chi^2 / [2 \times \ln(10)]$ .

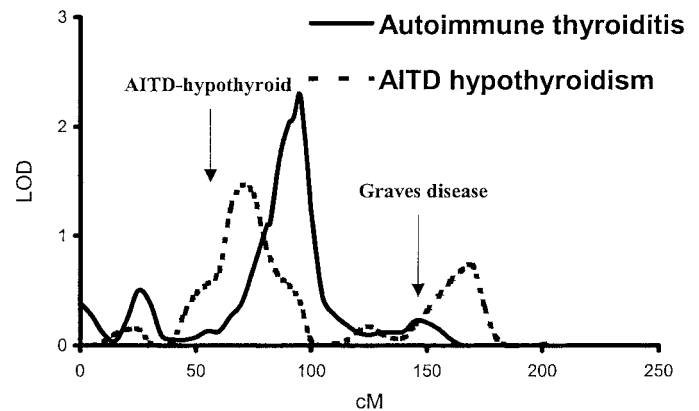
## Results

Figure 1 shows the distribution of TSH and AMA values in our Amish population. Based upon these data, subjects were classified as having AITD, defined by serum AMA levels of 1 IU/ml or greater (regardless of serum TSH level), or AITD-hypothyroidism, defined by serum AMA levels of 1 IU/ml or greater and serum TSH levels of 5 mU/liter or greater.

The prevalences of autoimmune thyroiditis and AITD-hypothyroidism in our study population were 22.7% and 9.1%, respectively (Table 1). Both AITD and AITD-hypothyroidism were more prevalent in females than males, consistent with studies by others (1, 2). More than one fifth of women aged 60 or older had AITD-hypothyroidism.

Heritability estimates ( $h^2$ ) were used to estimate the magnitude of the genetic effect on risk of AITD-hypothyroidism and AITD. Indeed, we found both of these traits to be significantly heritable. The  $h^2$  values for AITD for men, women, and men and women combined were 0.71, 0.58, and 0.48, respectively. The  $h^2$  estimates for AITD-hypothyroidism for women and men and women combined were 0.82 and 0.85, respectively. (The  $h^2$  estimate for men showed poor maximization and thus could not be accurately estimated.)

The genome-wide linkage results from analyses of all 22 autosomes and the X chromosome for AITD and AITD-hypothyroidism can be viewed at <http://medschool.umaryland.edu/Endocrinology/Amish/amlinkindex.html>. The genome-wide maximum LOD scores for AITD and AITD-hypothyroidism were both observed in close proximity to each other on chromosome 5q (Fig. 2). The maximum LOD score for AITD was 2.30 at 70 cM (closest marker, D5S647 on 5q11.2-q12), and that for AITD-hypothyroidism was 1.46 at 94 cM (closest marker, D5S428 on 5q14.3). Our genome-wide linkage analysis identified five other regions with a nominal  $P < 0.01$  (equivalent to  $LOD = 1.18$ ). AITD



**FIG. 2.** Results from multipoint linkage analysis of AITD and AITD-hypothyroidism on chromosome 5. The solid line indicates linkage to the trait AITD; the dotted line indicates linkage to the trait AITD-hypothyroidism. Arrows indicate the locations of maximum LOD scores for AITD-hypothyroidism and Graves' disease observed in a previous study using Japanese affected sibling pairs (15).

showed modest evidence for linkage to chromosomes 7p14–p15 ( $LOD = 1.42$ ;  $P = 0.005$ ), 11p13–p14 ( $LOD = 1.20$ ;  $P = 0.009$ ), 18p11.31 ( $LOD = 1.40$ ;  $P = 0.006$ ), and 22q12.2–q13.1 ( $LOD = 1.29$ ;  $P = 0.007$ ), and AITD-hypothyroidism showed modest evidence for linkage to chromosome 1q41–q42 ( $LOD = 1.27$ ;  $P = 0.008$ ; Table 2; also see <http://medschool.umaryland.edu/Endocrinology/Amish/amlinkindex.html>).

## Discussion

This study describes epidemiological characteristics of AITD in the Old Order Amish of Lancaster County, PA. Pauls *et al.* (8) reported that the prevalence of AMA in Amish over 31 yr of age was 22.2% in females and 10.6% in males. Our findings concur with this result and are consistent with epidemiological studies in other Caucasian populations (1, 2, 9). Also, consistent with other studies, there is pronounced familial aggregation of autoimmune hypothyroidism and AITD-hypothyroidism in the Amish, as determined by heritability estimates ( $h^2$ ). Thus, this cohort is an excellent resource for the pursuit of the loci and genes for AITD.

Our genome scan did not detect any regions that met or exceeded the threshold for genome-wide significance ( $LOD = 3.3$ ;  $P = 0.00005$ ), as proposed by Lander and Kruglyak (26). However, this level of statistical significance may be overly restrictive for mapping complex disease genes.

**TABLE 2.** Linkage signals with nominal *P* values < 0.01 from multipoint linkage analysis

Trait	Chromosome	Location (cM)	LOD	Nominal <i>P</i> value	Flanking markers
AITD-hypothyroidism	1	240	1.27	0.008	D1S425, D1S213
	5	70	1.46	0.005	D5S647, D5S424
AITD	5	94	2.30	0.0006	D5S424, D5S428
	7	60	1.42	0.005	D7S484, D7S510
	11	60	1.20	0.009	D11S1324, D11S935
	18	30	1.40	0.006	D18S452, D18S464
	22	40	1.29	0.007	D22S280, D22S283

Furthermore, simulations (not shown) indicate that our power to detect loci that have even moderate effects on trait variability in this sample is relatively low. Interestingly, our genome-wide linkage analysis in the Old Order Amish supports a recent observation of an AITD-hypothyroidism linkage at a cytokine gene cluster on chromosome 5q31–q33 in a Japanese study (15). Indeed, our observed LOD score of 2.3 ( $P = 0.0006$ ) satisfies the proposed criteria for a statistically significant replication (26). The linkage peak reported by Sakai and co-workers (15) for AITD-hypothyroidism is approximately 11 cM from our peak linkage for AITD-hypothyroidism and approximately 35 cM from our peak linkage for AITD. Given inherent inaccuracies in the ability of linkage analysis to localize susceptibility genes (27, 28), it is reasonable to hypothesize that these linkages represent the same locus. To our knowledge, this is the first replication of linkage of AITD to this region.

LOD scores with a nominal  $P < 0.01$  (equivalent to LOD = 1.18) were observed on five other chromosomes (7p, 11p, 18p, and 22q for AITD; 1q for AITD-hypothyroidism), as shown in Table 2. Interestingly, the linkage region on chromosome 18 is near a region of linkage for AITD-hypothyroidism reported in Japanese subjects (15).

Although there are consistencies (replications) between our study and those of others within two chromosomal regions, the four other regions showing modest evidence for linkage to AITD or AITD-hypothyroidism in our studies appear novel. These novel loci could represent false positives or true linkages not detected in other studies. Furthermore, previously reported loci of potential susceptibility for AITD-hypothyroidism, including cytotoxic T cell lymphocyte antigen 4, AITD-1, HT-1, and HT-2, did not show evidence for linkage in our study. This may be explained by genetic variability across ethnic groups, the influences of environmental triggers to modulate genetic expression, or, alternatively, type 1 or type 2 error. Genome-wide scans of AITD in other family studies and ultimately positional cloning of the susceptibility genes will be required to determine which of these linkages represent true positives.

We believe that AITD genes identified in the Amish will be relevant to the general population because the clinical characteristics of AITD and AITD-hypothyroidism in the Amish are indistinguishable from those of the general population. Secondly, the Amish gene pool is from that of Central Europe, and thus AITD genes in the Amish are likely to represent a subset of those present in the general Caucasian population. Our experience with type 2 diabetes in the Amish, in whom we have identified a susceptibility locus for type 2 diabetes on chromosome 1q21–q23 that is replicated in at least five other populations including Caucasians, Na-

tive Americans, and Chinese, provide additional support for generalizability of genetic findings in the Amish (29).

In conclusion, our genome-wide linkage analysis in the Amish provides evidence for replication of AITD loci on chromosomes 5q11.2–q14.3 and 18p11.31 as well as nominal evidence for linkage in four novel regions. These results combined with those of Sakai and co-workers (15) provide strong evidence for an AITD-related gene on the long arm of chromosome 5 and justify further investigation of the cytokine genes and other genes within this region.

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