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Clinical Research Article

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Limited Genetic Overlap Between Overt Hashimoto's Thyroiditis and Graves' Disease in Twins: A Population-based Study

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Abbreviations: A, additive genetic factors; AITD, autoimmune thyroid disease; C, environmental factors affecting both twins in a pair; D, dominance genetic factors; DZ, dizygotic; E, unique environmental factors not shared by twins; GD, Graves' disease; HR, hazard ratio; HT, Hashimoto's disease; ICD, International Classification of Diseases; MZ, monozygotic; NPR, National Patient Register; STR, Swedish Twin Registry; TGab, thyroglobulin; TPOab, thyroid peroxidase; TRab, thyroid-stimulating antibodies.

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Abstract

Context: Hashimoto's thyroiditis (HT) and Graves' disease (GD) are known to coaggregate in families, but the magnitude and nature of a shared etiology is unknown.
Objectives: To estimate the shared genetic influence on overt HT and GD and to examine if the heritability differs between men and women.
Design, setting, and patients: We used national health registries to identify cases of HT and GD in a cohort of 110 814 Swedish twins. By comparing intra-class and cross-twin cross-trait correlations in dizygotic and monozygotic twins, we calculated heritability and the proportions thereof shared between the diseases. Univariate estimates of heritability were calculated by sex.
Results: The heritability for HT and GD was 65% (95% CI, 61-70) and 63% (95% CI, 55-72), respectively. The genetic correlation was 0.35 (95% CI, 0.20-0.50) and shared genetic effects accounted for 8% of the variance for both HT and GD. Univariate heritability was significantly higher in men than in women for HT (90% vs 60%, $P < 0.001$) but not for GD (79% vs 63%, $P = 0.085$).

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Conclusions: From a genetic perspective, HT and GD appear to be only modestly related diseases. Hence, the term "autoimmune thyroid disease," used to cluster these disorders, may have limited validity in a genetic context. Moreover, the mechanisms contributing to HT are partly different for the sexes, with genetic components more important in men.

Key Words: hypothyroidism, hyperthyroidism, twin studies, genetics

Chronic autoimmune hypothyroidism, or Hashimoto's thyroiditis (HT), and autoimmune hyperthyroidism, or Graves' disease (GD) represent 2 of the most common forms of autoimmunity. The prevalence of autoimmune thyroid diseases (AITD) varies, but exceeds 5% among Caucasian women living in iodine-sufficient areas (1).

HT and GD are complex diseases caused by a combination of multiple genetic and environmental factors and, for both, heritability is considered high (2-4). HT and GD appear to be genetically related, with family studies demonstrating accumulation of both diseases in relatives of index cases (5-7), and with case reports on monozygotic (MZ) twin pairs where 1 twin had HT and the other has GD (8). Genetic factors predisposing for 1 of these disorders, are sometimes also found to increase the risk of the other disease (9, 10). Furthermore, autoantibodies directed against thyroglobulin (TGab) and thyroid peroxidase (TPOab), a hallmark of HT, are common in patients with GD as well. A consequence of this uniform view of AITD is that large-scale genetic studies sometimes treat HT and GD as etiologically homogenous (11).

There are, however, features that point to distinct and separate etiologies for HT and GD. Although HT is caused by cytotoxic destruction of the thyroid tissue, GD is considered a nondestructive disease, characterized by thyroid-stimulating antibodies (TRab), directed against the TSH receptor on the thyroid gland. Patterns of autoimmune clustering are also different for HT and GD (12). Still, without objective estimates, we do not know the extent of genetic and environmental overlap between HT and GD.

Both GD and HT are more common in women than in men, with female to male ratios of approximately 5 to 10:1 (1). This indicates that the underlying mechanisms leading to disease are partly different in men and women. Current estimates of heritability for HT and GD are based on mixed-sex twin cohorts, and heritability has not been examined for men and women separately (2-4), but 1 study reports a statistically significant difference in heritability for TGab between the sexes (3).

The aim of this study was to quantify shared and unique etiological sources for clinically overt HT and GD using a large cohort of Swedish twins. We also sought to explore if the heritability of the diseases is different for men and women.

Materials and Methods

This study was approved by the Regional Ethical Review Board in Stockholm, Sweden (Dnr: 2017/1546-32). Informed consent was waived by the ethics committee.

Registries

The Swedish Twin Registry (STR) is the world's largest twin resource. It contains information on individual twins born in Sweden from 1886 on. Zygosity has been determined for more than 85 000 twin pairs using a validated intra-pair similarity algorithm, DNA, or opposite sex. The Swedish National Patient Register (NPR) contains inpatient information dating back to 1964, with nationwide coverage since 1987. It includes hospital discharge records classified according to the International Statistical Classification of Diseases, versions 7 to 10 (ICD 7-10). As of 2001, data on hospital-associated outpatient care, but not primary health care, is also included. The Prescribed Drug Register, started in July 2005, collects data on all prescribed drugs dispensed in Sweden, including primary health care prescriptions.

Study Population

By combining information from the STR and the NPR, we retrieved diagnostic records on all twins from complete twin pairs born in Sweden between 1886 and 2006. To improve diagnostic precision and coverage, both twins were required to be alive (or not yet born) in 1976. In accordance with a prior study using the same data sources (4), HT was defined as a diagnosis of hypothyroidism without diagnostic records suggesting congenital, drug induced, infectious, postprocedural (surgery or radio-iodine treatment), or hypothyroidism secondary to iodine deficiency. For patients alive in 2006, we required multiple (≥2) dispensations of levothyroxine (ATC H03AA) for a diagnosis of HT. HT is often not ICD-coded when cooccurring with type 1 diabetes or Addison disease; in this setting, multiple (≥2) dispensations of levothyroxine, were considered indicative of HT in the absence of ICD codes indicating other thyroid disorders. GD was defined with a corresponding ICD code, whereas diagnostic records of other forms of hyperthyroidism were used as exclusions. For both GD and

HT, if patients had multiple (≥2) ICD codes indicating disease, multiple (≥2) exclusion codes were required for exclusion. Previous diagnoses of GD were used as an exclusion criterion for HT, mainly because of the frequent miscoding of postprocedural hypothyroidism as HT. Complete inclusion and exclusion criteria are listed in the Appendix (13). Overall, the quality of diagnoses in the NPR is high (14), but the validity of diagnostic records for AITDs has not been evaluated. However, a Danish registry-based study using nearly identical ICD codes and ATC codes to diagnose overt HT and GD, reported a misclassification of <2% when compared with clinical records, indicating high validity of diagnoses (15).

Statistical Analyses

Concordance and tetrachoric correlations

Probandwise concordance rates were estimated as the proportion of twins who had a disorder if their co-twin had the same disorder. This rate can be interpreted as a crude estimate of the probability that a co-twin of an affected twin will develop the disorder and may be contrasted to the population prevalence as an indicator of familial risk. Next, tetrachoric correlation across twins in pairs within as well as between disorders (so-called cross-twin cross-trait correlations) were calculated. The tetrachoric correlation is the statistical covariation between 2 dichotomous variables, calculated by assuming an underlying normal distribution whereby individuals with a liability beyond a certain threshold develop the disorder. Concordance rates and tetrachoric correlations were calculated separately by zygosity and by zygosity-sex-combinations.

Familial aggregation and coaggregation

The risks of twins developing HT or GD, given that their co-twins had either HT or GD, were calculated as hazard ratios (HR) using Cox regression models. Individuals were followed from start of observation until diagnosis, death, or end of follow-up. Using time-varying exposure; each individual was assumed unexposed until the date of diagnosis in the co-twin and exposed afterwards. The underlying timescale was attained age. We accounted for left truncation by allowing different ages of entry into analyses. This analysis was performed separately for MZ and dizygotic (DZ) twin pairs, adjusted for sex and birth year categories, and in subgroups of men and women, adjusted for birth year categories.

Quantitative genetic modeling

Quantitative genetic analyses were based on the classic twin assumptions that MZ twins are genetically identical, that DZ twins share on average 50% of their segregating alleles, that MZ and DZ twins share environment to a similar degree, and that there is no epistasis or dominance between genes and no interactions between genetic and environmental components. We used structural equation modeling, and fitted the models using weighted least squares.

Univariate estimates of heritability were analyzed for HT and GD separately, in subgroups of men and women. Observed population-variance was decomposed into additive genetic factors (A; the heritability), shared environmental factors affecting both twins in a pair (C), and unique environmental factors not shared by twins (E) using the ACE model. Some twin correlations indicated influences from dominance genetic factors (D), but we did not investigate this because of low power. In addition, A is considered a good approximation of total genetic effects A + D (16). For sex-specific estimates of heritability, same-sex pairs were used to test whether the magnitude of heritability differed between sexes (so-called quantitative genetic differences). To test for qualitative genetic differences (ie, whether genetic sources were different in males and females) opposite-sex twins were added to the models. ACE and AE models were fitted and compared using χ^2 tests and Akaike information criterion.

Next, a bivariate model was used to assess the extent to which HT and GD share genetic and environmental influences. Genetic (r_A) and environmental (r_C and r_E) correlations between the diseases were estimated. We then proceeded to calculate the proportion of genetic and environmental variance in HT that could be explained by genetic and environmental variance in GD and vice versa. Similar to the univariate analyses, a likelihood ratio test and Akaike information criterion calculations were used to test model fit.

Sensitivity analysis

We performed an additional analysis that did not incorporate within-individual tetrachoric correlation using a model we have previously described (17). Briefly, using a maximum likelihood model fitting procedure, the analysis relies on the same data as regular bivariate quantitative genetic analyses, but excludes contributions to the likelihood from the within-individual across disorder association (HT and GD in the same twin). Hence, the model does not estimate the r_E (ie, the individually unique contribution to phenotypic overlap). Finally, to investigate if results varied as a function of birth years, we fitted a model where the A, C, and E were allowed to vary over birth years. We fitted this model separately for HT and GD. Briefly, the model assumes a quadratic change in the contributions of A, C, and E over time, and has been described in detail elsewhere (18, 19).

All models were fitted using R (R-Development-Core-Team, 2010) using packages drgee, polycor, and OpenMx (20). All analyses were adjusted for birth year categories, and when appropriate for sex.

Results

Descriptive

A total of 120 286 individuals twins from complete twin pairs were identified in the STR. Of these, 3966 were excluded because of unknown zygosity. A further 3453 individuals deceased before 1976 were excluded along with 2049 co-twins of deceased twins. Two twin pairs (4 individuals) with ambiguous birth data were also excluded, yielding a final sample of 110 814 twins. In all, 1683 individual twins had HT (1.5%) and 558 had GD (0.5%), including 15 individuals affected by both diseases. Both HT and GD were more common in women than in men with a prevalence of 24.0/1000 (women) and 5.2/1000 (men) for HT and 8.9/1000 (women) and 1.8/1000 (men) for GD. HT and GD were present in 1545 and 536 twin couples, respectively. Sex, zygosity, and birth data are presented in Table 1.

Concordance and Tetrachoric Correlations

In total, 138 twin pairs were concordant for HT and 22 twin pairs were concordant for GD. In contrast, cross-trait concordance was present in 31 twin pairs. Probandwise concordance rates were consistently higher in MZ than in DZ pairs, with the highest concordance of found for HT in MZ same-sex male pairs (0.43, 95% CI 0.30-0.61), whereas no DZ same-sex male pairs or opposite-sex pairs were concordant for GD. Consequently, tetrachoric correlations in MZ twin pairs were higher than in DZ twin pairs, indicating considerable genetic influences on both HT and GD. Of note, tetrachoric correlations were higher in men than in women for both diseases. Concordance rates and tetrachoric correlations are presented in Table 2 (cross-trait concordances by sex and zygosity are presented in the Appendix (13)).

Familial Aggregation and Coaggregation

Both MZ and DZ twins were at increased risk of developing the disease present in their co-twin, with HRs generally higher in men than in women. In MZ men, the relative risks were very high (HR, 126.9 for HT and 113.9 for GD, adjusted for birth year and sex), in part reflecting a low population prevalence. Overall, adjusted HRs for cross-trait coaggregation were considerably lower, but still elevated in most groups (Table 3).

Quantitative Genetic Modeling

Although differences in model fit were marginal for most diseases, the AE models were universally preferred over the ACE models in both univariate and bivariate analyses (Table 4). Univariate estimates of heritability using the AE models were similar for HT (65%) and GD (63%) in analyses with both sexes combined. In the sex-separated analyses, heritability was statistically significantly higher in men than in women for HT (90% vs 60%, $P < 0.001$) but not for GD (79% vs 63%, $P = 0.085$) (Table 5). For HT, the genetic correlation between men and women was 1.00 (95% CI, 0.69-1.31; from best-fitting AE model), suggesting the same genetic factors contribute to disease, but with different proportions of observed variance explained. For GD, the cross-sex genetic correlation could not be calculated because of the lack of opposite sex twin pairs concordant with GD.

In the bivariate analysis, the additive genetic correlation (r_A) was estimated to 0.35 (95% CI, 0.20-0.50) and the

Table 1. Age, sex, and zygosity of twins, number of individuals (percent)

	Twin cohort	Twins with Graves' Disease	Twins with Hashimoto's Thyroiditis
All	110 814 (100)	558 (0.5)	1683 (1.5)
Sex			
Male	52 171 (47.1)	95 (17.0)	273 (16.2)
Female	58 643 (52.9)	463 (83.0)	1410 (83.8)
Zygosity			
Monozygotic	35 990 (32.5)	183 (32.8)	553 (32.9)
Dizygotic	74 824 (67.5)	375 (67.2)	1130 (67.1)
Birth year			
<1920	11 454 (10.3)	38 (6.8)	96 (5.7)
1920-1939	18 736 (16.9)	132 (23.7)	747 (44.4)
1940-1959	28 948 (26.1)	242 (43.4)	509 (30.2)
1960-1979	14 082 (12.7)	116 (20.8)	189 (11.2)
>1979	37 594 (33.9)	30 (5.4)	142 (8.4)

Table 2. Concordance rates and tetrachoric correlations

	Concordant Nonaffected Pairs	Discordant Pairs	Concordant Affected Pairs	Concordance Rate (95% CI)	Tetrachoric Correlation (95% CI)
Graves' disease					
MZ	17 831	145	19	0.21 (0.14-0.30)	0.68 (0.58-0.77)
Male-male	8069	23	3	0.21 (0.08-0.54)	0.73 (0.53-0.93)
Female-female	9762	122	16	0.21 (0.14-0.31)	0.65 (0.54-0.76)
DZ	37 040	369	3	0.02 (0.01-0.05)	0.16 (-0.01-0.33)
Male-male	10 579	38	0	0.00 (0.00-0.00)	…
Female-female	11 850	195	3	0.03 (0.01-0.09)	0.20 (0.00-0.40)
Female-male	14 611	136	0	0.00 (0.00-0.00)	…
Hashimoto's thyroiditis					
MZ	17 523	391	81	0.29 (0.25-0.35)	0.70 (0.65-0.75)
Male-male	8044	37	14	0.43 (0.30-0.61)	0.87 (0.79-0.95)
Female-female	9479	354	67	0.27 (0.23-0.33)	0.64 (0.57-0.70)
DZ	36 339	1016	57	0.10 (0.08-0.13)	0.39 (0.33-0.45)
Male-male	10 507	105	5	0.09 (0.04-0.20)	0.46 (0.28-0.64)
Female-female	11 486	528	34	0.11 (0.08-0.16)	0.35 (0.27-0.44)
Female-male	14 346	383	18	0.11 (0.07-0.16)	0.43 (0.33-0.54)
Cross-twin cross-trait					
MZ	17 375	605[a]	15[b]	…	0.28 (0.18-0.38)
DZ	35 995	1401[a]	16[b]	…	0.16 (0.08-0.25)

Abbreviations: DZ, dizygotic; MZ, monozygotic.
[a]Any combination of disease not including Hashimoto´s thyroiditis in 1 twin and Graves' disease in the co-twin.
[b]Hashimoto's thyroiditis in 1 twin and Graves' disease in the co-twin.

Table 3. Aggregation and coaggregation of Hashimoto's thyroiditis and Graves' disease

	Risk for HT When Co-twin Has HT		Risk for GD When Co-twin Has HT		Risk for GD When Co-twin Has GD		Risk for HT When Co-twin Has GD	
	aHR	(95% CI)	aHR	(95% CI)	aHR	(95% CI)	aHR	(95% CI)
Monozygotic twins								
All	11.8	(8.9-15.7)	3.3	(1.3-8.0)	33.8	(19.2-59.6)	3.5	(1.9-6.4)
Women	9.5	(7.1-12.8)	3.5	(1.4-8.7)	29.7	(16.5-53.7)	3.6	(2.0-6.6)
Men	126.9	(59.1-272.5)	…		113.9	(27.6-469.7)	…	
Dizygotic twins								
All	4.3	(3.2-5.7)	2.7	(1.2-6.1)	2.1	(0.7-6.7)	1.9	(1.0-3.6)
Women	3.7	(2.6-5.3)	1.8	(0.6-5.8)	3.0	(1.0-9.5)	1.8	(0.8-3.8)
Men	15.9	(5.5-45.5)	40.2	(8.5-190.3)	…		…	

Abbreviations: aHR, adjusted hazard ratio, adjusted for birth year categories and when appropriate for sex; HT, Hashimoto's thyroiditis; GD, Graves' disease.

unique genetic correlation (r_E) to –0.56 (95% CI, –0.89 to –0.22). Proportions of explained variance in HT by GD, and vice versa, are presented in Fig. 1 (complete models can be found in the Appendix (13)).

Sensitivity Analysis

In the bivariate quantitative genetic model that did not consider within-individual overlap between HT and GD, the estimates were similar to results in the main analyses. Importantly, the genetic correlation was estimated to precisely the same value (r_A = 0.35; 95% CI, 0.19-0.50; Appendix, page 4). When we modeled the A, C, and E contributions to vary over birth year, the estimates were mostly stable, with a slight increase in heritability, and decrease in unique environment, with increasing birth years in both HT and GD (supplemental Figs S1 and S2 (13)).

Discussion

In this study of more than 110 000 Swedish twins, we found that heritable factors explain most of the observed

Table 4. Model fitting results of univariate and bivariate analyses of Hashimoto's thyroiditis and Graves' disease

	Model	AIC	Diff-LL	Diff-df	*P*
Hashimoto's thyroiditis					
All	ACE	-221 584.51	0.08	1	0.78
	AE	**-221 586.43**			
Women	ACE	-87 765.70	0.00	1	>0.99
	AE	**-87 767.70**			
Men	ACE	-74 815.96	0.00	1	0.96
	AE	**-74 817.96**			
Graves' disease					
All	ACE	-221 585.34	0.00	1	>0.99
	AE	**-221 587.34**			
Women	ACE	-87 765.45	0.00	1	>0.99
	AE	**-87 767.45**			
Men	ACE	-74 827.48	0.00	1	>0.99
	AE	**-74 829.48**			
Bivariate model					
All	ACE	-443 156.09	0.06	3	>0.99
	AE	**-443 162.04**			

AE models provided the best fit for all traits.

Abbreviations: A, additive genetic factors; AIC, Akaike's information criterion (lower is better); C, environmental factors affecting both twins in a pair; Diff-LL, difference in –2log likelihood for AE compared with ACE models; Diff-df, difference in degrees of freedom for AE compared with ACE models; E, unique environmental factors not shared by twins; *P*, *P* value of likelihood ratio test for AE compared with ACE models.

Table 5. Univariate estimates of explained variance for Hashimoto's thyroiditis and Graves' disease

	Additive Genetic Effects			Nonshared Environmental Effects		
	A	95% CI	*P*	E	95% CI	*P*
Hashimoto's thyroiditis						
All	0.65	(0.60-0.70)	…	0.35	(0.30-0.40)	…
Women	0.60	(0.54-0.66)	<0.001	0.40	(0.34-0.46)	<0.001
Men	0.90	(0.82-0.97)		0.10	(0.03-0.18)	
Graves' disease						
All	0.63	(0.54-0.72)	...	0.37	(0.28-0.46)	…
Women	0.63	(0.52-0.73)	0.085	0.38	(0.27-0.48)	0.085
Men	0.79	(0.63-0.96)		0.21	(0.04-0.37)	

Models adjusted for age categories and sex when appropriate. Age categories were <1920, 1920-1939, 1940-1959, 1960-1979, >1979; except Graves' in men, where categories were collapsed to <1940, 1940-1959, >1959 because of low prevalence.

Abbreviations: A, additive genetic factors; E, unique environmental factors not shared by twins; *P*, *P* value for difference between men and women tested using a Wald test.

variance for overt HT and GD, but that only a minority of these factors are shared between the diseases. This contrasts to current dogma that HT and GD are closely related disorders. In HT, we also found that the underlying risk factors differ between men and women, with genetic factors explaining more of the observed variance in men.

Our results on the heritability for HT and GD are in line with previous estimates, but with twin concordance rates lower than previously reported. This difference is most evident for HT. In the present study, we report a probandwise concordance rate of 0.29 (95% CI, 0.25-0.35) for HT in MZ twins, at the lower end of what was reported by Brix et al (0.55; 95% CI, 0.23-0.82) (21) and Hansen et al (0.45; 95% CI, 0.22-0.67) (3) in studies on the Danish Twin Registry. However, their results were either based on small twin samples (21) or on titers of TPOab and/or Tgab in euthyroid subjects, rather than clinically overt HT (3), making direct comparisons difficult. Moreover, the genetic underpinnings of TPOab and overt HT may not be identical (22) further complicating the matter.

At present, the etiologic overlap between HT and GD is considered to be significant (9, 10), but a more uniform view of AITD, with HT and GD representing different

	Explained variance	Hashimoto's thyroiditis	Graves' disease
A	Total	0.65 (0.61-0.70)	0.63 (0.55-0.72)
	Shared	0.08 (0.01-0.15)	0.08 (0.01-0.14)
	Not shared	0.57 (0.49-0.66)	0.56 (0.44-0.67)
E	Total	0.35 (0.30-0.40)	0.37 (0.28-0.46)
	Shared	0.11 (-0.02-0.24)	0.11 (-0.02-0.25)
	Not shared	0.24 (0.10-0.37)	0.25 (0.09-0.41)

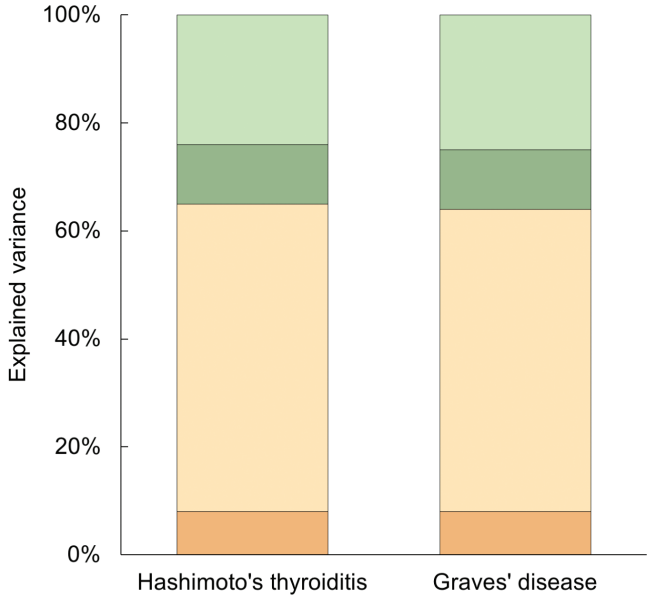

Figure 1. Explained variance and etiologic overlap in Hashimoto's thyroiditis and Graves' disease. A, additive genetic effects. E, environmental effects not shared by co-twins. A is equivalent to heritability.

manifestations on an autoimmune continuum rather than etiologically distinct entities, has also been suggested (23). This approach is supported by numerous reports on shared susceptibility loci for HT and GD (24). Most notably, HLA risk alleles corresponding to serotype DR3 predispose to both diseases (25). However, even though the HLA is the most important genetic determinant identified to date in both HT and GD, HLA variants explain less than 10% of disease heritability (26), and even less of the observed variance. Moreover, some loci implicated in HT and GD are shared with many autoimmune diseases, supporting a broad autoimmune tautology rather than a common genetic origin to AITDs alone (9, 10), and other loci, including HLA variants and alleles of the TSH receptor are not shared, but convey risk for 1 AITD only (27-29). Interestingly, in a recent study by Saevarsdottir et al, an allelic variant of the FLT3 gene is linked to an increased risk of AITD, but in separate analysis on a subset of patients diagnosed with either HT or GD, the risk appears to differ considerably between the diseases (30). Beyond case reports, epidemiological data on cross-twin cross-trait concordance for AITD is sparse. In a study from 2002 by Ringold et al, HT was recorded in 17% (5 of 29) of co-twins in MZ pairs discordant for GD (31). Familial coaggregation (first-degree relative of index case suffering from a different AITD) appears to be common, ranging from 5% to 38% in some studies (5-7). However, the cohorts used have often been collected at secondary or tertiary medical centers, potentially inflating estimates of coaggregation through selection bias, and diagnoses have sometimes relied on self-reported prevalence of thyroid autoimmunity (5), which does not always correlate with clinical records (7). The only unbiased study using population-based data that we have found reports a modest increase in risk of GD in probands if either a sibling or a parent has overt HT, in line with our reported HRs for cross-twin cross-trait coaggregation (32).

The sexual dimorphism in AITD is poorly understood. The effects of estrogen, skewed X-chromosome inactivation, and microchimerism have all been implicated, but there is little tangible evidence (33). A population-based

Danish twin study examining heritability of thyroid autoantibodies in euthyroid subjects reported a lower heritability for both TPOab and TGab in men compared with women, albeit statistically significant only for TGab (3). We could not replicate this finding for overt HT; to the contrary, our results indicate a significantly higher heritability in men than in women, reflected by much higher HRs for concordance for HT in MZ than in DZ men. It is important to remember that heritability represents a proportion of explained variance, and that all other variance is due to environmental factors and sampling errors. In a homogenous environment, heritability therefore tends to be higher. Consequently, the sex difference in heritability reported here does not necessarily equate to male-specific genetic factors in play, but could equally well be interpreted as a greater variance of important environmental factors among women. Our findings of a high genetic correlation for HT in men and women (r_A = 1.00), suggesting that the same genetic factors contribute to disease in both sexes (but with different proportions of explained variance) is consistent with this theory.

Iodine is known to affect the incidence of both GD and HT (34). With iodine fortification introduced in Sweden more than 50 years ago, intake is considered sufficient if not homogenous in the population. Other environmental factors of demonstrated importance in AITD, such as smoking, alcohol consumption, and selenium intake most likely explain part of the unique E factors observed (34). Of these factors, only smoking, which has been shown to reduce the risk of HT but increase the risk of GD (35), is consistent with the negative unique environmental correlation (r_E) reported here. However, the most probable explanation to this negative correlation is that GD was used as an exclusion criterion for HT.

The prevalence of GD in this study was modest compared with most international cohorts, but the incidence of GD in the Swedish population is reportedly low (36). For HT, our prevalence of 2.4% among women is slightly higher than previous estimates on middle-aged and elderly Swedish women, indicating acceptable coverage (37). The true prevalence of HT is likely somewhat higher, but to avoid decreased diagnostic specificity, which would distort heritability estimates (38), we chose not to base diagnoses of HT on prescription patterns alone.

Hypothyroidism and hyperthyroidism are distinct phenotypes, but transition from 1 condition to the other occurs. This may reflect an overlap between HT and GD, but shifts from blocking to stimulating TRabs, or vice versa, representing variants of GD, albeit rare, do occur (39). However, up to 25% of patients treated with antithyroid drugs for GD reportedly use levothyroxine replacement on long-term follow-up (40). To what extent this represents a shift from GD to HT is unclear.

Limitations

The use of diagnostic records to ascertain AITDs entails difficulties that must be addressed. In Sweden, GD is usually treated by endocrinologists in hospital-associated outpatient clinics, and measurements of TRab are routinely performed as part of the diagnostic workup. HT is usually diagnosed in a primary health care setting, based on thyroid hormone levels and TPOab status, but inclusion in the NPR requires that a diagnosis of HT is at some point recorded during hospital-associated inpatient or outpatient care. Still, the lack of biochemical data, most notably TPOab, is a limitation, and despite using multiple exclusion criteria, some misclassifications cannot be ruled out. However, the magnitude of such errors would have to be unreasonably large for results to come close to supporting the current notion of a large genetic overlap between HT and GD. In a registry-based context, diagnostic codes of both GD and HT in the same individual may represent erroneous ICD coding. Radioiodine treatment of GD is difficult to detect in a registry-based setting, and the ensuing hypothyroidism is often miscoded as HT. We used prior or concurrent GD as an exclusion criterion for HT. This likely increased the diagnostic accuracy of HT, but also influenced the r_E, which should be interpreted with caution. The results also need to be viewed in the light of the inherent assumptions in the twin model used. These include the equal environments assumption, which states that co-twins share nongenetic sources of similarity to an equal degree regardless of zygosity. If this assumption is violated, and there are nongenetic contributions to similarity in HT, GD, and/or their overlap, which is more prominent in MZ than in DZ pairs, the heritability will be biased upwards.

Heritability estimates are dependent on the population in which they are performed. Both GD and HT are, from a global perspective, heterogeneous conditions, with evidence of partly different genetic risk factors in populations of differing ethnicity (10). Hence, the results presented here may not be transferrable across ethnicities. Environmental factors, such as iodine status, most likely affect external validity as well, and our results may not be representative of populations residing in iodine insufficient regions.

To conclude, using registry-based data on a large twin cohort, we find evidence of modest genetic and environmental overlap between overt HT and GD. Our data contradict the present notion of a substantial common genetic origin to AITD. Moreover, the mechanisms contributing to disease appear to be partly different between the sexes. Our results warrant replication in studies using similar design as well

as with molecular genetic data, but suggest that research on the genetic underpinnings of HT and GT could benefit from considering them as separate entities.

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References

1. Taylor PN, Albrecht D, Scholz A, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol*. 2018;**14**(5):301-316.
2. Brix TH, Kyvik KO, Christensen K, Hegedüs L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *J Clin Endocrinol Metab*. 2001;**86**(2):930-934.
3. Hansen PS, Brix TH, Iachine I, Kyvik KO, Hegedüs L. The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity: a study of healthy Danish twins. *Eur J Endocrinol*. 2006;**154**(1):29-38.
4. Skov J, Eriksson D, Kuja-Halkola R, et al. Co-aggregation and heritability of organ-specific autoimmunity: a population-based twin study. *Eur J Endocrinol*. 2020;**182**(5):473-480.
5. Boelaert K, Newby PR, Simmonds MJ, et al. Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. *Am J Med*. 2010;**123**(2):183.e1-183.e9.
6. Tomer Y, Ban Y, Concepcion E, et al. Common and unique susceptibility loci in Graves and Hashimoto diseases: results of whole-genome screening in a data set of 102 multiplex families. *Am J Hum Genet*. 2003;**73**(4):736-747.
7. Strieder TG, Tijssen JG, Wenzel BE, Endert E, Wiersinga WM. Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the Thyroid Events Amsterdam (THEA) score. *Arch Intern Med*. 2008;**168**(15):1657-1663.
8. Aust G, Krohn K, Morgenthaler NG, et al. Graves' disease and Hashimoto's thyroiditis in monozygotic twins: case study as well as transcriptomic and immunohistological analysis of thyroid tissues. *Eur J Endocrinol*. 2006;**154**(1):13-20.
9. Simmonds MJ. GWAS in autoimmune thyroid disease: redefining our understanding of pathogenesis. *Nat Rev Endocrinol*. 2013;**9**(5):277-287.
10. Lee HJ, Li CW, Hammerstad SS, Stefan M, Tomer Y. Immunogenetics of autoimmune thyroid diseases: a comprehensive review. *J Autoimmun*. 2015;**64**:82-90.
11. Cooper JD, Simmonds MJ, Walker NM, et al.; Wellcome Trust Case Control Consortium. Seven newly identified loci for autoimmune thyroid disease. *Hum Mol Genet*. 2012;**21**(23):5202-5208.
12. Wiebolt J, Achterbergh R, den Boer A, et al. Clustering of additional autoimmunity behaves differently in Hashimoto's patients compared with Graves' patients. *Eur J Endocrinol*. 2011;**164**(5):789-794.
13. Skov J, Calissendorff J, Eriksson D, et al. Data from: limited genetic overlap between Hashimoto's thyroiditis and Graves' disease in twins: a population-based Twin study. Figshare. com. Deposited 6 January 2021. http://doi.org/10.6084/m9.figshare.13528076.
14. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health*. 2011;**11**:450.
15. Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16 249 patients. *Thyroid*. 2002;**12**(5):411-419.
16. Keller MC, Medland SE, Duncan LE. Are extended twin family designs worth the trouble? A comparison of the bias, precision, and accuracy of parameters estimated in four twin family models. *Behav Genet*. 2010;**40**(3):377-393.
17. Song J, Kuja-Halkola R, Sjölander A, et al. Specificity in etiology of subtypes of bipolar disorder: evidence from a Swedish population-based family study. *Biol Psychiatry*. 2018;**84**(11):810-816.
18. Purcell S. Variance components models for gene-environment interaction in twin analysis. *Twin Res*. 2002;**5**(6):554-571.
19. Medland SE, Neale MC, Eaves LJ, Neale BM. A note on the parameterization of Purcell's G × E model for ordinal and binary data. *Behav Genet*. 2009;**39**(2):220-229.
20. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2012. ProMED-mail website. http://www R-project org.
21. Brix TH, Kyvik KO, Hegedüs L. A population-based study of chronic autoimmune hypothyroidism in Danish twins. *J Clin Endocrinol Metab*. 2000;**85**(2):536-539.
22. Inoue N, Watanabe M, Yamada H, et al. Associations between autoimmune thyroid disease prognosis and functional polymorphisms of susceptibility genes, CTLA4, PTPN22, CD40, FCRL3, and ZFAT, previously revealed in genome-wide association studies. *J Clin Immunol*. 2012;**32**(6):1243-1252.
23. Bliddal S, Nielsen CH, Feldt-Rasmussen U. Recent advances in understanding autoimmune thyroid disease: the tallest tree in the forest of polyautoimmunity. *F1000Res*. 2017;**6**:1776.
24. Hwangbo Y, Park YJ. Genome-wide association studies of autoimmune thyroid diseases, thyroid function, and thyroid cancer. *Endocrinol Metab (Seoul)*. 2018;**33**(2):175-184.

25. Jacobson EM, Huber A, Tomer Y. The HLA gene complex in thyroid autoimmunity: from epidemiology to etiology. *J Autoimmun*. 2008;**30**(1-2):58-62.
26. Chu X, Pan CM, Zhao SX, et al.; China Consortium for Genetics of Autoimmune Thyroid Disease. A genome-wide association study identifies two new risk loci for Graves' disease. *Nat Genet*. 2011;**43**(9):897-901.
27. Zeitlin AA, Heward JM, Newby PR, et al. Analysis of HLA class II genes in Hashimoto's thyroiditis reveals differences compared to Graves' disease. *Genes Immun*. 2008;**9**(4):358-363.
28. Li L, Ding X, Wang X, et al. Polymorphisms of IKZF3 gene and autoimmune thyroid diseases: associated with graves' disease but not with Hashimoto's thyroiditis. *Cell Physiol Biochem*. 2018;**45**(5):1787-1796.
29. Dechairo BM, Zabaneh D, Collins J, et al. Association of the TSHR gene with Graves' disease: the first disease specific locus. *Eur J Hum Genet*. 2005;**13**(11):1223-1230.
30. Saevarsdottir S, Olafsdottir TA, Ivarsdottir EV, et al. FLT3 stop mutation increases FLT3 ligand level and risk of autoimmune thyroid disease. *Nature*. 2020;**584**(7822):619-623.
31. Ringold DA, Nicoloff JT, Kesler M, Davis H, Hamilton A, Mack T. Further evidence for a strong genetic influence on the development of autoimmune thyroid disease: the California twin study. *Thyroid*. 2002;**12**(8):647-653.
32. Hemminki K, Li X, Sundquist J, Sundquist K. The epidemiology of Graves' disease: evidence of a genetic and an environmental contribution. *J Autoimmun*. 2010;**34**(3):J307-J313.
33. Brix TH, Hegedüs L. The complexity of the etiology of autoimmune thyroid disease is gravely underestimated. *Thyroid*. 2011;**21**(12):1289-1292.
34. Wiersinga WM. Clinical relevance of environmental factors in the pathogenesis of autoimmune thyroid disease. *Endocrinol Metab (Seoul)*. 2016;**31**(2):213-222.
35. Wiersinga WM. Smoking and thyroid. *Clin Endocrinol (Oxf)*. 2013;**79**(2):145-151.
36. Abraham-Nordling M, Byström K, Törring O, et al. Incidence of hyperthyroidism in Sweden. *Eur J Endocrinol*. 2011;**165**(6):899-905.
37. Petersen K, Lindstedt G, Lundberg PA, Bengtsson C, Lapidus L, Nyström E. Thyroid disease in middle-aged and elderly Swedish women: thyroid-related hormones, thyroid dysfunction and goitre in relation to age and smoking. *J Intern Med*. 1991;**229**(5):407-413.
38. Bishop SC, Woolliams JA. On the genetic interpretation of disease data. *PLoS One*. 2010;**5**(1):e8940.
39. Gonzalez-Aguilera B, Betea D, Lutteri L, et al. Conversion to Graves disease from Hashimoto thyroiditis: a study of 24 patients. *Arch Endocrinol Metab*. 2018;**62**(6):609-614.
40. Sjölin G, Holmberg M, Törring O, et al. The long-term outcome of treatment for graves' hyperthyroidism. *Thyroid*. 2019;**29**(11):1545-1557.