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Further Evidence for a Strong Genetic Influence on the Development of Autoimmune Thyroid Disease: The California Twin Study

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To determine the heritable component of Graves' disease (GD) more precisely, a disease survey questionnaire completed by 13,726 California-born twin pairs over the age of 37 years was used as the foundation of this study. On the basis of this survey, each member of pairs reporting a past diagnosis of GD was then sought for an extensive telephone interview to seek diagnostic confirmation. Successful diagnostic evaluation occurred in 108 cases, of which 99 affected twin pairs form the basis of this report. The results indicate that the estimated pairwise concordance for is 17% in monozygotic (MZ) twins, and 1.9% in dizygotic (DZ) twins, which are in close agreement with a recent report from a Danish twin population. Moreover, the reported 3.9% occurrence of GD found in the first-degree relatives of affected twin pairs supports these findings. In contrast, only 0.45% of all twins, 0.27% of the spouses of twins, and approximately 0.16% of the first-degree relatives of unaffected twins were reported to have GD. Additionally, among the unaffected MZ twins of patients with GD, 17% reported having chronic thyroiditis and 10% other nonthyroid autoimmune conditions such as lupus erythematosus, pernicious anemia, or idiopathic thrombocytopenic purpura. Thus, a genetic predisposition appears to be shared for both thyroid and some nonthyroid autoimmune diseases. While it seems that GD is a strongly and nonspecifically heritable condition, the relatively low level of twin concordance indicates that this disease likely requires a nonheritable etiologic determinant(s) as well.

Introduction

Since Parry (1825) and Graves (1835) first described what is now generally termed Graves' disease (GD), its etiology has remained obscure despite increasing understanding of its pathogenesis. Currently, GD is considered to be an autoimmune disease in which autoantibodies reactive to the host's thyrotropin receptor (TSHR) are responsible for producing typical phenotypic findings of diffuse goiter, thyroid hormone hypersecretion, and thyrotoxicosis (1,2). This disease complex is also occasionally accompanied by evidence of infiltrative ophthalmopathy and, rarely, with pretibial myxedema (3). However, the presentation is variable both in terms of severity and course, which on occasion may culminate in an apparent complete clinical remission (3).

While there is clear evidence of genetic and environmental contributions to the genesis of GD, the specific factors and the nature of their interaction are unclear. Heritability has been suggested by early observations of familial clustering (4–6) and a high degree pairwise concordance approximating 50% in initial reports of monozygotic (MZ) twins (7–8), which is substantially higher than the 0%–9% range found in like-gender dizygotic (DZ) twins. Thus, it has been generally presumed that GD is not only familial, but a heritable disease with a high degree of identity between genotype and phenotype. The genetic underpinnings of GD may also be extended to other autoimmune thyroid diseases, as evidenced by case reports of MZ twin pairs in which one twin has GD and the other has chronic thyroiditis (9–13), suggesting a common heritable factor(s) in etiology of thyroid autoimmune thyroid diseases as a group. This evidence supporting the inheritance of autoimmune thyroid diseases has provided the rationale for the search to identify the responsible genes (14).

This presumption of an overwhelmingly genetic predisposition for GD has been challenged by a recent report from the Danish Twin Registry in which pairwise concordance was observed to be 15% in a group of 39 MZ twin pairs with self-described GD and 22% in a subgroup of 18 pairs in whom the disease was validated through medical records (15). The reason for the discrepancy between this recent observation and the substantially higher concordance rates

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found in a previous report in a similar Danish twin population was ascribed to subject selection biases and diagnostic inaccuracies in the earlier study (8).

Because future investigations into the etiology of GD may depend on precisely defining genetic and environmental determinants, we undertook the present investigation into the familiality and twin concordance of GD using a large set of representative native resident California twins. The results of this study reaffirm the role of genetics in GD with a similar concordance rate as seen in the recent Danish Twin Registry study. It also underscored the possibility that nonheritable factors are necessary to express this GD phenotype. Additionally, this study sought to explore the often postulated common genetic association between GD and CT as well as with other systemic autoimmune diseases. Our findings, suggest that there is a strong common genetic basis for both autoimmune thyroid diseases and with other nonthyroid autoimmune diseases as well.

Subjects and Methods

Study population

The California Twin Program was established by creating a file of all twins born in California between 1908 and 1982. Linking this file with the Department of Motor Vehicles file of active drivers' licenses in 1991 produced a roster of 102,000 native resident California twins. Only nondrivers and some females born before approximately 1940 are underrepresented on this roster because the latter drivers' license files with maiden names were not computerized until the mid-1960s. A 16-page instrument was devised to include sections on family background, growth and development, employment and education, reproductive history, personal and immediate family medical history, diet, and other aspects of lifestyle. GD was specifically addressed by questions about diagnoses made about the respondent, his or her co-twin, other first-degree relatives, and his or her spouse. Provision was also made for an open-ended response to a request for any "other conditions" suffered. Questionnaires were only sent to subjects 40 years of age or older in order to better ascertain cumulative incidence rates. The initial instrument was followed by a reminder and a second copy sent to nonrespondents after 3 months. A total of 19,934 instruments were completed and returned, equivalent to 55% of those sent. Small follow-up surveys to respondents and nonrespondents led to the conclusion that approximately 67% of those who actually received the questionnaire responded. Of those returned, 556 (2.8%) were unusable for technical reasons, leaving 19,378 respondents representing 13,708 twin pairs. The mean ages of male MZ, female MZ, male DZ, female DZ, and unlike-gender DZ respondents at the time of survey were 50.4, 44.2, 50.0, 44.4, and 47.7 years, respectively. Respondents were generally representative of all native California residents with respect to race, social class, and California subregion.

Zygosity

Self-reporting of zygosity by adult twins has in the past repeatedly been shown to be highly predictive of true zygosity (16) and results of self-reports followed by DNA "fingerprinting" of more than 40 pairs of twins in this center has further reinforced this perception (17). Of the final set of 19,378 respondents, identical females accounted for 15.5% of the respondents and identical males for 16.5%; 16.8% were from like-gender fraternal female pairs, 20.2% from like-gender fraternal male pairs, and 30.9% from unlike-gender fraternal pairs. Furthermore, in only 0.1% of cases was zygosity in doubt or disagreement. Based on California twin birth records for the period, the expected frequency for these five categories should be approximately 16.5%, 16.5%, 16.5% 16.5%, and 33%, respectively.

Concordance

Pairwise concordance is defined in this study as the proportion of all affected pairs in which both twins are affected. Probandwise concordance, which estimates the contingent probability that the unaffected twins of a case will be affected, counts each concordant pair twice on the assumption that each such twin has been independently ascertained. The latter measure can then be compared directly to cumulative incidence.

Validation

Each twin reporting a diagnosis of GD, hyperthyroidism or a "thyroid condition," was scheduled for a telephone interview in which a more detailed information of the signs, symptoms, procedures, and treatment associated with establishing a specific clinical diagnosis of GD was obtained. This telephone interview was carried out using a formatted questionnaire in which the interviewers (D.A.R. and M.K.) systematically elicited relevant historical information including age at diagnosis, clinical features of Graves' eye disease, and other diagnostic labels that had been applied to their condition such as "overactive or underactive thyroid," hyperthyroidism, toxic thyroid or goiter, autoimmune thyroiditis, Hashimoto's thyroiditis, or "any thyroid trouble." The duration of their condition, clinical course, and therapeutic measures used were also recorded including the use of antithyroid medications (methimazole and propylthiouracil [PTU]), radioiodine, and surgery as well as current use of hormone preparations, time of initiation, dose, and therapeutic response. This clinical information was then cross-checked with their twin for additional validation. Additionally, the interviewers attempted to elicit other symptomatology that might have further relevance in validating the diagnosis. This data was then reviewed blinded to individual identity and zygosity (J.T.N.) and a final determination was made as to whether sufficient information was available to establish a likely clinical diagnosis of GD. No attempt was undertaken to communicate with the attending physician or review medical records. Additional historical information relevant to first-degree relatives who may have had GD or other thyroid or nonthyroid autoimmune diseases (i.e. lupus, rheumatoid arthritis, vitiligo, pernicious anemia, etc.) was also ascertained.

Results

Study population and validation

A past diagnosis of GD in 118 individual twin respondents was reported by the members of 110 twin pairs (220 individuals). In 62 of these pairs, self-reports were available from

all 124 individuals, and 70 were reported to be affected. A single twin responded to the original questionnaire from each of 48 other pairs; 35 of them reported GD in themselves, and 14 did so in reference to their nonrespondent co-twin. Each of the 220 individuals, including the 118 reported cases, was sought for interview. Of the 118 initial case reports of GD, 105 were available for review. Of the 110 identified pairs, 11 co-twins of cases were never at risk of GD because of death, thyroidectomy, or invalid reporting. Two of the remaining 99 identified pairs were uncertain of their zygosity and DNA "fingerprinting" was offered to and accepted by them (GeneLex, Seattle, WA). The final zygosity distribution of the 99 identified pairs was 36 MZ (29 females, 7 male), and 63 DZ (25 like-gender female, 5 like-gender male, and 33 unlike-gender pairs). A history consistent with the occurrence of Graves' eye disease was reported on in 42 (45%) of the affected population (15 MZ and 27 DZ). The average age at confirmatory interview of affected pairs was 52.0 (MZ) and 48.7 (DZ) years while the average age at the time of diagnosis of validated GD cases was 36.6 (MZ) and 34.0 (DZ) years. Of the 99 identified pairs, 8 were of Latino, 5 of African, 3 of Asian, and the remainder of European heritage. The gender ratio (F/M) of the final rosters of both the 106 identified and 96 confirmed cases was 2.4/1.

Age-specific cumulative incidence

Cumulative incidence through a specific age provides a rough measure of the risk to a population. Of the 106 identified cases derived from the 13,708 twins surveyed, 77 were female and 29 were male, resulting in age-specific cumulative incidence estimates of 0.61% and 0.20% at roughly age 40, respectively, and an overall gender-adjusted estimate of 0.40%.

Familial cases

In the initial questionnaire, 12 male and 26 female spouses of respondents were reported to have been diagnosed with GD, indicating a gender-adjusted cumulative incidence of 0.20% in adult persons from presumably unaffected families (Table 1). However, no conjugal cases were reported in the spouses of affected twins. In addition, 19 of the twin pairs who reported GD in themselves indicated that GD had occurred in at least one first-degree relative. This provides a cumulative incidence (recurrence rate) of 3.9%, which is more than 10 times that found in the families of unaffected twins. Familial cases were reported by two (both MZ) of the seven twin pairs concordant for GD. GD in one or more members of the immediate family was reported by 90 twin pairs who were themselves unaffected (Table 1).

Concordance

All 14 cases in 7 concordant pairs were confirmed by interview, and no other concordant pairs were identified or projected to exist. Six MZ pairs (5 female, 1 male) were concordant for GD; whereas one concordant DZ pair (a male-female pair) was found. The average age at diagnosis of concordant pairs was 33 years (range, 12–46); 4 of the 14 diagnoses were established as teenagers. The average interval between diagnoses in each pair was 11.6 years, although two pairs were diagnosed within a year of each other. Based on the identified pairs, the pairwise and probandwise concordance for the 36 MZ pairs were 0.17 and 0.29 and for the 62 DZ pairs were 0.019 and 0.036, respectively (Table 2).

Occurrence of chronic thyroiditis

Five (17%) of the GD-unaffected MZ twins and 1 (2.1%) of the GD-unaffected DZ co-twins reported a history consistent with chronic thyroiditis. In addition, 4 (4.2%) of the 96 validated cases of GD themselves also reported a history consistent with this condition.

Occurrence of nonthyroid autoimmune diseases

Among the 29 unaffected identical co-twins of GD cases interviewed, 3 (10.2%) reported having a nonthyroid autoimmune disease. Two reported having been diagnosed with systemic lupus erythematosus (SLE) and 1 reported both idiopathic thrombocytopenic purpura (ITP) and pernicious anemia (PA). One of the 47 (4.2%) unaffected fraternal twins of GD cases interviewed also reported ITP, and among the 96 validated cases of GD were 2 with ITP, 1 with SLE, and 1 with PA. In contrast, among the 27,108 twins in respondent pairs unaffected by GD, 83 or 0.3% reported having had either SLE, ITP, or PA, a significantly lower rate than that observed in all GD cases, or in the MZ twins of GD cases (95% $p = <0.001$). However, there was no apparent excess of multiple sclerosis or inflammatory bowel disease reported, and self-reports of rheumatoid arthritis and insulin-

Table 1. Graves' Disease in the Families of California Twins

	# *Cases*	*Persons at risk*	*Age-specific cumulative incidence*
All male twins in respondent pairs	30	14,756	0.0020
All female twins in respondent pairs	76	12,660	0.0060
All twins gender-adjusted (equal numbers by gender)			0.0040
Male spouses of married respondents	12	7,044	0.0017
Female spouses of married respondents	26	7,031	0.0037
As above, gender-adjusted (equal numbers by gender)			0.0027
Spouses of validated twin cases	0	99	0
First-degree relatives of unaffected pairs	90	78,433	0.0011
First-degree relatives of affected pairs	19	488	0.039

Table 2. Concordance for Graves' Disease and Graves' Disease and Chronic Thyroiditis Combined

Twins	*Second twin diagnosis*	*Concordant pairs*	*All pairs*	*Pairwise concordance*	*Probandwise concordance*
MZ	Confirmed GD	6	35	17%	29%
DZ	Confirmed GD	1	54	1.9%	3.6%
MZ	GD or chronic thyroiditis	11	35	31%	48%
DZ	GD or chronic thyroiditis	2	54	3.7%	7.1%

MZ, monozygotic; DZ, dizygotic.

dependent diabetes were considered unreliable. No attempt was made to validate these other reported nonthyroid autoimmune conditions.

Discussion

Age-specific cumulative incidence

No direct comparisons with our estimates of cumulative incidence of GD are currently available in the medical literature, however, studies have estimated the prevalence and the annual incidence rates. The Whickham study estimated the prevalence of hyperthyroidism to be 1.1% in a cohort from Great Britain (18). This study population was basically similar to the present one but comprised 56% females compared to 46% in the present study. The difference in the gender distribution of subjects as well as the other study's apparent lack of differentiation between GD and nonautoimmune hyperthyroidism likely contribute to some of the discrepancy in the rate of GD.

In another study reported from Olmstead County, Minnesota, a relatively constant annual incidence rates 22.6 per 100,000 (averages, 15–30 years) would lead to an estimated cumulative incidence for both sexes of 0.56% (19). This value is comparable to cumulative estimates of 0.40% seen in the present study and would support the validity of the survey used in the present investigation.

Familiality and concordance

MZ twins always share the entire genome, and to the extent that concordance is incomplete, there is an inferred contribution of nonheritable factors. The interpretation of nonheritable influences rests on the assumption that environmental and/or random (stochastic) elements play an important etiological role in the development of the phenotype. The known random aspect of the immune system response is consistent with the concept that stochastic processes are most likely involved.

The precise nature of the genetic contribution to GD largely remains an unanswered question. Initial attempts to identify the specific genetic locus or loci responsible for the heritability of GD focused on the HLA region on chromosome 6, a credible possibility because of the role played in the pathogenesis of other autoimmune diseases (20,21). Although the haplotype containing DR3 (DR17) has been observed to segregate with GD (22), this linkage was not consistent in all ethnic groups (23–25). Loci on other chromosomes that have been given attention include those regulating the production of the thyrotropin receptor (TSH-R) (26), thyroid peroxidase (27), an interleukin-1 (IL-1) antagonist (28), and cytotoxic T lymphocyte Antigen 4 (CTLA-4) (29). The first two of these are logical candidates if the thyroid gland itself plays an essential role. However, to date, study of these loci has produced no evidence of linkage to GD. The genes responsible for producing the IL-1 antagonist, which contributes to the regulation of IL-1 activity, and the CTLA-4 gene product, which modifies T-cell activation and proliferation, have both been linked to GD. However, whether variations in these two loci serve as the principle source of the genetic predisposition to GD remains to be defined. More recently, linkage of GD to a locus (GD-1) on chromosome 14 near the gene encoding for the TSH-R has also been proposed (30). The impact of this newer genetic information on the interpretation of the present investigation would reinforce the concept that the heritable aspects of GD are most likely polygenetic. Indeed, this may well account, in part, for the 10-fold higher concordance rates for GD between MZ and DZ observed in this study and others (15).

Chronic thyroiditis and other autoimmune diseases

A somewhat unexpected reported finding was the high prevalence of chronic thyroiditis and other nonthyroid autoimmune diseases in GD cases in the unaffected identical twins. The observed cumulative incidence of 17% chronic thyroiditis and 10% (nonthyroid autoimmune disease) in cotwins both approximate the 16.7% pairwise concordance observed for GD itself and is much higher than the prevalence in all twins or the DZ twins. Combining the concordance data for GD with the cumulative incidences of CT produces pairwise concordance rates of 31% and 3.7% in MZ and DZ twins, respectively (Table 2). This finding strongly suggests that these diseases share some common genetic foundation. While a common genetic determinant for these two autoimmune thyroid diseases has been previously postulated (24), definitive proof of such genetic commonality remains elusive. There are many case reports in the literature that describe twins and families in which both diseases coexist (9–13). Additionally, this study and as well as numerous other reports demonstrate instances where GD and chronic thyroiditis occur in the same patient, further suggesting commonality between these disorders (2,31–33). A recent study preformed on a Japanese population demonstrated a specific CTLA-4 polymorphism to be more common in patients with GD and chronic thyroiditis compared to control subjects (34). However, genetic linkage analysis has yet only produced one common locus for GD and chronic thyroiditis that is near to, but distinct from, the HLA region (35). One possible explanation for this apparent discrepancy is that these disorders are polygenetic and no single gene alteration is sufficient or

necessary for the expression of disease. In this situation it may be difficult to detect specific susceptibility loci by family-based linkage analysis as this method is better suited for detecting genes that exert a dominant influence on a disease.

In addition, SLE (36), ITP (37), and PA (38) have all been previously reported to be associated with GD, but their excessive occurrence in the MZ twins of cases strongly suggests that the coincidence is due to shared heritability. These findings also underscore the need to undertake a more focused and detailed future investigation to verify the validity of these observations.

Nonheritable determinants

Because a sizable majority of persons with a susceptible genome go through life unaffected by symptomatic GD, the correspondence between genotype and phenotype is not high. While the factors that determine the development of a clinical phenotype may be in whole, or in part a purely stochastic (random) immunologic process, it is rational to assume that environmental factors are also likely to be involved.

The idea that environmental factor(s) might initiate GD is not new. Caleb Hiller Parry in 1803, first proposed a putative link between environmental stress and GD (39) that more recently has been reinterpreted as a stress-induced immune suppression followed by a "rebound" of hyperactivity (14,40). Supporting this concept is a reported GD "outbreak" in Eastern Serbia between 1992 and 1995 attributed to the stress brought on by civil war (41). Also, variations in dietary iodine intake have been implicated (42,43) but have been questioned by others (44,45). Recently, smoking tobacco has been shown to be a risk factor for GD, especially for ophthalmopathy (46). Sex hormonal influences have also been proposed to explain the female preponderance in GD, as in other autoimmune diseases. The amelioration of GD during pregnancy and the postpartum exacerbation suggests a possible role for sex hormones as well as prolactin (47). However, a more plausible mechanism has centered on X-inactivation and the establishment of a T-cell repertoire inappropriately directed at the thyroid receptor (48).

Some recent attention has also been given to the possibility that some infectious agent or agents may play a role in the etiology of GD. Reports of apparent "outbreaks" of GD have further implicated such a possibility (49,50). Although one population-based search failed to find evidence that GD incidence is nonrandomly clustered in time and space (51), such infectious agents may be environmentally omnipresent making clustering unlikely. Some studies of specific agents have implicated viral agents, including retroviruses (52–54). Among bacteria, particular attention has been focused on the widely disseminated enteric bacterium, *Yersinia enterocolitica*, on the basis that serum antibodies that react with TSHR found in patients with GD also cross-react against this organism (55–57). Although such molecular mimicry of the TSHR by *Yersinia* proteins provides a plausible mechanism for GD causation, at this stage the relationship between cause and effect remains largely hypothetical (3).

Despite the evidence presented above regarding potential environmental triggers, it also appears reasonable that random events alone may be responsible for the clinical development of GD. Presumably, such a process would involve an intrinsically determined variability in the immunologic response to autoantigens that would be randomly expressed over time in a population genetically susceptible to GD. Supporting this view are the close concordance values observed in DZ twins (3.2%) and in first-degree relatives (3.9%) suggesting that an environmental component is not likely to be etiologically important. The similar findings of the present California-based study and that reported in the Danish survey (15) adds further support to this concept as well. In essence, the assumption of the Graves phenotype may simply prove to result from stochastic events occurring in genetically susceptible individuals.

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