



Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study

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Background Autoimmune mechanisms are thought to have a major role in the pathogenesis of multiple sclerosis. We aimed to identify coexisting autoimmune phenotypes in patients with multiple sclerosis from families with several members with the disease and in their first-degree relatives.

Methods A total of 176 families (386 individuals and 1107 first-degree relatives) were characterised for a history of other autoimmune disorders. Family-based or case-control analyses were done to assess the association of cytotoxic T-lymphocyte-antigen 4 (*CTLA4*) and protein tyrosine phosphatase (*PTPN22*) variants with susceptibility to multiple sclerosis.

Findings 46 (26%) index cases reported at least one coexisting autoimmune disorder. The most common were Hashimoto thyroiditis (10%), psoriasis (6%), inflammatory bowel disease (3%), and rheumatoid arthritis (2%). 112 (64%) families with a history of multiple sclerosis reported autoimmune disorders (excluding multiple sclerosis) in one or more first-degree relatives, whereas 64 (36%) families reported no history of autoimmunity. Similar to index cases, Hashimoto thyroiditis, psoriasis, and inflammatory bowel disease were also the most common disorders occurring in family members. A common variant within *CTLA4* was strongly associated with multiple sclerosis in families who had other autoimmune diseases ($p=0.009$) but not in families without a history of other autoimmune disorders ($p=0.90$).

Interpretation The presence of various immune disorders in families with several members with multiple sclerosis suggests that the disease might arise on a background of a generalised susceptibility to autoimmunity. This distinct multiple-sclerosis phenotype, defined by its association with other autoimmune diseases, segregates with specific genotypes that could underlie the common susceptibility.

Introduction

Much indirect evidence lends support to the concept that multiple sclerosis is an organ-specific autoimmune disease.¹ However, the possibility that the disease might arise from a general susceptibility to autoimmunity has received little attention. Clustering of multiple autoimmune diseases within individual families has been described² and such families are increasingly being recognised as a valuable resource for understanding the genetic factors that lead to autoimmunity. Strong evidence for familial clustering has been shown for autoimmune thyroid disease (especially Hashimoto thyroiditis), rheumatoid arthritis, and type-1 diabetes mellitus.^{3,4} Associations between multiple sclerosis and other autoimmune disorders have also been suggested. Some large studies and case reports have reported the coexistence of multiple sclerosis in individuals with systemic lupus erythematosus, psoriasis, rheumatoid arthritis, type-1 diabetes mellitus, autoimmune thyroid disease,^{5–9} ankylosing spondylitis,¹⁰ pemphigus vulgaris,¹¹ scleroderma,¹² and primary biliary cirrhosis.¹³ Broadley and colleagues¹⁴ investigated the prevalence of autoimmune disease in first-degree relatives of individuals with and without multiple sclerosis. An excess of other autoimmune diseases within families with multiple sclerosis was reported, whereas the prevalence of other chronic, but non-autoimmune, diseases was not increased.

The most important genetic locus associated with susceptibility to most autoimmune diseases is the MHC. Different autoimmune phenotypes have long been reported to be associated with distinct MHC haplotypes.¹⁵ More recently, two non-MHC candidate genes, cytotoxic T-lymphocyte-antigen 4 (*CTLA4*) and protein tyrosine phosphatase (*PTPN22*, also called *LYP*), have been proposed as susceptibility factors for autoimmunity.¹⁶ *CTLA4*, located on chromosome 2q33, is a cell-surface molecule implicated in the down regulation of T-cell responses. A functional single-nucleotide polymorphism within *CTLA4* (designated CT60 or rs3087243) has been convincingly associated with autoimmune thyroid disease and type-1 diabetes mellitus¹⁷ and is also a potential contributor to other autoimmune diseases, including Addison's disease, coeliac disease, primary biliary cirrhosis, systemic lupus erythematosus, and rheumatoid arthritis.^{18,19} Similarly, a functional variant within *PTPN22* (rs2476601), located on chromosome 1p13, confers risk of developing diverse autoimmune diseases, including type-1 diabetes mellitus, rheumatoid arthritis, autoimmune thyroid disease, and systemic lupus erythematosus.²⁰ Notably, recent studies, including our own, have not shown a role for either *CTLA4* or *PTPN22* in multiple sclerosis.^{21,22}

We aimed to identify patterns of coexisting autoimmune phenotypes in people with multiple

sclerosis belonging to a large and well-characterised dataset of families with multiple family members with the disease and in their first-degree relatives. Additionally, we investigated whether variants within *CTLA4* and *PTPN22* were associated with multiple sclerosis in families with a history of additional autoimmune diseases.

Methods

Patients and procedures

The dataset consisted of 184 US families, which contained two or more members with stringently ascertained and clinically characterised multiple sclerosis.²³ Index cases from each family were contacted, unless they were deceased in which case a sibling was contacted to obtain the history of first-degree relatives. Information was obtained from the one index case per family with a comprehensive questionnaire sent by mail. A follow-up phone call was made to index cases who had not responded within 6 weeks, at which time the option of completing the survey over the phone with the assistance of an investigator was offered. The survey requested information about 31 recognised autoimmune or immune-mediated disorders (table 1),^{24–29} selected because of their high prevalence, association with diagnostic autoantibodies that would be useful for further studies, and to investigate both positive and negative findings in previous reports.¹⁴ Inflammatory bowel disease (both Crohn's disease and ulcerative colitis) were included because of previous studies linking these disorders with multiple sclerosis.^{30,31} Atopic asthma was also included because of inconsistent reports of a reduced prevalence of asthma in patients with multiple sclerosis.^{32,33} Age of onset was ascertained to help differentiate between type-1 and type-2 diabetes mellitus. Careful attention was also paid to reported diagnoses of rheumatoid arthritis to distinguish this disorder from osteoarthritis. Type-2 diabetes and osteoarthritis are not deemed autoimmune disorders and were not included in this study. Published prevalence estimates for the 31 disorders in this study are included in table 1. Because of substantial differences in study design these estimates cannot be directly compared with results from this study, but are provided for reference.

Respondents were asked to identify members of their immediate family, including self, parents, siblings, and children, with any history of one or more of these disorders. For each disease, respondents were asked whether a specific diagnosis had been determined by a physician. Participants were encouraged to contact all family members to reduce the possibility of under-reporting due to unawareness of a certain illness. A detailed explanation of each disorder, including general clinical symptoms and common treatment approaches (each averaging 40–50 words), was included with the questionnaire. On receipt of each completed survey, index cases reporting a history of any autoimmune

disease were contacted for a telephone interview. Investigators further clarified self-reported diagnoses by asking specific questions about each reported disease, including age of disease onset, whether specific lab tests were obtained, and the use of particular medication. All first-degree relatives reported to have a history of autoimmune disease were then contacted by telephone for confirmation. Where there was any doubt about a

	Prevalence*
Any autoimmune disease†	5–8%
Addison disease	0.005–0.014%
Adult still disease	≤0.001%
Alopecia areata	0.1–0.2%
Ankylosing spondylitis	0.13–0.2%
Asthma	5.3–9%
Autoimmune hepatitis	≤0.001%
Behçet syndrome	≤0.001%
CREST syndrome	≤0.001%
Diabetes (type 1)	0.19–0.3%
Graves' disease	1.2%
Guillain-Barré syndrome	≤0.001%
Hashimoto thyroiditis	0.8%
Idiopathic thrombocytopenic purpura	≤0.001%
Inflammatory bowel disease‡	0.25–0.65%
Meniere's disease	0.2%
Mixed connective tissue disease	0.002–0.02%
Myasthenia gravis	0.005%
Pemphigus vulgaris	0.001–0.005%
Pernicious anaemia	0.15%
Polymyositis/dermatomyositis	0.005%
Primary biliary cirrhosis	0.004%
Psoriasis	0.5–1.0%
Raynaud's disease	>4%
Reiter syndrome (reactive arthritis)	0.001–0.003%
Rheumatoid arthritis	0.50–1.0%
Sarcoidosis	0.04–0.15%
Sjögren's syndrome	0.1–0.5%
Systemic lupus erythematosus‡	0.04–0.12%
Systemic sclerosis (scleroderma)	0.004%
Uveitis/iritis	0.002%
Vasculitis	0.015%

*There is a lack of reliable figures for prevalence of autoimmune disease; prevalence estimates were derived from several sources and are not specific for sex or ethnic origin.^{14,24–29} A direct comparison cannot be made between published prevalence estimates and the results obtained in this study because of the significant differences in study design. These figures are just provided for reference. †Current estimates of the prevalence of all autoimmune diseases (now more than 80 recognised disorders) range from 5–8% of the US population. This corresponds to around 14–24 million people based on August, 2004, census bureau figures (NIH, Autoimmune Diseases Coordination Committee. Autoimmune Diseases Research Plan, March 2005). Previous estimates of 3–5% prevalence were based on fewer than 24 diseases.^{24–26} ‡Includes both systemic lupus erythematosus and discoid lupus. §Includes both Crohn's disease and ulcerative colitis.

Table 1: Autoimmune and other immune-mediated diseases included in the survey of families with multiple sclerosis

particular diagnosis, for example if a physician had not made the diagnosis, a conservative approach in which the reply was assumed to be negative was applied to prevent the inclusion of false-positive diagnoses.

Two large, well-characterised, multiple-sclerosis datasets,^{15,34} including 537 nuclear multiple sclerosis families with one case (total $n=2075$ individuals including 355 trios and 375 discordant sibpairs; all individuals and known ancestors were non-Hispanic whites of European descent) and an African-American case-control dataset (518 cases and 234 controls), were also used to investigate associations with the *CTLA4* single-nucleotide polymorphism (rs3087243). The multiple-sclerosis dataset for *PTPN22* analyses has been described previously.²²

All studies were approved by the Committee of Human Research (University of California at San Francisco) and the Committee of Protection of Human Subjects (University of California at Berkeley), and written informed consent was obtained from all participants.

Laboratory procedures

Genomic DNA was extracted from white-blood cells by standard procedures. Genotyping for *CTLA4* rs3087243 was undertaken with a Taqman Assay By Design (Applied Biosystems, Foster City, CA, USA) and the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Genotypes for the *PTPN22* rs2476601 variant were determined as previously described.²² Both assays were validated using samples of known genotype based on DNA sequencing. *HLA-DRB1* genotypes were determined as previously described.¹⁵

Statistical analysis

The prevalences of autoimmune diseases in multiple-sclerosis index cases, in all cases overall, and in cases stratified by sex were determined by direct counting of each observation. Bootstrap estimation was used to obtain the prevalence of each autoimmune disease in first-degree relatives. For each disease, the proportion of affected individuals was computed within each family (index cases were excluded). New samples of the same size were generated by randomly selecting, with replacement, from the total proportions (each family) 2000 times. The sampling was done with R version 1.9.1. An estimate of the mean frequency was taken from the resulting estimated sample distribution. The reported 95% CIs represent the 2.5 and 97.5 percentiles of the distributions generated for each disease.

P values, odds ratios (ORs), and 95% CIs for the χ^2 or Fisher's exact test were used to test autoimmune-disease distributions in multiple-sclerosis cases and families and for testing differences in *PTPN22* single-nucleotide-polymorphism allele distributions between multiple-sclerosis cases and control individuals. Values were derived with *PROC FREQ* (SAS version 9.1, Cary, NC). All family genotypes for *HLA-DRB1*, *CTLA4*, and *PTPN22*

were examined for Mendelian inconsistencies and any discrepancies were addressed. Suspect genotypes were reread or rerun. Additionally, genotypes from unrelated control individuals and all family founders were tested for Hardy Weinberg equilibrium using the exact test implemented in the PYPOP program. Family-based association analysis was done with the sum statistic from the pedigree disequilibrium test (PDT) version 5.1. The PDT can use data from related nuclear families within extended pedigrees, including multiple trios and discordant sibpairs, and is valid even when there is population substructure. Differences in mean sibship size, number of offspring, or total number of first-degree relatives between families categorised according to presence or absence of autoimmune-disease history were tested using *PROC TTEST* (SAS version 9.1, Cary, NC, USA).

Results

A total of 176 families participated in the study. Eight families were excluded because they did not respond to repeated attempts to contact them either by mail or telephone ($n=6$) or because they declined to participate ($n=2$). Data were gathered for 176 index cases, 1317 first-degree relatives (1107 without multiple sclerosis, 210 with multiple sclerosis) including 656 siblings (368 sisters and 288 brothers), 309 children (161 daughters and 148 sons), and 352 parents. This dataset included 366 patients with disease relapsing from onset and 20 primary-progressive cases. All individuals with primary-progressive multiple sclerosis were from different families. 45 families were large extended pedigrees with more than two multiple sclerosis cases. In these families, individuals with multiple sclerosis were not restricted to one nuclear family, although only one nuclear family (containing the index case or individual through whom the family was originally ascertained and regular contact has been maintained) was selected for investigation of autoimmune-disease history in first-degree relatives. The overall female to male ratio in multiple-sclerosis index cases ($n=176$) was 2.9:1 and was 2.6:1 in all multiple sclerosis cases ($n=386$), whereas for multiple sclerosis index cases with other coexisting autoimmune disorders the proportion of women was greater (4.2:1). Strong evidence for sex dimorphism was not observed in first-degree relatives without multiple sclerosis (1:1) or without other autoimmune disease (0.9:1). The mean age for all multiple-sclerosis index cases (53.5 years, SD 9.4 years) and index cases with coexisting autoimmune disease (52.7 years, SD 8.6 years) was closely similar, whereas the mean age for all first-degree relatives without multiple sclerosis or other autoimmune disorders (61.7 years, SD 15.9) was slightly higher. Differences in mean sibship size, number of offspring, or total number of first-degree relatives between families categorised according to presence or absence of autoimmune-disease

For more on the Python for Population Genetics see <http://allele5.biol.berkeley.edu/pypop>

For more on the PDT analysis program see <http://www.chg.duke.edu/research/pdt.html>

For R Foundation for Statistical Computing see <http://www.R-project.org>

history were not observed (all $p > 0.10$; data not shown), suggesting that ascertainment bias for the presence of other autoimmune disorders due to family composition was not present.

A total of 46 (26%) multiple-sclerosis index cases reported at least one coexisting autoimmune disorder; 38 (21%) reported one, four (2%) reported two, and four (2%) reported three or more coexisting autoimmune disorders (data not shown). Figure 1 shows the overall distribution for each individual autoimmune disease in all multiple sclerosis cases stratified by sex (n=386). The results obtained for multiple sclerosis index cases (n=176) were very similar (data not shown). Among the most common diseases present in the index cases and all cases overall were Hashimoto thyroiditis (10% and 7%), psoriasis (6% and 5%), inflammatory bowel disease (3% and 3%), and rheumatoid arthritis (2% and 2%). Most patients with multiple sclerosis who had co-occurring Hashimoto thyroiditis, rheumatoid arthritis, and inflammatory bowel disease were female; however, a large proportion of patients with psoriasis were male. Raynaud's disease was reported by 3% of multiple sclerosis index cases (4% in all cases). Around 8% of all patients with multiple sclerosis also reported having asthma. Other clinical phenotypes, including mean age of onset for multiple sclerosis symptoms, disability (measured by the multiple sclerosis severity score, as previously described³⁵), and proportion of patients with relapsing multiple sclerosis were similarly distributed in those with and without coexisting autoimmune diseases (data not shown).

A total of 112 (64%) multiple-sclerosis families reported at least one case or first-degree relative with an autoimmune disease (102 [58%] families, if restricted to non-multiple sclerosis members only), whereas 64 (36%) families had no history of autoimmunity other than multiple sclerosis. In addition to multiple sclerosis, 48 (27%) families reported one, and 31 (18%) reported two autoimmune diseases. Remarkably, 24 (14%) families reported three or more autoimmune diseases; in three families six or more disorders were identified in first-degree relatives of the index case. One family reported four different autoimmune diseases in first-degree relatives (nine cases), including Hashimoto thyroiditis (n=6), rheumatoid arthritis (n=1), psoriasis (n=1), and pernicious anaemia (n=1).

We estimated an overall prevalence for each individual disease using all first-degree relatives of multiple-sclerosis index cases (n=1317 individuals; figure 2). As seen in patients with multiple sclerosis, Hashimoto thyroiditis was the most common autoimmune disease in family members (3%), followed by psoriasis (2%), inflammatory bowel disease (2%), and rheumatoid arthritis (2%). Family members with multiple sclerosis were also stratified by sex to look at differences in frequency (data not shown). Hashimoto thyroiditis and

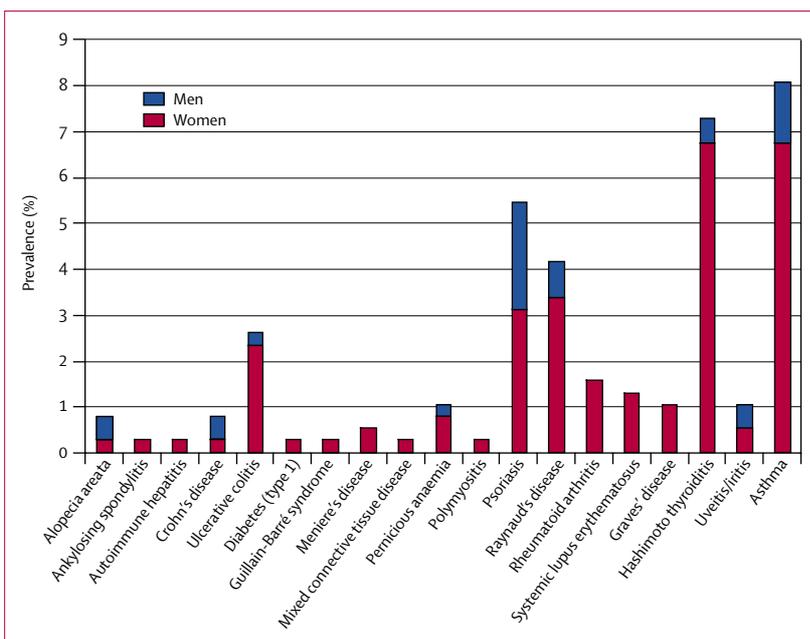


Figure 1: Autoimmune diseases reported in patients with multiple sclerosis from 176 families

rheumatoid arthritis were both more common in female than in male first-degree relatives, as expected (5% vs 2% and 3% vs 0.4%, respectively). Ankylosing spondylitis was present (0.3%) in male family members only.

Overall, the occurrence of any autoimmune disease in first-degree family members was 3.5 times more likely when index multiple-sclerosis cases also reported a coexisting autoimmune disease (OR 3.5, 95% CI 1.6–7.6; $p = 0.002$). A few specific autoimmune diseases also seemed to be more common in first-degree relatives

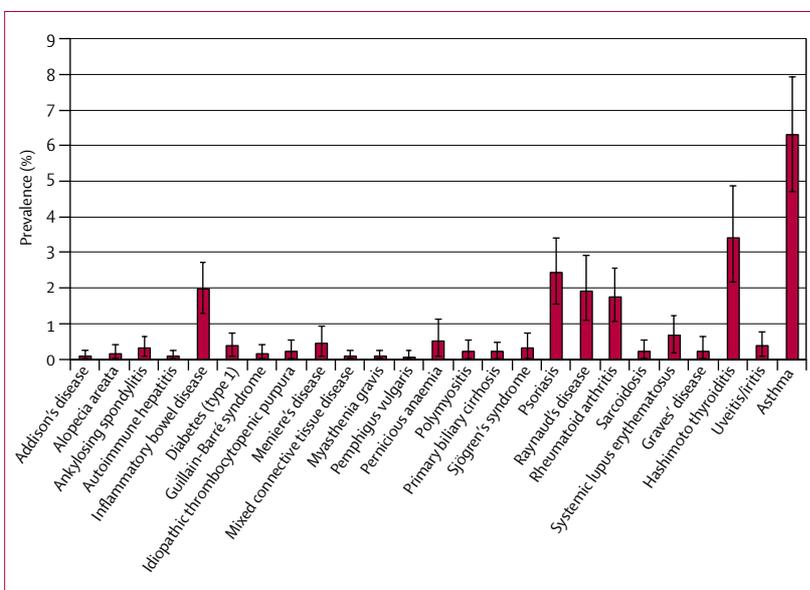


Figure 2: Estimated prevalence of autoimmune disease among first-degree relatives

	Families (n)	Trios (n)	Trios ratio of T:NT	p
All multicase MS families†	185	208	256:232	0.11
Multicase MS families without AD history‡	34	66	70:69	0.90
Multicase MS families with AD history	46	93	127:106	0.02
Multicase MS families with AD history; MS cases with coexisting disorders excluded from analysis	36	52	76:58	0.009
Single-case MS families§	537	355	384:382	0.73

T=transmitted. NT=non-transmitted allele counts. MS=multiple sclerosis. AD=autoimmune disease. *CTLA4 rs3087243 (G→A) single-nucleotide polymorphism minor-allele frequency=0.42 (unrelated multicase MS family founders). †A total of 185 multicase MS families (208 trios and 1068 discordant sibpairs) were available for genetic analysis of the CTLA4 rs3087243 single-nucleotide polymorphism. Analyses were done with the pedigree disequilibrium test, or PDT version 5.1. Results are shown for analyses of all MS families including trios and discordant sibpairs. Results were very similar when analyses of CTLA4 were restricted to trio families only (p=0.09). ‡Autoimmune disease history and CTLA4 single-nucleotide polymorphism data were available for 159 families: 101 (64%) with positive and 58 (36%) with negative history. Families were stratified according to AD history for analysis of CTLA4 rs308724. Because many siblings without MS also had other autoimmune disorders, PDT analyses were restricted to MS trios only to reduce heterogeneity (discordant sibpairs were not included) in order to examine transmission of the G allele specifically to MS cases. Multiple trios were present in MS families due to the presence of multiple affected sibs within a sibship (see Methods). §Results were obtained for CTLA4 rs3087243 with 537 single-case MS families (355 trios and 375 discordant sibpairs; shown above), and were very similar when analyses were restricted to trio families only (p=0.91).

Table 2: Results from PDT analyses of CTLA4 rs3087243* in families with multiple sclerosis

	Individuals (n)	Alleles	2N (%)
All MS cases from multicase families	369	C	684 (93%)
		T	54 (7%)
Index cases from multicase families	169	C	314 (93%)
		T	24 (7%)
All cases without AD history	123	C	238 (97%)
		T	8 (3%)
All cases with AD history	246	C	446 (91%)
		T	46 (9%)
All cases with family history of seven AD†	151	C	274 (91%)
		T	28 (9%)
All cases with family history of seven AD‡	113	C	206 (91%)
		T	20 (9%)
MS cases from single-case families	563	C	1016 (90%)
		T	110 (9%)
Controls§	374	C	676 (90%)
		T	72 (10%)

MS= multiple sclerosis; AD=autoimmune disease. *Data for the PTPN22 rs2476601 (C→T) variant was available for 169 multicase MS families and 374 single case MS families as previously described.²² †Analyses were restricted to MS cases with family history of seven specific AD that have shown strong evidence for association with PTPN22 (Hashimoto thyroiditis, Graves' disease, Addison's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, and type-1 diabetes). ‡Analyses were restricted to MS cases with family history of seven specific AD that have shown strong evidence for association with PTPN22 (Hashimoto thyroiditis, Graves' disease, Addison's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, and type-1 diabetes); however, cases with coexisting conditions were removed from the analyses. §PTPN22 rs2476601 control frequencies were derived from non-transmitted parental chromosomes present in the single-case MS families. For this calculation, independent trios with parental genotyped data were derived from the larger single-case family dataset.

Table 3: MS case and control frequencies for the PTPN22 (rs2476601) C→T single-nucleotide polymorphism*

when present in index cases. When individuals with multiple sclerosis reported Hashimoto thyroiditis or psoriasis, the odds that these individual conditions would be reported for family members were 4.8 times (OR 4.8, 95% CI 1.7–13.5; p=0.005) and 4.4 times (4.4, 1.1–16.7; p=0.04) greater than for families of cases in which these diagnoses were not present.

We investigated whether common *CTLA4* (CT60 or rs3087243) or *PTPN22* (rs2476601) polymorphisms associated with other autoimmune disorders were associated in multiple-sclerosis families or multiple-sclerosis cases, respectively (table 2). No evidence for an association with *CTLA4* was observed when all multicase families were considered (p=0.11). Similarly, results from analyses of both families with only a single case of multiple sclerosis (p=0.73) and the African-American multiple sclerosis case-control dataset (518 cases and 234 controls; data not shown, p=0.47) were also negative. However, when families with several cases of multiple sclerosis were stratified by presence or absence of other autoimmune diseases, over-transmission of the CT60 variant (SNP allele 1 or G) was present in the autoimmune-prone families (p=0.02) and remained significant even after patients with multiple sclerosis who had coexisting autoimmune diseases were removed from the analyses (p=0.009). The association was not present in families with a negative history of autoimmune disease in either patients with multiple sclerosis or first-degree family members (p=0.90).

We have previously reported that there is no association between multiple-sclerosis susceptibility and *PTPN22* R620W.²² Because of the low frequency of this variant, the current study lacked sufficient power for a family-based association analysis stratified by presence or absence of a history of autoimmune disorders. However, allele frequencies from patients with multiple sclerosis, grouped according to history of autoimmunity, were examined. As shown in table 3, allele frequencies for the *PTPN22* Arg620Trp variant were similar in individuals with multiple sclerosis from single-case families and in population controls (10%). This variant was less common in people with multiple sclerosis without a history of other autoimmune disorders (3% in all cases, n=123, and 4% in index cases only, n=59) than in controls. However, this difference was only nominally significant (p=0.05).

By contrast with results observed for *CTLA4* (table 2), significant evidence for over-transmission of the multiple sclerosis-associated *HLA-DRB1*15* allele was present in all multicase family strata (data not shown). Results for families with no family history of autoimmune disease (31 families, 61 trios; p=0.03) and those with a family history of autoimmune disease (52 families, 105 trios; p=0.0008) were very similar, taking into account the differences in family sample size. Furthermore, no evidence for over-transmission of other *DRB1* alleles (total=15) in either family group was observed.

Discussion

A well-characterised familial dataset, recruited for the presence of two or more cases of clinically definite multiple sclerosis, was investigated to determine whether additional autoimmune diseases were present in cases and first-degree relatives. About a quarter of individuals

with multiple sclerosis reported a diagnosis of at least one other autoimmune disease. Hashimoto thyroiditis, psoriasis, inflammatory bowel disease, and rheumatoid arthritis were commonly present in patients with multiple sclerosis. Although an accurate prevalence estimate for autoimmune disease in familial cases of multiple sclerosis would require a much larger number of study participants and careful adjustment for age and sex, overall, the proportion of multiple sclerosis cases with coexisting autoimmune diseases, and frequencies for particular disorders reported in this study, were higher than expected when compared with population-based data (table 1).^{14,24–29} Additional support for an autoimmunity-prone background in familial multiple sclerosis was obtained by characterisation of other autoimmune diseases in first-degree relatives; more than 60% of families contained at least one member (case, parent, sibling of child) who reported another autoimmune disease diagnosis. This study relies on comparisons with historical epidemiological data, thus a weakness is a lack of a contemporary control group.

Although this was the first large study of autoimmune disease in familial multiple-sclerosis patients, several earlier studies in unselected cases of multiple sclerosis, including a large outpatient cohort³⁶ and two small case-control studies,^{9,37} have suggested a higher than expected prevalence of autoimmunity. Several studies have also reported that autoimmunity occurs in nuclear families of multiple-sclerosis cases.^{14,37,38} A study in the UK reported no difference in autoimmune disease prevalence rates in patients with multiple sclerosis compared with controls or population data.¹⁴ However, only 32 of the 571 (11%) UK index multiple sclerosis cases were derived from families with several cases of multiple sclerosis, by contrast with the current multicase study. Thus, an inherent susceptibility to multiple autoimmune diseases seems to be a characteristic of familial, but not sporadic, multiple-sclerosis cohorts.

Hashimoto thyroiditis was the most prevalent autoimmune disease in index cases and in first-degree family members; patients with multiple sclerosis with a diagnosis of Hashimoto thyroiditis were also more likely to have first-degree relatives with this disease—a total of 42 (24%) families reported one or more diagnoses of Hashimoto thyroiditis. Increased rates of autoimmune thyroid disease in patients with multiple sclerosis and family members have also been reported elsewhere.^{14,36,38,39} Additionally, individuals with multiple sclerosis and family members from the current study had significantly higher titres of thyroid-related autoantibodies than healthy control individuals with no history of multiple sclerosis (unpublished). In this regard, it is noteworthy that a phase-2 trial of an anti-CD52 monoclonal antibody (CAMPATH-1H) that targets T and B lymphocytes produced autoimmune thyroid (Graves) disease as an apparent side-effect of treatment in a third of patients treated for multiple sclerosis, whereas this complication

was not observed in non-multiple sclerosis individuals who were given this drug for other indications.⁴⁰ Collectively, this evidence strongly suggests a specific relation between multiple sclerosis and autoimmune thyroid disease.

An increased risk of psoriasis within multiple-sclerosis families, previously suggested by others,^{2,9,14} was also identified in the current study. As with Hashimoto thyroiditis, patients with multiple sclerosis with a diagnosis of psoriasis were more likely to have first-degree relatives with this disorder; 18% (n=32) of families had one or more diagnoses of psoriasis, providing additional evidence for an association between these two diseases. A total of 13% (n=22) of families reported rheumatoid arthritis and 17% (n=30) reported inflammatory bowel disease. Previous studies of multiple sclerosis with inflammatory bowel disease have provided results that lend support to an association,^{2,41,42} although these results are conflicting.¹⁴ Most recently, a population based case-control study undertaken in the University of Manitoba IBD database,⁴³ and both retrospective cohort and cross-sectional studies from the General Practice Research Database in the UK,⁴⁴ strongly lend support to an association between multiple sclerosis and inflammatory bowel disease. Ulcerative colitis, specifically, shows the most compelling association with multiple sclerosis and other demyelinating diseases, including optic neuritis.⁴⁴ Asthma was also common overall in the multiple-sclerosis family cohort, similar to a recent study,³⁶ and was reported more often by families with a history of autoimmune disease (47/112 [42%] families) than by families with no history (11/64 [17%] families). Much evidence suggests that asthma and autoimmunity could share a common pathophysiological mechanism^{33,45} and the current data potentially extend these associations to multiple sclerosis. The association between multiple sclerosis, traditionally considered a Th1-mediated disease, and asthma, a Th2-mediated disease, was unexpected. Further studies will be needed to fully clarify this finding.

A diagnosis of type-1 diabetes was reported in only one patient with multiple sclerosis and was present in very few first-degree relatives, despite the large numbers of individuals who were surveyed. These results are in contrast with those from studies of multiple sclerosis in Sardinia where diabetes prevalence is high in both individuals with multiple sclerosis and those from families with several members with multiple sclerosis.⁴⁶ Although other reports also lend support to an association between type-1 diabetes and multiple sclerosis,⁴⁷ a high frequency of the *HLA-DR2* haplotype (*DRB1*15* allele) found in northern Europeans and, in particular, observed in the families with multiple cases of multiple sclerosis studied here (around 70% carrier frequency in patients)²³ would be expected to confer protection for this disease.⁴⁸ The multiple-sclerosis patient with type-1 diabetes observed in this study carried the type-1 diabetes risk *HLA-DRB1*03/04* genotype.

The *CT60* variant within *CTLA4* has been well established as a genetic risk factor for Graves' disease, type-1 diabetes, and rheumatoid arthritis.^{17,19} A significant association was observed here for *CT60* with multiple sclerosis, and was restricted to those families with a history of autoimmune disease in first-degree relatives. By using varying approaches, including large family-based and meta-analytical strategies, most studies of *CTLA4* have not been able to establish any consistent role for this locus in multiple sclerosis.^{21,49,50} Although some investigations have focused on disease course and severity in addition to risk, none to date has stratified families or cases according to family history of other autoimmune diseases.

Familial clustering of autoimmune diseases within families could be explained by shared genotypes, shared environmental exposures, or some combination of both. Independent genome-wide linkage searches of several autoimmune disorders, in addition to multiple sclerosis, have been reported, revealing complex patterns compared with traditional linkage studies of monogenic diseases. Clinical or phenotypic heterogeneity has also probably contributed to the disparity observed between linkage screens in multiple sclerosis where different loci might be contributing to particular disease phenotypes. Although results obtained from the largest linkage screen in families with several cases of multiple sclerosis indicate that only very modest genetic effects are operating in multiple sclerosis,⁵¹ consideration of even a stringent diagnosis of multiple sclerosis as a single phenotype might not be optimal. In genome screens of families with several members affected by systemic lupus erythematosus stratified by distinct phenotypic features such as the presence of vitiligo or rheumatoid arthritis, additional prominent regions of linkage have also been identified and await confirmation.^{52,53}

The problem of heterogeneity represents a key challenge to elucidating the cause of multiple sclerosis. The current study indicates that a distinct multiple-sclerosis phenotype could be defined by a general susceptibility to autoimmunity and affected by *CTLA4*. Careful characterisation of comorbid autoimmune disorders in multiple sclerosis cases and clustering of particular diseases in family members seems to be important for defining the multiple sclerosis phenotype. Large family-based or case-control studies that incorporate this clinical information will be extremely valuable for disease-gene identification and genotypic-phenotypic correlations in multiple sclerosis.

Contributors

L F Barcellos, J R Oksenberg, and S L Hauser were involved in all aspects of the design and execution of the study, including data analysis, interpretation, and writing of the manuscript. B Kamdar participated in the study design and data collection. P P Ramsay, S Schmidt, J Haines, and M Pericak-Vance undertook data analysis. R Lincoln prepared, stored, and databased samples. C DeLoa assisted with data generation. S Caillier assisted with genotyping for the study.

Conflicts of interest

We have no conflicts of interest.

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