Analysis of Families in the Multiple Autoimmune Disease Genetics Consortium (MADGC) Collection: the *PTPN22* 620W Allele Associates with Multiple Autoimmune Phenotypes

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Autoimmune disorders constitute a diverse group of phenotypes with overlapping features and a tendency toward familial aggregation. It is likely that common underlying genes are involved in these disorders. Until very recently, no specific alleles—aside from a few common human leukocyte antigen class II genes—had been identified that clearly associate with multiple different autoimmune diseases. In this study, we describe a unique collection of 265 multiplex families assembled by the Multiple Autoimmune Disease Genetics Consortium (MADGC). At least two of nine "core" autoimmune diseases are present in each of these families. These core diseases include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), multiple sclerosis (MS), autoimmune thyroid disease (Hashimoto thyroiditis or Graves disease), juvenile RA, inflammatory bowel disease (Crohn disease or ulcerative colitis), psoriasis, and primary Sjögren syndrome. We report that a recently described functional single-nucleotide polymorphism (*rs2476601*, encoding R620W) in the intracellular tyrosine phosphatase (PTPN22) confers risk of four separate autoimmune phenotypes in these families: T1D, RA, SLE, and Hashimoto thyroiditis. MS did not show association with the PTPN22 risk allele. These findings suggest a common underlying etiologic pathway for some, but not all, autoimmune disorders, and they suggest that MS may have a pathogenesis that is distinct from RA, SLE, and T1D. DNA and clinical data for the MADGC families are available to the scientific community; these data will provide a valuable resource for the dissection of the complex genetic factors that underlie the various autoimmune phenotypes.

Introduction

Autoimmune diseases share a number of characteristics that suggest common etiologic pathways or mechanisms, including reactivity to self-antigens by the humoral and/or cellular immune system, as well as genetic associations with human leukocyte antigen (HLA). Although familial clustering of autoimmunity has long been recognized, the patterns of aggregation across different autoimmune diseases have not been clearly delineated. Certain rare familial clusters of autoimmune phenotypes, such as the polyglandular autoimmune endocrinopathies, are known to have a common genetic basis (Eisenbarth and Gottlieb 2004). Among the more common autoimmune disorders, probably the best evidence for familial clustering among different autoimmune diseases involves rheumatoid arthritis (RA [MIM 180300]), autoimmune thyroid disease (AITD) (especially Hashimoto thyroiditis [MIM 140300]), and type 1 diabetes (T1D [MIM 222100]) (Torfs et al. 1986; Lin et al. 1998). However, the degree and significance of familial clustering among the larger group of autoimmune disorders is uncertain; only a few large or well-controlled epidemiological studies of this issue have been reported (Torfs et al. 1986; Ginn et al. 1998; Lin et al. 1998; Broadley et al. 2000), in part, because many of these diseases are relatively uncommon and have heterogeneous phenotypes that are difficult to confirm in population surveys.

Clustering of diseases within families may be explained by shared environmental exposures, shared genes, or interactions between genetic and environmental factors. A growing body of evidence suggests that exposure to cigarette smoke, for example, may increase the risk of multiple autoimmune diseases, including AITD (Vestergaard 2002), RA (Heliovaara et al. 1993; Symmons et al. 1997;

Table 1

Demographic Characteristics of 265 Multiplex Autoimmune
Families

Characteristic	Finding
Mean no. of affected individuals per family (range)	3.2 (2–9)
Mean total no. of individuals[a] enrolled per family (range)	5.7 (2–19)
Ethnicity[b] (% white)	96.6
No. from recruitment source (%):	
Patient advocacy group/publication	128 (48)
Other	80 (30)
Local recruitment[c]	57 (22)

[a] Affected plus unaffected.

[b] Other ethnicities included Hispanic (1.1%), mixed ethnicity (1.9%), and unknown ethnicity (0.4%).

[c] Enrollment at these sites included recruitment onsite (e.g., clinics) and offsite (e.g., patients known to investigators through other research activities).

Uhlig et al. 1999), systemic lupus erythematosus (SLE [MIM 152700]) (Hardy et al. 1998; Bengtsson et al. 2002), and multiple sclerosis (MS [MIM 126200]) (Riise et al. 2003). Exposure to crystalline silica (Steenland and Goldsmith 1995) and to Epstein-Barr virus infection (Harley and James 1999; James et al. 2001) also appears to influence the risk of multiple autoimmune diseases. In addition to these environmental factors, there is now increasing evidence that common genes may underlie autoimmunity. Becker and colleagues (1998) have described a pooled analysis of genome-screening results and have identified 18 distinct clusters with evidence of genetic linkage to two or more autoimmune or immune disorders. However, evidence that specific risk alleles are associated with multiple autoimmune diseases is relatively sparse. Even within the HLA system, different alleles are associated with different autoimmune phenotypes (Todd et al. 1988; Hall et al. 1996; Tan and Arnett 1998; Barcellos et al. 2003), and, indeed, it is not always clear that the HLA alleles are the only relevant risk factor within the major histocompatibility complex (MHC) (Jawaheer et al. 2002). Until very recently, *CTLA4* was the only gene that had been convincingly associated with two different autoimmune diseases—AITD and T1D—but the strength of these associations is rather modest, with odds ratios (ORs) of ~1.45 and ~1.15, respectively (Ueda et al. 2003).

In this study, we describe a unique registry of 265 multiplex families that have been specifically identified as having two or more different autoimmune diseases in the same family. Detailed clinical data for each family member—along with a blood sample—have been collected by the Multiple Autoimmune Disease Genetics Consortium (MADGC), under a contract with the National Institute of Allergy and Infectious Diseases (NIAID). Genetic analysis of these families reveals that

PTPN22 is associated with several different autoimmune disorders, confirming and extending several recent reports (Begovich et al. 2004; Bottini et al. 2004; Kyogoku et al. 2004). For the first time, we demonstrate an association with Hashimoto thyroiditis, a common form of AITD. This work establishes PTPN22 as the first major genetic variant that clearly confers substantial risk of multiple different autoimmune phenotypes, and it points the way to understanding common disease mechanisms. The MADGC collection of multiplex families is likely to provide an important scientific resource for further studies in this area.

Material and Methods

Multiplex Families: Entry Criteria and Approach to Recruitment

At the request of NIAID, investigators with expertise in the genetics of a wide range of autoimmune diseases were asked to serve as consultants for this effort and met with the study investigators at the National Institutes of Health on November 1, 1999 (see the "Acknowledgments" section for the names of participants). The primary goal of that meeting was to determine which autoimmune diseases should be included in the collection, on the basis of a variety of considerations, including strength of evidence for genetic susceptibility, clustering with other autoimmune diseases, population prevalence, impact on public health, and feasibility of collection. Eight "core" autoimmune diseases were targeted initially: adult RA, SLE, MS, AITD (Graves disease [MIM 275000] or Hashimoto thyroiditis), T1D, psoriasis (MIM 177900), inflammatory bowel disease (IBD [MIM 266600]) (Crohn disease or ulcerative colitis), and juvenile rheumatoid arthritis (JRA [MIM 604302]). Subsequently, primary Sjögren syndrome (SS [MIM 270150]) was added to this core list of diseases. To be eligible for inclusion in the registry/repository, the multiplex families were required to have at least two members affected with at least two of these nine core diseases. Furthermore, at least two of the affected members had to be informative for linkage (e.g., a single affected parent/offspring pair would not meet enrollment criteria).

Families were recruited from a variety of sources, including patient advocacy groups and publications (e.g., the American Autoimmune Related Diseases Association [AARDA] and the *Multiple Sclerosis Quarterly Report*), as well as other forms of publicity and direct mailing of flyers and posters to physicians' offices. Once a family was deemed eligible for inclusion, all individuals affected with any autoimmune disease (see the MADGC Web site) were invited to participate, as were all unaffected individuals who were likely to be useful for genetic link-

age or association studies. Informed consent was obtained from all enrolled subjects, and the study was approved by the institutional review boards at each of the three participating centers: North Shore University Hospital, University of Minnesota, and University of California San Francisco (UCSF).

Demographic and Clinical Information

Basic demographic information was collected from all enrolled subjects via telephone or mailed questionnaire. All subjects were also asked about the presence of any of the nine core diseases or other autoimmune diseases. Subjects who reported at least one of these conditions provided additional clinical information over the telephone, including date of diagnosis; name, address, and specialty of treating physician(s); and a history of the symptoms and treatment received for the disease(s). Specific lists of symptoms and treatments were developed for each of the nine core diseases with the assistance of the aforementioned consultants; these lists are available either by request from the authors or on the MADGC Web site. For other autoimmune diseases (i.e., noncore diseases), similar information was collected using a standardized form that was designed to be more general.

Confirmation of Autoimmune Disease Diagnoses

Autoimmune disease diagnoses were confirmed either by the treating physician or on the basis of a review of medical records by one of the study investigators or consultants. Criteria for diagnostic confirmation were those established either by the appropriate professional body (e.g., American College of Rheumatology) or on the advice of consultants. Standardized diagnostic confirmation forms were developed for each of the nine core diseases and were completed by the confirming physician/investigator. For use in documenting diagnosis of other autoimmune diseases, a more general form was developed for completion by the study physician/investigator. All subjects who reported an autoimmune disease that could not be confirmed were considered "unknown" for that disease, unless it was clear from the available information that the disease was not present. Subjects could be confirmed for more than one autoimmune disease, or they could be confirmed for one or more diseases and considered unknown for other autoimmune diseases.

Collection of Biological Material

All enrollees were asked to provide blood for processing and storage at a centralized biorepository located at the North Shore Long Island Jewish Research Institute. Samples were processed and were stored as plasma and as frozen viable peripheral blood mononuclear cells (PBMCs). After shipment of frozen PBMCs in batches,

immortalized lymphoblastoid cell lines were successfully established from the majority of samples at a second site (University of Minnesota). As a security precaution, all samples are stored in at least two geographically separate sites (Manhasset and Minneapolis).

White control subjects ($n = 2,064$) were derived from several sample sets. The PTPN22 genotyping data for the majority of these control subjects have all been reported elsewhere (Begovich et al. 2004; Kyogoku et al. 2004). Additional PTPN22 typing for this study was performed on 103 white subjects derived from the New York Cancer Project collection (Mitchell et al. 2004).

Genotyping

To confirm family relationships, all samples were genotyped, as described elsewhere (Baechler et al. 2003), for a set of ~100 polymorphic markers (ABI, version 2.5; marker sets 1–7) and were analyzed using the programs Relative (Göring and Ott 1997) and PedCheck (O'Connell and Weeks 1998). *HLA-DR* typing was performed at Roche Molecular Systems, as described elsewhere (Erlich et al. 1991). Genotyping of the *PTPN22* SNP (*rs2476601*, 1858C→T, R620W) was performed using a PSQ HS 96A Pyrosequencer. A quantity of 2 ng of DNA was amplified by PCR in a 10-μl reaction by use of the following primers: 5′-GTTGCGCAGGCTAG-TCTTGA-3′ (forward), *5′-**GCTGCTCCGGTTCATAG-ATT**GGATAGCAACTGCTCCAAGG-3′* (reverse), and 5′-Biotin-GCTGCTCCGGTTCATAGATT-3′ (Univ1_B). The addition of specific sequences to the 5′ end of the reverse primer (shown in bold italics) allowed the use of a biotinylated universal primer, Univ1_B. These primers were used at a ratio of 1:9 (reverse primer:universal primer). PCR conditions were as follows: 95°C for 12 min; 50 cycles at 95°C for 45 s, at 56.4°C for 45 s, and at 72°C for 45 s; and 72°C for 10 min. The amplicon was denatured with NaOH, separated, washed, and neutralized. To detect the polymorphism, the sequencing primer 5′-AAATGATTCAGGTGTCC-3′ was used in combination with appropriate Pyrosequencing substrates and enzymes, in accordance with manufacturer instructions. Genotyping of *PTPN22* R620W on control subjects was performed as described elsewhere (Begovich et al. 2004).

Data Management and Statistical Analysis

All demographic and clinical information was entered into a Microsoft Access database. Pedigrees were created for each family by use of the program Cyrillic, version 2.1 (Cherwell Scientific), and the pedigree data were imported into the Access database. These core data are also available upon request as downloadable Microsoft Excel files (see the MADGC Web site). Information about bi-
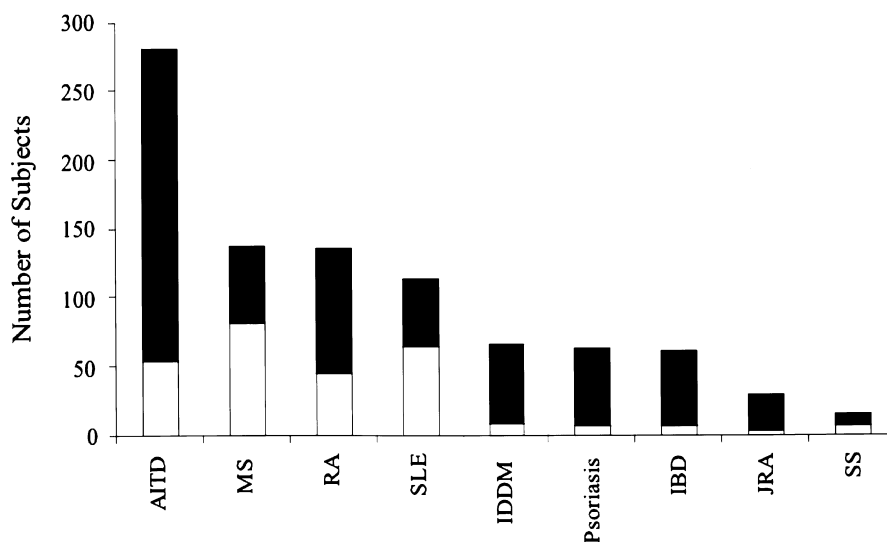
Figure 1   Distribution of the core autoimmune diseases among 806 affected members of 265 multiplex autoimmune families. Probands are shown in the lower (*unblackened*) portion of bars; other affected individuals are shown in the upper (*blackened*) portion of bars. A total of 907 instances of these core autoimmune diseases are tabulated, since 97 individuals had two or more such diseases (most frequently involving AITD [see text]). IDDM = insulin-dependent diabetes mellitus.

ological material—such as sample status, sample location, and genotyping results—is managed with a custom-designed relational database in SQL at the North Shore Long Island Jewish Research Institute.

Basic summary statistics were generated using the program SAS, version 8.02 (SAS Institute). ORs and 95% CIs were computed to describe associations between specific autoimmune diseases (and groups of autoimmune diseases) and the presence of the minor allele of *PTPN22*, on the basis of a comparison with control subjects. All of the affected individuals in MADGC families were used in these case-control association analyses. Since affected siblings obtained from the same parental mating type exhibit the same genotype and allele frequencies (W.L. and P.K.G., unpublished results), the inclusion of more than one affected sibling per family will not bias the estimates of ORs, as long as no particular parental mating type is over- or underrepresented in the sample. The inclusion of affected relatives other than siblings has an even smaller effect on an association analysis, because the genotype correlation among distant relatives is much lower.

Results

Demographic and Disease Characteristics of the MADGC Family Collection

The demographic and disease characteristics of the MADGC family collection reflect, in part, the recommendations of an expert advisory panel that was convened in late 1999 (see the "Material and Methods" section). The

entry criteria for these families include a requirement for at least two affected individuals per family and at least two of the nine core diseases in these affected individuals.

Demographic characteristics of the currently enrolled 265 families with multiplex autoimmune diseases are summarized in table 1. The majority of families were white, with only nine families from other ethnic backgrounds. Families consisted of an average of 3.2 affected individuals, but 11 families each had between 6 and 9 affected individuals. Nearly half of the families (48%) were recruited through patient advocacy groups, primarily the AARDA and the National Multiple Sclerosis Society. Approximately 20% of the families were ascertained by one of the three major recruitment sites for the MADGC: North Shore University Hospital, the University of Minnesota, and UCSF. Since the emphasis of research at these sites has been primarily on population-genetics studies of SLE and/or RA, there is a relative enrichment of probands with these rheumatic diseases. The remaining families were recruited using a variety of other approaches, including articles in the lay press. Thus, by virtue of the criteria for entry and the modes of recruitment, these families are not—and were not intended to be—a representative sample of families with multiple autoimmune diseases in the general population.

The distribution of the nine core diseases among all 806 affected individuals, as well as among the 265 probands in these families, is shown in figure 1. Note that the total number of core diseases tallied in figure 1 (907) exceeds the number of affected individuals (806) because

97 subjects were affected with two or more different core diseases. Overall, AITD is the most common disorder among all affected family members. This is expected, given that it is the disorder with the highest population prevalence of the nine core diseases. MS, RA, and SLE were the next most common diseases present in these families. The high prevalence of RA in the families is not unexpected, given the overall ~1% estimated prevalence of this disease in the general population (Gabriel 2000). However, both SLE and MS are substantially overrepresented—both among all affected individuals and among the probands—compared with their low population prevalence (Broadley et al. 2000; Gabriel 2000). This undoubtedly reflects the ascertainment bias inherent in the modes of recruitment discussed above—namely, that existing collections of patients with RA, SLE, and MS were used as a source of recruitment for ~20% of the MADGC families.

Despite the ascertainment bias in this collection, we also examined the patterns of disease aggregation in the MADGC families. In an attempt to reduce the effects of ascertainment bias—as well as for simplicity of interpretation and data presentation—we stratified the families by the seven most common diseases present in the probands, and then we examined the distribution of these seven diseases in the siblings of these probands. We also calculated ORs to determine whether there was any significant enrichment of particular autoimmune diseases among siblings in families selected by proband diagnosis, compared with the distribution of these diseases among all the affected siblings in the entire set of 265 families. The results are shown in table 2. For example,

the data show that AITD is present in 69 (30%) of the 232 siblings in the 265 families (first row). Among the families in whom the proband has AITD, 19 (48%) of the 40 siblings also have AITD (second row). This represents a nominally significant enrichment of AITD in these siblings, compared with siblings overall in these families (OR 2.13; 95% CI 1.08–4.2; $P = .043$ [P value uncorrected]).

Similar trends toward disease aggregation among siblings can be seen in table 2. However, with the exception of families with multiple siblings with RA, none of these associations remains significant after correcting for multiple comparisons. The reason for the enrichment of sibling pairs with RA in these families is that a significant number of these families were originally recruited from pre-existing collections of sibling pairs with RA (Jawaheer et al. 2004). Therefore, although this collection offers a rich resource of families with multiple autoimmune diseases, it does not provide anything more than suggestive information concerning the normal patterns of familial aggregation of autoimmune diseases in the general population.

High Degree of Female Predominance in RA and MS

As expected for autoimmune diseases in general, there was a predominance of females among the affected members of these families. Overall, 82% of affected individuals were female, compared with 54% of unaffected individuals who were contacted directly for the study. When all unaffected individuals in the family are considered, regardless of direct contact, 43% are female. This suggests

Table 2

Comparison of Frequency of Autoimmune Diseases in Siblings in Family Groups, Stratified by Diagnosis of Proband

Family Group[a] (No. of Probands)	No. of Affected Siblings	No. (%) of Siblings Affected with						
		AITD	RA	MS	SLE	T1D	IBD	Psoriasis
All probands (265)	232	69 (30)	36 (16)	16 (7)	19 (8)	23 (10)	15 (6)	25 (11)
Probands with:								
AITD (43)	40	*19 (48)*[b]	9 (23)	2 (5)	3 (8)	6 (15)	3 (8)	0 (0)
RA (46)	51	15 (29)	*20 (39)*[c]	2 (4)	2 (4)	3 (6)	5 (10)	6 (12)
MS (82)	64	17 (27)	5 (8)	3 (5)	6 (9)	9 (14)	7 (11)	*12 (19)*[d]
SLE (65)	53	*23 (43)*[b]	9 (17)	4 (8)	6 (11)	4 (8)	1 (2)	4 (8)
T1D (10)	9	2 (22)	0 (0)	0 (0)	1 (11)	*3 (33)*[e]	0 (0)	1 (11)
IBD (7)	8	2 (25)	1 (13)	*3 (38)*[f]	0 (0)	2 (25)	0 (0)	0 (0)
Psoriasis (8)	8	1 (13)	2 (25)	1 (13)	0 (0)	0 (0)	0 (0)	*3 (38)*

NOTE.—Bold italics indicate $P < .1$ (two-sided Fisher's exact test).

[a] Stratified by proband diagnosis.

[b] AITD in siblings of probands with AITD: OR 2.13, 95% CI 1.08–4.2, $P = .043$; AITD in siblings of probands with SLE: OR 1.81, 95% CI 0.98–3.34, $P = .073$.

[c] RA in siblings of probands with RA: OR 3.5, 95% CI 1.8–6.8, $P = .0003$.

[d] Psoriasis in siblings of probands with MS: OR 1.9, 95% CI 0.90–4.0, $P = .09$; psoriasis in siblings of probands with psoriasis: OR 4.96, 95% CI 11.1–22.0, $P = .053$.

[e] T1D in siblings of probands with T1D: OR 4.54, 95% CI 1.06–19.40, $P = .06$.

[f] MS in siblings of probands with IBD: OR 8.1, 95% CI 1.77–37.0, $P = .018$.

Table 3

Sex Distribution among 806 Affected Members of 265 Multiplex Autoimmune Families, by Core Autoimmune Disease

DISEASE (NO. OF AFFECTED SUBJECTS[a])	% FEMALE	
	All Affected Individuals	Probands Only
AITD (282)	88	100
MS (138)	85	93
RA (137)	89	93
SLE (113)	94	94
T1D (67)	57	100
Psoriasis (63)	68	100
IBD (61)	66	86
JRA (30)	83	100
SS (16)	94	100

[a] The total number of individuals exceeds 806 because of the presence of more than one autoimmune disease in some individuals.

an overall increase in females among participants versus nonparticipants in the study. Nevertheless, the percentage of females varied by autoimmune disease, ranging from 57% of individuals with T1D to 94% of individuals with SLE and individuals with SS (see table 3). The degree of enrichment for females was unexpectedly high for diseases such as RA and MS, which typically exhibit 3:1 and 2:1 female-to-male ratios, respectively, in sporadic cases. The enrichment of females among all diseases is even more dramatic if only probands are considered (table 3). This may reflect a tendency for female subjects to be more knowledgeable about family history and therefore more likely to participate in family research studies. However, it is possible that the female predominance in our families may also have biological explanations, perhaps reflecting enhanced penetrance in females when multiple risk genes are present, as may be the case in these families.

HLA Typing Reveals Typical Patterns of Disease Association

First-level DNA-based *HLA-DR* typing was performed for all members of these multiplex families. Table 4 summarizes the results for the five core diseases that have widely recognized *HLA-DR* associations in whites—namely, *DR4* for RA, *DR2* and *DR3* for SLE, *DR3* for AITD, *DR2* for MS, and *DR3* and *DR4* for T1D. The frequencies of these *HLA-DR* allelic groups in a white control population ($n = 926$) from the New York Cancer Project cohort are shown for comparison, with calculated ORs. The observed HLA associations are consistent with previous observations in white subjects with these disorders (Hall et al. 1996; Tan and Arnett 1998; Todd et al. 1988; Barcellos et al. 2003). Of course, recent studies have indicated that additional loci, other than HLA-*DRB1,* within the MHC also contribute to risk of several autoimmune diseases (Graham et al. 2002; Jawaheer et al. 2002; Harbo et al. 2004; Vandiedonck et al. 2004).

Other Autoimmune Diseases

One or more other (noncore) autoimmune diseases were confirmed among 80 individuals (67 families). The most frequent of these diseases were pernicious or hemolytic anemia (MIM 170900 and MIM 205700, respectively) (confirmed in 19 members of 14 families), idiopathic thrombocytopenic purpura (MIM 188030) (12 individuals), antiphospholipid syndrome (MIM 107320) (7 individuals), mixed or undifferentiated connective tissue disease (6 individuals), scleroderma (MIM 181750) (6 individuals), and vitiligo (MIM 193200) (7 individuals).

Patterns of Disease Aggregation in Single Individuals

In the families we studied, 128 affected individuals had more than one autoimmune disease. This includes the 97 individuals with two or more core diseases, as summarized in figure 1. The co-occurrence of AITD with another disease was by far the most common pattern and was present in 84 (65.6%) of the 128 subjects. These included 13 subjects with MS/AITD, 23 subjects with RA/AITD, 12 subjects with T1D/AITD, and 16 subjects with SLE/AITD.

Association of the PTPN22 R620W Polymorphism with Multiple Autoimmune Diseases

To begin to use this family resource to study genetic polymorphisms that may underlie multiple autoimmune diseases, we genotyped 249 white families in this initial collection for a case-control study of the *PTPN22*

Table 4

HLA-DR Associations with Selected Autoimmune Diseases among 256 White Multiplex Autoimmune Families

DISEASE (NO. OF INDIVIDUALS) AND DR ALLELE	DR-ALLELE FREQUENCY (%[a]) IN		OR (95% CI)[b]
	Cases	Controls	
RA (129):			
DR4	58	28	2.02 (1.62–2.42)
SLE (107):			
DR2	35	29	1.2 (.91–1.59)
DR3	45	20	2.24 (1.75–2.87)
MS (135):			
DR2	53	29	1.84 (1.54–2.24)
AITD (270):			
DR3	38	20	1.89 (1.58–2.30)
T1D (65):			
DR3	57	20	2.89 (2.24–3.65)
DR4	69	28	2.44 (2.0–2.96)

NOTE.—*DR* typing results are shown by use of serologic equivalence. *DR2* contains both DRB1*15 and *DRB1*16 alleles, and *DR4* contains all *DRB1*04 subtypes.

[a] Frequency (%) is rounded to the nearest integer.

[b] Compared with a group of 926 white controls from the New York metropolitan area.

R620W polymorphism. Recent studies have indicated that *PTPN22* is associated with T1D, RA, SLE, Graves disease, and autoimmune Addison disease. No information is currently available for other autoimmune disorders, although unpublished data indicate that there is no evidence of association with MS (Begovich et al. 2005). Using a Pyrosequencing assay, we determined the prevalence of the minor (T) allele (*rs2476601*, encoding tryptophan at amino acid position 620 of the full-length protein) in 746 affected members of these families, compared with 2,064 healthy white controls (the majority of whom had been genotyped previously) (Begovich et al. 2004; Kyogoku et al. 2004).

The results, given in table 5, show striking variation in genetic association for different diseases, with ORs ranging from 0.35 to 2.49. When all 746 affected individuals were compared with controls, the combined OR was 1.35 ($P = .002$). Notably, in addition to T1D and SLE, Hashimoto thyroiditis was also significantly associated with the 620W risk allele (OR 1.63; 95% CI 1.24–2.17). The association with RA was positive but was of borderline significance. The OR for Graves disease was >1 but was not statistically significant. When all individuals in disease groups with an OR of >1 (T1D, SLE, AITD, and RA) were combined ($n = 483$), the cumulative OR was 1.59 ($P < .0001$). The very low sample sizes for some disease subgoups make it impossible to draw definitive conclusions for all diseases. Nevertheless, the absence of any evidence of association of *PTPN22* with MS is striking, given that 120 patients with MS were available for study, and this confirms recent data (Begovich et al. 2005). Positive associations with T1D,

RA, SLE, Graves disease, and autoimmune Addison disease have been reported elsewhere (Begovich et al. 2004; Bottini et al. 2004; Kyogoku et al. 2004; Smyth et al. 2004; Velaga et al. 2004). We also examined PTPN22 associations in individuals with more than one autoimmune disorder (data not shown). Since most of these individuals had AITD as one of these diseases (see above), it is not surprising that the OR for this group (OR 1.73; 95% CI 1.17–2.55) was very similar to that seen for Hashimoto thyroiditis alone.

The significant association with Hashimoto thyroiditis is a novel observation. To address any concern about including multiple affected members from the same family in a case-control analysis, we performed an association analysis by restricting the cases with Hashimoto thyroiditis to one affected sibling per sibship (165 individuals). A particular random sampling of one affected sibling per sibship led to a T-allele frequency of 13.9%, an OR of 1.70, a 95% CI of 1.20–2.42, and a *P* value of .003. We repeated this random sampling 100 times; the averaged T-allele frequency is 13.91%, with an SD of 0.37%, which is consistent with the result given in table 5.

Discussion

Autoimmune diseases constitute a diverse set of related phenotypes that are presumed to have common underlying mechanisms and, thus, some degree of shared genetic predisposition (Becker 2001). The diversity of phenotypes among autoimmune diseases makes them difficult to study as a unified group, and their relative rarity

Table 5

Association of the *PTPN22* Missense SNP with Four Autoimmune Diseases in the White MADGC Families (249 Families, 746 Affected Individuals)

Autoimmune Disease(s)	No. of Cases	T-Allele Frequency (%)	OR (95% CI)[a]
T1D	61	18.9	2.49 (1.56–3.97)
Hashimoto thyroiditis	194	14.2	1.77 (1.31–2.40)
SLE[b]	101	12.9	1.58 (1.04–2.43)
RA[c]	122	11.9	1.45 (.97–2.16)
Graves disease	58	10.3	1.24 (.67–2.27)
MS	120	8.3	.98 (.61–1.56)
Psoriasis	51	7.8	.91 (.44–1.89)
JRA	27	7.4	.86 (.31–2.39)
IBD	41	7.3	.85 (.37–1.96)
SS	16	3.1	.35 (.05–2.54)
T1D, AITD, SLE, and RA	483	12.9	1.59 (1.28–1.98)

[a] The OR was calculated on the basis of a T-allele frequency of 8.5% in 2,064 white control subjects; 95% CIs are not corrected for the complex patterns of relatedness of cases.

[b] Thirty-two cases from affected sib pair families reported elsewhere (Kyogoku et al. 2004).

[c] Forty-three cases from affected sib pair families reported elsewhere (Begovich et al. 2004).

as individual diseases poses a challenge for accumulating sufficient sample sizes that contain specific disease subgroups or subphenotypes. In general, with the exception of the HLA region, attempts to identify genes that confer genetic predisposition across the different autoimmune diseases have been unsuccessful or, in the case of *CTLA4,* have provided only modest and incomplete evidence that a common allele underlies different forms of autoimmunity (Ueda et al. 2003). Several recent reports suggest that another member of the *CTLA4* family of T-cell regulatory receptors, PD-1, may also predispose to several autoimmune diseases (Prokunina et al. 2002, 2004; Nielsen et al. 2003; Lin et al. 2004).

The MADGC collection of families was originally conceived to be a resource for identifying the common genetic elements involved in autoimmunity and was based on the idea that certain alleles may act to predispose to multiple autoimmune diseases. We posited that genes acting across autoimmune phenotypes would be enriched in families in whom multiple different autoimmune phenotypes are present. These might be common genes with low penetrance, and, thus, they would probably also be found in sporadic disease or in families with only one autoimmune phenotype. Alternatively, more rare and highly penetrant genes for autoimmunity may also be present in the population, and these will likely only be detected by focusing on larger multiplex families. The MADGC collection provides a valuable resource for identifying both types of autoimmunity genes.

In this study, we show that a relatively common variant of PTPN22, 620W, confers susceptibility for four different autoimmune disorders: T1D, RA, SLE, and Hashimoto thyroiditis. There have been initial reports of separate associations of *PTPN22* with three of these diseases (Begovich et al. 2004; Bottini et al. 2004; Kyogoku et al. 2004). This is the first reported association with Hashimoto thyroiditis. The OR for Graves disease was >1, but our sample size was inadequate to definitively address this question. However, two recent reports have shown clear evidence of association with Graves disease (Smyth et al. 2004; Velaga et al. 2004). In contrast, the lack of association with MS is of interest, since the sample size of MS cases in this data set ($n = 120$) provides a reasonable likelihood of detecting such an association. For this analysis, a case sample size of 120 has 62% power to detect an OR of 1.8, under the assumption of a control risk-allele frequency of 0.085. Furthermore, this lack of association has recently been confirmed in a larger MS data set (Begovich et al. 2005).

These results have implications for the diversity of pathogenic mechanisms that are likely to underlie autoimmune diseases. It is clear that PTPN22 is directly involved in setting thresholds for T-cell receptor (TCR) signaling and, in this context, is acting, in part, through binding to intracellular kinases such as Csk (Gregorieff et al. 1998; Cloutier and Veillette 1999). These interactions control the phosphorylation state of regulatory tyrosines on key signaling molecules in the TCR complex, such as Lck and ZAP70 (Mustelin et al. 2004). PTPN22-knockout animals display rather subtle changes in a number of immune parameters (Hasegawa et al. 2004)—most prominently, in the enhanced T-cell proliferation in the memory-effector compartment. In the older animals (aged 6 mo), there was an expansion in the number of T cells and an enlargement of the spleen and the lymph nodes. This was accompanied by the spontaneous formation of germinal centers and higher levels of several Ig isotypes. Thus, there was an up-regulation of the humoral immune response in these animals that appeared to be largely secondary to lowered thresholds for signaling in T cells (Hasegawa et al. 2004).

The existence of humoral abnormalities in the PTPN22-knockout mice is consistent with the fact that autoantibody production is a prominent feature of all the human diseases that are associated with *PTPN22.* Indeed, SLE, RA, T1D, and Hashimoto thyroiditis are all characterized by the frequent development of autoantibodies, often before the onset of clinical disease (Arbuckle et al. 2003; Strieder et al. 2003; Hoppu et al. 2004; Nielen et al. 2004). We also have recently shown that the *PTPN22* association is almost exclusively with seropositive RA (Begovich et al. 2004; Lee et al., in press). In addition, the formation of ectopic germinal centers can be seen in both RA and AITD (Weyand et al. 2001), and increased germinal center formation is observed in autoimmune mouse models for SLE and T1D (Luzina et al. 2001). In contrast, human MS does not appear to have a prominent humoral component (Haffler 2004). These combined observations suggest that the *PTPN22* 620W risk allele may act primarily as a predisposing factor for the development of autoantibodies in these disorders. It will therefore be of great interest to examine (1) whether the *PTPN22* risk allele is also associated with the presence of autoantibodies in individuals who do not display a clinical phenotype and (2) whether *PTPN22* confers additional risk of the eventual development of clinical autoimmunity in these individuals. The MADGC families should be useful for such an investigation, since we have recruited many unaffected individuals in these families and plasma is available for additional antibody testing of all subjects.

The absence of an association with MS appears to imply that lowered thresholds for TCR signaling may not have an important role in the predisposition to MS, at least at stages of T-cell differentiation in which PTPN22 levels are critical for regulation. This is somewhat surprising, given the wealth of data implicating T cells in the pathogenesis of this disease (Haffler 2004). However, knowledge is still very incomplete concerning exactly which T-cell subsets depend on PTPN22 for regulation.

Overall, phosphatases have a negative regulatory function in T cells, and there may be other phosphatases, such as PTP-PEST (another Csk-binding phosphatase), that might serve a function that is redundant to PTPN22 in certain cell types (Mustelin et al. 2004). It should also be pointed out that PTPN22 is broadly expressed in hematopoietic cells, and, thus, the mechanism for disease susceptibility may extend beyond T cells (Begovich et al. 2004). Knowledge of the regulatory circuits controlled by phosphatases in T lymphocytes (or other cell types), combined with population-genetics data such as we describe here, may eventually make it possible for researchers to identify the critical lymphocyte subsets and pathogenic mechanisms that are involved in the different autoimmune diseases.

The overall goal of the MADGC is to collect family data that will facilitate the identification of genes that predispose to multiple different autoimmune diseases. Data from additional families are currently being collected to increase the statistical power for future studies of putative disease-susceptibility alleles, as they are discovered. We intend to pursue a more detailed analysis of intermediate phenotypes in this family collection, including a comprehensive analysis of autoantibodies in both affected and unaffected individuals. Data from these families can also be used to examine other biomarkers that may be relevant to autoimmunity, including patterns of gene expression (Baechler et al. 2003). Numerous other questions need to be addressed, including the significance of female predominance among the affected individuals in these families and the possible role of environmental factors in accounting for familial aggregation. The very large extended pedigrees in this collection may be useful in linkage analyses for the identification of rare, high-penetrance genes. Finally, the data from these families may prove to be particularly useful for discovering gene-gene interactions, under the assumption that the final autoimmune phenotype depends on an interaction of common autoimmunity genes with more disease-specific susceptibility alleles segregating in these families. It is likely that very large sample sizes will be required to successfully perform such studies, since stochastic and environmental factors will complicate the elucidation of these relationships.

Electronic-Database Information

The URLs for data presented herein are as follows:

MADGC, http://www.madgc.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RA, Hashimoto thyroiditis, T1D, SLE, MS, Graves disease, psoriasis, IBD, JRA, SS, pernicious or hemolytic anemia, idiopathic thrombocytopenic purpura, antiphospholipid syndrome, scleroderma, and vitiligo)

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