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Association of a Functional Polymorphism of *PTPN22* Encoding a Lymphoid Protein Phosphatase in Bilateral Meniere's Disease

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Objectives/Hypothesis: Bilateral Meniere's disease (BMD) is a severe disease that usually results in bilateral severe or profound sensorineural hearing loss and chronic disequilibrium with loss of vestibular function. We examined single nucleotide polymorphisms (SNPs) in the *PTPN22* and *CTLA4* genes in Caucasian patients with BMD to assess the possible association between these polymorphism and the predisposition and clinical expression of this disease.

Study Design: A case control study.

Methods: The functional protein tyrosine phosphatase type 22 (*PTPN22*) SNP (rs2476601, 1858C/T) and *CTLA4* SNP (rs231775, 49A/G) were analyzed in 52 patients with BMD and 348 healthy controls by a TaqMan 5′ allelic discrimination assay. Data were analyzed by a χ^2 test with Fisher exact test.

Results: No association was found between the +49A/G *CTLA4* genotype and BMD patients. However, the heterozygote *PTPN22* 1858C/T genotype was present at a significantly higher frequency in BMD patients than in controls (odds ratio = 2.25, 95% confidence interval: 1.09–4.62; $P = .04$).

Conclusions: These results suggest that the *PTPN22* 1858C/T genotype may confer differential susceptibility to BMD in the Spanish population and support an autoimmune etiology for BMD.

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INTRODUCTION

Bilateral Meniere's disease (BMD) is a severe disease affecting the inner ear characterized by attacks of vertigo associated with hearing loss, usually preceded by tinnitus and aural fullness, leading to bilateral severe or profound sensorineural hearing loss with chronic disequilibrium. Although the cause of Meniere's disease (MD) (MIM 156000) is unknown, an immune mediated mechanism has been posited in the pathophysiology of MD,[1] because elevated autoantibodies titters and a clinical improvement with steroid therapy is observed in some patients with MD.[2] The elevation of levels of serum circulating immune complexes (CIC) found in patients with MD suggests that CICs may be involved in the pathogenesis of this disease, either as a direct cause of damage, or as a product of an underlying autoimmune abnormality.[3,4]

The T cell regulatory gene encoding the cytotoxic T lymphocyte-associated protein 4 (*CTLA4*) has been consistently associated with autoimmunity.[5] *CTLA4* is a homologue of CD28 and binds the CD80/86 ligands of T cells. Whereas the CD28 ligand interaction plays a critical role in increasing and maintaining the T cell response initiated through engagement of the T cell receptor (TCR), the *CTLA4* ligand interaction has an inhibitory effect on T cell activation and might contribute to peripheral tolerance. *CTLA4* single nucleotide polymorphisms (SNPs) within the gene have consistently been found to be associated with Graves' disease (GD),[5] type 1 diabetes (T1D),[5] autoimmune hypothyroidism (AIH),[6] systemic lupus erythematosus (SLE),[7] and Addison's disease.[8] Several polymorphisms have been described in *CTLA4*, including −1722T/C and −319C/T, both of which are located within the promoter region, +49A/G in exon 1 (rs 231775),[9] a microsatellite $(AT)_n$

polymorphism, and a CT60A/G, both of which are within the 3′-untranslated region.

Phosphorylation of protein tyrosine kinases is mediated by protein tyrosine phosphatases (PTPs). Many PTPs play a negative role in TCR signalling, including lymphoid tyrosine phosphatase (LYP), a protein encoded by the *PTPN22* gene, expressed in the cytosol and nucleus of T cells.[10] LYP is a strong negative regulator of T cell activation, either independently or through binding to a variety of adaptor molecules including c-Cbl, Csk kinase and Grb2. *PTPN22* is expressed primarily in T cells, B cells, monocytes, neutrophils, dendritic cells, and natural killer cells, which raise the possibility that the association of *PTPN22* with autoimmune diseases like rheumatoid arthritis (RA),[11] could also be a result of functional changes in these cells.

More recently, LYP has been involved in several autoimmune diseases including T1D, GD, SLE, AIH and RA.[11,12] An SNP has been identified within *PTPN22* at position 1858 in codon 620 that results in an Arg to Trp (C to T) shift, and this change disrupts interaction between LYP and Csk kinases, disinhibiting T cell activation,[8] and perhaps thereby increasing susceptibility to autoimmune disease.[9] In vitro experiments have shown that the T allele of *PTPN22* bind less efficiently to Csk than the C allele does, suggesting that T cells expressing the T allele may be hyper-responsive, and consequently, individuals carrying T allele may be prone to autoimmunity.[11,13]

Both of these two genes (*CTLA4* and *PTPN22*) encode molecules that are integral to the immune system and are actively involved in the process of T cell activation.[14] The purpose to this study is to investigate the association of the *CTLA4* +49A/G and *PTPN22* 1858T SNPs in BMD patients.

TABLE I.
Demographics and Clinical Characteristics in 52 Patients with Bilateral MD.

Variables	Bilateral MD	
Age, mean ± SD	57 ± 10	
Sex, women (%)/men (%)	32 (61)/20 (38)	
Hearing loss at diagnosis, pure tone average, mean ± SD	Left	Right
	61.6 ± 24.8	54.5 ± 20.4
Headache, n (%)	29 (56)	
Psychiatric disorder, n (%)	18 (35)	
Cardiovascular disease, n (%)	20 (38)	
Osteoarticular disease, n (%)	21 (50)	
Hearing stage, n (%)*		
1	0	
2	2 (4)	
3	30 (58)	
4	20 (38)	
Time-course (mo.), mean ± SD	108 ± 89	
Class, n (%)		
A	23 (44)	
B or C	29 (56)	
Functional scale, n (%)		
1	9 (17)	
2	12 (23)	
3	14 (27)	
4	0	
5	14 (27)	
6	3 (6)	
Sodium-free diet, n (%)	49 (94)	
Drug therapy for MD, n (%)	38 (73)	

*Hearing stage calculated for the worst ear in bilateral MD.
MD = Meniere's disease; SD = standard deviation.

MATERIALS AND METHODS

Patients and Controls

We analyzed 52 patients with BMD and 348 healthy volunteer donors. All patients were diagnosed according to the diagnostic scale for MD of the American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS).[15] Three centers recruited patients for this study: Hospital La Fe from Valencia, Hospital Virgen de las Nieves from Granada, and Hospital de Poniente from El Ejido, Almeria. The subject's informed consent was obtained to participate in the study according to the Declaration of Helsinki, and the local institutional ethics review board approved the study. The clinical features of patients with BMD are shown in Table I. Patients with unilateral MD or probable BMD, according to the AAO-HNS were excluded.

Genotyping

Total genomic DNA was automatically isolated from peripheral blood of patients and healthy controls using the GenoVision M-48 robot (Qiagen, Venlo, The Netherlands) and the MagAttract DNA Blood Mini M48 (192) kit from Qiagen.

Genotyping of +49A/G *CTLA4* (rs231775) was performed using a TaqMan 5′ allelic discrimination assay by a custom TaqMan SNP Genotyping Assay method (Applied Biosystems, Foster City, CA). Primers and TaqMan probes were obtained from Applied Biosystems. The TaqMan minor groove binder (MGB) probe sequences were 5′-GGCACAAGGCTCAGCTGAA CCTGGCT[A/G]CCAGGACCTGGCCCTGCACTCTCCT. The first probe (A) was labelled with the fluorescent dye VIC and the second (G) with FAM.

Samples were genotyped for *PTPN22* 1858C/T variants (rs2476601) with a TaqMan 5′ allelic discrimination Assay-by-Design method (Applied Biosystems), as previously described.[16] The primers sequences were 5′-CCAGCTTCCTC AACCACAATAAATG (forward) and 5′-CAACTGCTCCAAGGA TAGATGATGA (reverse), and the TaqMan MGB probes sequences were 5′-CAGGTGTCC[A/G]TACAGG. The probes were labelled with VIC and FAM, respectively. Allelic discrimination using TaqMan was performed using 10 ng of sample DNA in a 25 μL reaction containing 12.5 μL TaqMan Universal polymerase chain reaction mix (Applied Biosystems) 300 nM primers, and 200 nM TaqMan MGB probes (Applied Biosystems). Reaction conditions consisted of preincubation at 50°C for 2 minutes and 95°C for 10 minutes; and cycling for 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Amplifications were performed in an ABI Prism 7750 machine (Applied Biosystems) for continuous fluorescence monitoring.

Statistical Analysis

Data were analyzed using the SPSS Software (SPSS Inc., Chicago, IL). For association studies, a χ^2 test with Fisher exact

TABLE II.
Frequency of CTLA4 Alleles and Genotypes Among BMD Patients and Healthy Controls.

	Bilateral MD N = 52 (%)	Controls N = 348 (%)	OR (95% CI)	*P* Value
CTLA4 Genotype				
A/A	30 (57.7)	184 (52.1)	1.21 (0.67–2.19)	.62
A/G	21 (40.4)	153 (43.7)	0.86 (0.30–1.56)	.74
G/G	1 (1.9)	11 (3.1)	0.60 (0.07–4.76)	1.00
Allele	2n = 104 (%)	2n = 696 (%)	1.25 (0.76–2.07)	.45
A	82 (78.8)	521 (74.9)		
G	22 (21.2)	175 (25.1)		

BMD = bilateral Meniere's disease, MD = Meniere's disease; OR = odds ratio; CI = confidence interval.

test was performed. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to compare the observed frequencies between patients with BMD and their controls. *P* values <.05 were considered significant.

RESULTS

Our cohort showed a high prevalence of headache and osteoarticular disease, which could be symptoms of an underlying autoimmune disease. Thirty-four of 52 patients were tested for antinuclear antibodies (ANA) by indirect immunofluorescence, but only 4/34 (11.7%) had positive titers (2 cases at 1/160, one at 1/320, and another at 1/640, respectively).

Table II lists the allelic distribution of +49A/G *CTLA4* polymorphism in patients with BMD and controls. The allelic distribution in controls was similar to that obtained in other Caucasian populations. After comparing patients with the control population, we found no significant deviation in the distribution of the alleles or genotypes of +49A/G polymorphism.

Table III shows the allele and genotype frequencies of *PTPN22* polymorphism in patients with BMD and healthy subjects. The CT genotype was significantly observed at a higher frequency in BMD patients than in controls ($P = .04$; OR 2.25 [95% CI, 1.09-4.62]), whereas the CC genotype was less frequent in BMD patients than in controls ($P = .05$; OR 0.46 [95% CI, 0.22-0.94]), and the TT genotype is visually nonexistent. Marginal differences were also observed when allele frequencies are compared, with the T allele being present at a higher frequency in BMD patients ($P = .06$; OR 1.98 [95% CI, 1.01-3.89]).

We have studied the association of *PTPN22* polymorphism with clinical parameters. However, we did not found any association with sex, age of onset of MD, time course, headache, or severity of disease such as hearing loss, frequency of vertigo, or AAO-HNS functional scale (all $P > .05$).

DISCUSSION

Meniere's disease is a multifactorial disease, and population studies are necessary to search for risk alleles. It has long been speculated that MD might be an immune-mediated condition. So, antiheat shock protein 70 antibodies, tumor necrosis factor alpha, ANA, antiphospholipid antibodies, and erythrocyte sedimentation rate have been studied for possible association with autoimmunity in MD.[17] Despite the many unique clinical and serological features associated with specific autoimmune diseases, the frequent overlap of these conditions in individuals or families, coupled with growing evidence that different autoimmune diseases share susceptibility loci, predict commonalities in their genetic etiologies.[12] This prediction is borne out by studies showing that susceptibilities to T1D, RA, SLE, GD, and other autoimmunity disorders are associated with functional polymorphisms in *CTLA4* and *PTPN22*.

In this work, we investigated for the first time the role of +49A/G *CTLA4* polymorphisms in BMD susceptibility, and no evidence of association was found. The relevance of *CTLA4* in the counter-regulation of CD28 T cell antigen receptor activation of T cells is well established[18]; however, the molecular basis of the interactions between *CTLA4* and CD28 is uncertain. *CTLA4*, by

TABLE III.
Frequency of PTPN22 Alleles and Genotypes Among BMD Patients and Healthy Controls.

	Bilateral MD N = 52 (%)	Controls N = 348 (%)	OR (95% CI)	*P* Value
PTPN22 Genotype				
T/T	0	1 (0.3)		
C/T	12 (23.1)	41 (11.8)	2.25 (1.09–4.62)	.04
C/C	40 (76.9)	306 (87.9)	0.46 (0.22–0.94)	.05
Allele	2n = 104 (%)	2n = 696 (%)	1.98 (1.01–3.89)	.06
T	12 (11.5)	43 (6.2)		
C	92 (88.5)	653 (93.8)		

BMD = bilateral Meniere's disease, MD = Meniere's disease; OR = odds ratio; CI = confidence interval.

means of its ligand-binding domain, may compete with CD28 for the receptors (CD80 and CD86) that they share. *CTLA4* might also deliver a negative signal to each T cell by inhibiting the TCR signalling complex, an activity that would not necessarily depend on ligand binding.[5] Cell surface expression of *CTLA4* is influenced by polymorphisms in its sequence. The G allele at position +49 of exon 1 of *CTLA4* gene affects the *CTLA4* function, and was correlated with a higher risk of various autoimmune diseases, including GD, T1D, and HT.[19] However, our analyses of the +49A/G *CTLA4* SNP showed no allelic or genotypic association with BMD. This lack of association was also observed with vitiligo,[20] RA,[21] and inflammatory bowel disease.[22]

Protein tyrosine phosphatases are critical regulators of T cell signal transduction. In conjunction with protein tyrosine kinases, PTPs regulate the reversible phosphorylation of tyrosine residues, and thereby play important roles in many different aspects of T cell physiology.[10] Abnormalities in tyrosine phosphorylation have been shown to be involved in the pathogenesis of numerous human diseases, from autoimmunity to cancer. Thus, T cells displaying dysregulated tyrosine phosphorylation would be expected to mediate the pathologic process in autoimmunity. *PTPN22* has been found to be associated with most autoimmune disease studied so far, specifically with some of the most prevalent ones, such as GD, RA, SLE, and T1D. In addition, the functional effect of the *PTPN22* 1858 variation in the binding of LYP to Csk has been confirmed,[13] suggesting that the association of this polymorphism with autoimmunity may be due to the role of *PTPN22* in the negative regulation of T cell activation.

This is the first study analyzing the *PTPN22* gene in BMD, and we could substantiate an association between *PTPN22* and this disease. We find an association of BMD with the functional *PTPN22* 1858T allele and the heterozygous C/T genotype. The frequency of the C/T genotype in the northern and western European population is 28.3%, and in Spanish Caucasian subjects it is 12.3%.[16] Our population is from the same area in Spain, and we found a frequency of 11.8% for the C/T genotype in control individuals. This findings are in agreement with the results obtained for others authors in different autoimmune diseases,[20] and support the association for the *PTPN22* 1858 T allele in susceptibility to BMD and other autoimmune diseases. Indeed, the association of several autoimmune diseases with the *PTPN22* 1858C/T SNP has been considered as indicative of the existence of an inflammatory process common to many autoimmune diseases.[16] According to this idea, the positive association with that gene would support the autoimmune etiology for BMD.

We selected patients with bilateral ear involvement for this study because they have several common clinical features (headache, vestibular and hearing loss progression, worst perceived health-related quality of life),[23] and a higher frequency of ANA titers was described for BMD patients.[4] However, this *PTPN22* SNP should be evaluated in patients with unilateral MD.

CONCLUSION

The genotype C/T of the functional SNP at position 1858 of the *PTPN22* gene is associated with BMD, supporting the possible autoimmune etiology of BMD. The +49A/G *CTLA4* SNP did not show an allelic or genotypic association with BMD.

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