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BRIEF COMMUNICATION

Osteopontin polymorphisms and disease course in multiple sclerosis

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Osteopontin (OPN), also known as early T-cell activating gene (Eta-1), has been recently shown to be a critical factor in the progression of experimental autoimmune encephalomyelitis, and perhaps multiple sclerosis (MS). Here we investigated whether the 327T/C, 795C/T, 1128A/G or 1284A/C single-nucleotide polymorphisms in the OPN gene were correlated with susceptibility or any of the several clinical end points in a cohort of 821 MS patients. Overall, we observed no evidence of genetic association between the OPN polymorphisms and MS. Although not reaching statistical significance, a modest trend for association with disease course was detected in patients carrying at least one wild-type 1284A allele, suggesting an effect on disease course. Patients with this genotype were less likely to have a mild disease course and were at increased risk for a secondary-progressive clinical type.
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Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by an autoimmune response against myelin proteins, which results in progressive neurological dysfunction.[1] In Caucasian populations it is the most common cause of acquired neurological dysfunction arising during early and mid-adulthood, affecting more than 1 million people in North America and Western Europe. The course of the disease may consist of recurrent attacks each followed by a variable degree of recovery (relapsing-remitting) or, in a minority of patients, of a progressive course from onset (primary-progressive). Intermediate phenotypes are also common. A large body of research supports a multifactorial etiology, with an underlying genetic susceptibility likely acting in concert with undefined environmental exposures.[2] In a recent report, transcriptional profiling using large-scale sequencing of expressed tagged sequences (ESTs) from MS lesions has identified a number of genes that are potentially involved in disease pathogenesis.[3] One of the genes showing maximal differential expression between patient samples and controls was osteopontin *(OPN)*. Microarray analysis of spinal cord RNA from rats with experimental allergic encephalomyelitis (EAE) also revealed increased *OPN* transcripts. In addition, *OPN*-deficient mice were resistant to MOG-induced progressive EAE and had frequent remissions[3] suggesting a significant role for this gene in determining disease course.

OPN maps to 4q21–q25 and codes for a 60 kDa secreted phosphoprotein with pleiotropic functions, including roles in tissue remodeling, cell survival and cellular immunity.[4,5] *OPN* costimulates T-cell proliferation,[4] and is classified as a T helper cell-1 (Thl) cytokine, upregulating interferon-gamma and interleukin-12 production, while downregulating that of interleukin-10.[5] Here we investigated whether four single-nucleotide polymorphisms (SNPs) in the *OPN* gene were correlated with susceptibility to MS or any of several clinical end points in a well-characterized cohort of 821 patients.

Results and discussion

The data set studied consisted of 184 multicase families including 434 MS patients (total $n = 1312$ genotyped individuals). In addition, we studied a second, independent, data set ($n = 387$) composed primarily of patients with no family history of MS, giving a total of 821 clinically definite MS patients. This group was comprised of 617 females and 204 males (sex ratio = 3.0 : 1), with an overall mean age of onset of 30.0 ($\pm$ 8.7) years and mean disease duration of 14.1 ($\pm$ 10.1) years. All patients were non-Hispanic Caucasian and of European descent. Diagnostic criteria, ascertainment protocols, and other clinical and demographic characteristics of MS patients and families have been summarized elsewhere.[6]

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Table 1 Global PDT *P*-values for allele and genotype results for *OPN* polymorphisms in multicase MS families

OPN SNPs[a]	*All families (n=184)*	*DR2 all*[b] *(n=105)*	*DR2 none*[c] *(n=43)*
327T/C, rs4754, exon 6 Ala/ Ala	0.51 (0.54)	0.75 (0.87)	0.40 (0.62)
795C/T, rs1126616, exon 6 Asp/Asp	0.51 (0.68)	0.78 (0.92)	0.40 (0.62)
1128A/G, rs1126772, exon 7 UTR	0.72 (0.27)	0.66 (0.66)	0.53 (0.53)
1284A/C, rs9138, exon 7 UTR	0.34 (0.70)	0.82 (0.96)	0.40 (0.62)

[a]SNP nomenclature is based on distance from transcriptional start site in the *OPN* sequence found at NCBI RefSeq NM_000582. Genotyping was performed using Pyrosequencing AB technology.[11,12] Genomic DNA was amplified and genotyped using the following primers: 327T/C forward: CCA AGT AAG TCC AAC GAA AG, reverse: AAA TCA GTG ACC AGT TCA TC, sequencing primer: GGC TGT CCA CAT GGT; 795C/T forward: TAC CCT GAT GCT ACA GAC GA, reverse: AAT TCA CGG CTG ACT TTG GA, sequencing primer: GCT CAT TGC TCT CAT CA; 1128A/G forward: TAC TGC ATC TTC TGA GGT CAA T, reverse: TTA GTT TAC AGG GAG TTT CCA T, sequencing primer: AAA CTA ATT ATC AAA CAC AT; 1284A/C forward: TTC ATG GAA ACT CCC TGT AAA C, reverse: ATT GAC ACC ACC AAA TTC TTA T, sequencing primer: GGG TAA AAG TAT TTT GTT TG. All forward primers are biotinylated for PCR purification using Dynabeads M-280 Streptavidin (Dynal, Norway). Genotype PDT results are shown in parentheses.
[b]*DR2* all refers to families in which all affected members carry at least one copy of *HLA-DR2* haplotype.
[c]*DR2* none refers to families in which no affected members carry *HLA-DR2*. HLA typing for *DRB1* and *DQB1* loci was performed using a nonradioactive PCR-based sequence-specific oligonucleotide probe reverse line-blot assay (PCR-SSOP) (Dynal, Norway).
Generation of all genotypes was performed blind to pedigree structure and clinical status of the individual. PEDCHECK was used to check for Mendelian consistency within all MS families.

Table 2 Global TRANSMIT *P*-values for *OPN* polymorphisms in multicase MS families

OPN SNPs	*All families (n=184)*
327T/C	0.16
795C/T	0.16
1128A/G	0.45
1284A/C	0.21
OPN SNPs 4-SNP haplotype	0.13

Table 3 Allele frequencies for *OPN* SNPs in MS index patients and Caucasian controls

OPN SNPs	*Normal controls*[a] *(n=96)*	*Unaffected founders*[b] *(n=91)*	*MS index cases (n=184)*
327T/C	T=67.7%	T=71.4%	T=72.1%
	C=32.3%	C=28.6%	C=27.9%
795C/T	T=67.2%	T=78.5%	T=72.6%
	C=32.8%	C=21.5%	C=27.4%
1128A/G	T=74.0%	T=79.7%	T=78.2%
	C=26.0%	C=20.3%	C=21.8%
1284A/C	A=67.7%	A=70.8%	A=75.0%
	C=32.3%	C=29.2%	C=25.0%

Fisher's exact test $P > 0.05$ for all comparisons (SAS v. 8.2).
[a]The unrelated control group included Caucasian individuals with no history of autoimmune diseases.
[b]One unaffected founder was selected at random from each multicase family.

A strong association to the *HLA-DR* locus overall ($P = 1.9 \times 10^{-6}$), and specifically with the *HLA-DR2* haplotype (*DRB1**1501–*DQB1**0602) ($P = 1.6 \times 10^{-7}$) was observed (data not shown), as previously reported in a subset of this population.[6]

To test the *OPN* polymorphisms for association with MS, two complimentary analytical approaches were used considering the diversity in pedigree structure of the studied data set. The multicase families were analyzed using the pedigree disequilibrium test (PDT)[7,8] for both allelic and genotypic effects (Table 1). Alleles and haplotypes were also examined using the likelihood-ratio test (Table 2) implemented in TRANSMIT.[9] No significant associations were observed for *OPN* allele or genotype in all families or subgroups stratified by *HLA-DR2* status.

TRANSMIT can consider transmission of multiple marker haplotypes even in the presence of phase uncertainty and missing parental genotypes. Haplotype assignments revealed strong associations between alleles at the different SNP sites within *OPN*, resulting in two major haplotypes: one comprised of all wild-type SNP alleles or '1111' with a frequency of 72.0%, and a second comprised of all rare alleles or '2222' with a frequency of 22.0%. All other *OPN* haplotypes were present at frequencies $\leq 5.0\%$. The four SNP haplotypes as well as all pairwise haplotype combinations were analyzed, and no significant results were observed. When analyzed separately, all possible nuclear families from the multicase data set ($n = 292$; total affecteds = 459), as well as only one nuclear family selected from each multicase family ($n = 184$), yielded comparable results (data not shown).

We also carried out case–control testing using randomly selected familial index cases and a control group, which did not reveal significant *OPN* allelic associations (Table 3). *OPN* SNP allele distributions for controls, patients and unaffected founders were similar.

Table 4 *OPN* genotype–clinical phenotype correlations in MS patients

		OPN SNP 327 T/C			*OPN SNP 1284 A/C*		
Clinical phenotype		*OR*[e]	*95% CI*	*P-value*	*OR*	*95% CI*	*P-value*
Mild MS[a]	wt/wt	0.6	0.2, 1.8	0.33	0.5	0.2, 1.3	0.15
	het	0.5	0.1, 1.6	0.22	0.3	0.1, 1.1	0.08
Severe MS[b]	wt/wt	1.1	0.3, 4.1	0.94	1.1	0.3, 4.1	0.94
	het	0.8	0.2, 3.4	0.76	0.8	0.2, 3.4	0.76
Disease course(l)[c]	wt/wt	1.3	0.4, 4.4	0.65	1.4	0.4, 4.7	0.57
	het	1.1	0.3, 3.7	0.93	1.1	0.3, 3.9	0.84
Disease course(2)[d]	wt/wt	1.8	0.8, 4.2	0.18	2.0	0.9, 4.9	0.11
	het	2.1	0.9, 4.9	0.09	2.4	1.0, 5.9	0.05

[a]Benign or 'mild MS' as compared to 'nonmild'. Patients in this group can walk normally or have mild gait disability only after $\geq$15 years from disease onset. Analyses were restricted to all patients with a disease duration of 15 or more years ($n = 40$ or 12.6% of affecteds).
[b]'Severe MS' as compared to 'nonsevere'. Patients in this group require bilateral assistance to walk or are wheelchair dependent in $\leq$10 years from disease onset. Analyses were restricted to all patients with a disease duration of 10 or less years onset ($n = 32$ or 9.9% of affecteds).
[c]Disease course (1) refers to patients with initial relapsing-remitting course ($n = 709$ or 92.9%) compared to primary and relapsing-progressive patients ($n = 54$ or 7.1%).
[d]Disease course (2) refers to relapsing–remitting ($n = 482$ or 63.2%) *vs* secondary-progressive patients ($n = 227$ or 29.7%); here, all primary and relapsing-progressive patients have been removed from analyses. For analyses of disease course, duration of disease from time of onset was used as covariate in the model. Gender, age of onset and *HLA-DR2* status were included as covariates for all analyses.
[e]Odds ratios are reported for homozygous *OPN* wild-type and heterozygous genotypes; *OPN* genotypes homozygous for less frequent SNPs were used as reference groups.

Given the striking phenotypic effect of *OPN*-deficient mice,[3] we hypothesize that genomic variations in *OPN* are responsible, at least in part, for the clinical heterogeneity in MS. Therefore, we examined the effect of *OPN* genotypes on four well-defined clinical phenotypes such as disease course and severity in the combined MS patient data set using logistic models estimated by generalized estimating equations (GEE), which take into account any correlation between family members (Table 4). As a result of the strong disequilibrium observed between SNPs within the *OPN* locus, only two of the four original *OPN* polymorphisms were selected to study potential genotype–phenotype correlations, 1284A/C and 327T/C. Patients were coded by genotype (wild-type homozygous '11', heterozygous '12' and homozygous '22'), and gender, age of onset, *HLA-DR2* status, and disease duration (when appropriate) were included as covariates in all analyses. Patients were also categorized by disease course (see Table 4). Disability was also assessed at entry with the Expanded Disability Status Scale (EDSS).[10] Mild (benign) and severe disease patient classifications based upon EDSS scores maintained over or achieved within designated time intervals were also used in this study.[6]

A modest but interesting trend was observed in the data set. Patients carrying at least one wild-type *OPN* SNP 1284A allele were less likely to have a mild course (OR = 0.3, $P = 0.08$), and exhibited an increased risk for a secondary-progressive clinical course (OR = 2.4, $P = 0.05$). Similar results were also observed for *OPN* SNP 327T/C genotypes. The results, however, require careful interpretation because of multiple comparison effect.

Overall, we observed no evidence of genetic association between the *OPN* polymorphisms and MS susceptibility. However, the paucity of patients with mild disease carrying at least one wild-type A allele in the 1284A/C SNP suggests that there may be an effect of *OPN* in disease pathogenesis that, conceivably, may be regulated through downstream molecules in the *OPN*-mediated immunological cascade. These findings will require confirmation in an independent population.

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Electronic-database information

URLs for data in this article are as follows:

Center for Human Genetics, http://wwwchg.mc.duke.edu/ software/pdt.html for Pedigree Disequilibrium Test computer program. A beta-version of the geno-PDT program is available upon request (emartin@chg.mc.duke.edu).

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