Susceptibility and outcome in MS

Associations with polymorphisms in pigmentation-related genes

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Abstract—Multiple sclerosis (MS) risk is determined by environment and genes. The authors investigated in 419 cases and 422 controls if polymorphism in the vitamin D receptor (VDR), melanocortin-1 receptor (*MC1R*), and tyrosinase (*TYR*) genes is linked with MS risk and outcome. VDR *ff* was associated with reduced (odds ratio [OR] = 0.59) and *MC1R* His^{294}-encoding alleles with increased (OR = 2.21) risk. *MC1R* Glu^{84}/Glu^{84} was linked with disability (OR = 5.65). These preliminary data suggest a role for these genes in MS pathogenesis.

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Multiple sclerosis (MS) risk is determined by interaction between genes and environment.[1] Studies using various approaches suggest that latitude is a relevant factor. For example, studies of migrants from Northern Europe to South Africa and from the West Indies and Asia to the United Kingdom indicate the importance of latitude particularly during childhood.[1,2] Further, MS incidence decreases along a north–south gradient in Europe and in Australia from south to north. It has been suggested that this apparent effect of latitude is mediated by the extent of exposure to ultraviolet radiation (UVR).[3] Indeed, the US Veterans Study reported a closer correlation between MS risk and indexes of annual and winter sunshine than with latitude,[4] and recently sunlight exposure between 6 and 16 years has been shown to relate to MS risk.[5]

Although the mechanism for the protective effect of UVR on MS risk is unclear, it is presumed to be mediated by UVR effects on vitamin D synthesis. Individual variation in pigmentation influences vitamin D synthesis in response to UVR and could therefore influence MS risk.[6] 1,25-Dihydroxyvitamin D exerts its effects via the vitamin D receptor (VDR). Melanin synthesis determines pigmentation; rate-limiting steps are catalyzed by tyrosinase (TYR), which is influenced by melanocyte-stimulating hormone (MSH) acting on the melanocortin-1 receptor (MC1R). Accordingly, we have further investigated the hypothesis that UVR mediates MS risk by determining whether polymorphisms in *MC1R*, *TYR,* and *VDR* are associated with susceptibility and outcome in MS patients.

Methods. *Subject recruitment.* Four hundred nineteen unrelated Northern European Caucasian subjects with clinically definite MS according to Poser criteria were recruited with ethics committee approval and informed consent. Disability was assessed by the Expanded Disability Status Scale (EDSS). Outcome was stratified into mild/moderate (EDSS 0 to 5.5) or severe (EDSS 6 to 10) disability; this cut-off is a defined point requiring at least unilateral support to walk. Cases were recruited between 1995 and 1997. None was receiving disease-modifying agents. Four hundred twenty-two unrelated Northern European Caucasian subjects with noninflammatory and nonmalignant conditions from the same area were used as controls.

Identification of genotypes. DNA was isolated from peripheral blood leukocytes. We identified variants in *MC1R* ($Arg^{151}Cys$, $Arg^{160}Trp$, $Val^{92}Met$, $Asp^{294}His$, $Asp^{84}Glu$), *TYR*A1* and *A2* (codon 192), *VDR T* and *t* exon 9 (Taq I), and *F* and *f* exon 2 (Fok I) using published assays.[7]

Statistical methods. Genotype frequencies were compared in cases and controls for susceptibility and within cases for outcome. For *MC1R,* we compared wild-type homozygotes with heterozygotes and mutant homozygotes combined (termed *MC1R* phenotype) because mutant allele frequencies are generally small and both heterozygotes and mutant homozygotes are associated with UVR-sensitive phenotypes. Data were analyzed using χ^2 testing and logistic regression models (Stata statistical package, version 6.0; Stata Corp., College Station, TX) with correction for sex, disease duration, and age at onset, which independently influence outcome. An additional analysis of the association of genotypes with outcome was performed in only cases with disease duration of over 10 years as outcome is better established after this length of follow-up. Linkage disequilibrium between the *MC1R* alleles and *VDR* alleles and haplotype frequencies were determined using Estimating Haplotype Frequencies software (ftp://linkage.rockefeller.edu/software/eh). As these studies are exploratory, *p* values were uncorrected. Although this risks type I errors, correction for multiple testing increases risk of type II errors.[8]

Results. *Subject characteristics.* The case group had a mean age of 43.8 ± 11.2 (SD) years and mean age at disease onset of 31.0 ± 9.8 (SD) years. It comprised 105 (25.1%) men,

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Table *Susceptibility analysis: genotype frequencies presented with logistic regression analysis correcting for age and sex with factorization as shown*

Gene	Genotype	Controls, n (%)	MS patients, n (%)	Odds ratio	95% CI	*p*
MC1R	Asp^{84}/Asp^{84}	265 (96.7)	383 (97.0)	1.00		
	Asp^{84}/Glu^{84}	9 (3.3)	12 (3.0)	1.48	0.47–4.71	0.50
	Glu^{84}/Glu^{84}	0	0			
	Total	274	395			
	Val^{92}/Val^{92}	282 (81.5)	317 (84.3)	1.00		
	Val^{92}/Met^{92}	57 (16.5)	56 (14.9)	0.78	0.48–1.26	0.31
	Met^{92}/Met^{92}	7 (2.0)	3 (0.8)			
	Total	346	376			
	Arg^{151}/Arg^{151}	165 (83.8)	337 (80.4)	1.00		
	Arg^{151}/Cys^{151}	30 (15.2)	77 (18.4)	1.40	0.84–2.35	0.20
	Cys^{151}/Cys^{151}	2 (1.0)	5 (1.2)			
	Total	197	419			
	Arg^{160}/Arg^{160}	182 (82.3)	351 (84.0)	1.00		
	Arg^{160}/Trp^{160}	38 (17.2)	37 (16.0)	0.71	0.43–1.18	0.18
	Trp^{160}/Trp^{160}	1 (0.5)	0			
	Total	221	418			
	Asp^{294}/Asp^{294}	404 (95.7)	386 (92.8)	1.00		
	Asp^{294}/His^{294}	18 (4.3)	29 (7.0)	2.21	1.03–4.76	0.042
	His^{294}/His^{294}	0	1 (0.2)			
	Total	422	416			
VDR	*FF*	83 (35.5)	155 (38.2)	1.00		
	Ff	105 (44.9)	196 (48.3)	1.00	0.66–1.51	0.99
	ff	46 (19.6)	55 (13.5)	0.59	0.34–1.02	0.059
	Total	234	406			
	TT	86 (37.2)	140 (34.8)	1.00		
	Tt	106 (45.9)	203 (50.5)	1.10	0.73–1.65	0.65
	tt	39 (16.9)	59 (14.7)	0.97	0.56–1.69	0.91
	Total	231	402			
TYR	*A1A1*	29 (13.1)	48 (11.7)	1.00		
	A1A2	106 (48.0)	202 (49.3)	1.09	0.60–1.97	0.78
	A2A2	86 (38.9)	160 (39.0)	1.10	0.60–2.01	0.77
	Total	221	410			

and median EDSS score of 5.0. One hundred ten (26.3%) of the control subjects were male with mean age of 50.1 years.

MC1R, VDR, *and* TYR *genotypes and MS susceptibility.* Control and case genotype frequencies did not significantly depart from Hardy–Weinberg equilibrium (table). We did not identify linkage dysequilibrium between the *MC1R* or *VDR* alleles.

Using logistic regression analysis, we identified no significant associations between MS risk and the *MC1R* Asp^{84}/Glu^{84}, Val^{92}/Met^{92}, Arg^{151}/Cys^{151}, and Arg^{160}/Trp^{160} phenotypes or individual *VDR TT*, *Tt,* and *tt* or *TYR* genotypes. By contrast, the frequency of the phenotype based on the His^{294}-encoding allele was significantly greater (odds ratio [OR] = 2.21, $p = 0.042$) in cases than controls (see the table). The frequency of *VDR ff* was lower in cases than controls, but this difference was not statistically significant (OR for disease = 0.59, $p = 0.059$).

We determined if these associations were gender related. The *MC1R* Asp^{294}/His^{294} and His^{294}/His^{294} phenotype was associated with increased risk in females ($p = 0.043$, OR = 2.44, 95% = CI 1.03 to 5.78) but not males ($p = 0.67$, OR = 1.43, 95% CI = 0.27 to 7.61). *VDR ff* was associated with reduced risk in males (*ff* vs *FF*: $p = 0.010$, OR = 0.19, 95% CI = 0.05 to 0.67) but not females (*ff* vs *FF*: $p = 0.42$, OR = 0.78, 95% CI = 0.42 to 1.43). No additional associations were found between risk and *MC1R* or *VDR* haplotypes (data not shown).

MC1R, VDR, *and* TYR *genotypes and MS severity.* Logistic regression analysis was used to identify genotype associations with MS severity. Phenotypes with one or more

MC1R Glu^{84}-encoding allele were associated with a worse outcome ($p = 0.036$, OR = 5.65, 95% CI = 1.12 to 28.5).

In cases with a disease duration of >10 years (n = 210; 89 cases mild/moderate, 121 cases severe outcome), *MC1R* Asp^{84}/Glu^{84} was associated with a more severe disease course than Asp^{84}/Asp^{84} ($p = 0.024$ and $\chi_1^2 = 5.12$). An OR for this association could not be calculated, as there were no cases with the Glu^{84}-encoding allele with EDSS score of 0 to 5.5. No other associations were seen.

Discussion. We describe studies to determine whether *MC1R*, *TYR*, and *VDR* genotypes are associated with MS susceptibility and outcome. We studied a case group from an area with a stable and homogeneous population. The case group was hospital based, although the distribution of EDSS scores follows the expected bimodal pattern (data not shown).

We did not identify associations between *TYR* genotypes and MS risk or outcome. *TYR*A2* is not known to have functionally different capacity from *TYR*A1* though is in linkage disequilibrium with functional mutations associated with reduced production of melanin and pigmentation. An association between *TYR A2A2*, extent of exposure to UVR, and prostate cancer risk has been reported.[7] Prior studies of the *VDR* gene have shown conflicting results for association with MS. We found associations between *VDR ff* and increased risk particularly in males. This genotype appears to be associated with less effective function. However, although the *VDR* genotype data are compatible with the UVR hypothesis, our results for *MC1R* phenotypes are not; thus, phenotypes associated with sun-sensitive skin and presumably a better ability to synthesize vitamin D under conditions of limited UVR exposure were linked with increased MS risk and worse outcome. The frequency of the *MC1R* Glu^{84} allele is low, and it is not likely to be a major factor in mediating disease course in most patients. It is possible that the associations between *MC1R* phenotypes and risk reflect effects on the immune system. MSH has antipyretic and anti-inflammatory effects in the brain and immune system that may be mediated by *MC1R*. Indeed, serum levels of vitamin D may determine MS risk by influencing immune responses. Thus, vitamin D metabolites prevent experimental allergic encephalomyelitis.[9]

Studies in prostate cancer also suggest the importance of UVR.[7,10] Indeed, these show that men with the lowest quartile of exposure are at increased risk of the cancer. This finding may be relevant in MS risk. Thus, vitamin D deficiency is widespread even around the Mediterranean basin because of indoor lifestyles and sun avoidance. This may explain why some hot countries such as Sardinia show a high MS incidence comparable with that in temperate countries such as England.

References

1. Sawcer S, Robertson N, Compston A. Genetic epidemiology of multiple sclerosis. In: Thompson AJ, Polman C, Hohlfeld R, eds. Multiple sclerosis; clinical challenges and controversies. London: Martin Dunitz, 1997: 13–34.
2. Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. J Neurol Neurosurg Psychiatry 1997;63:565–568.
3. Hayes CE, Cantorna MT, DeLuca HF. Vitamin D and multiple sclerosis. Proc Soc Exp Biol Med 1997;216:21–27.
4. Acheson ED, Bachrach CA, Wright FM. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation and other variables. Acta Psychiatr Scand 1960;35:132–147.
5. van der Mei IAF, Ponsonby AL, Dwyer T, et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. Br Med J 2003;327:316–322.
6. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1982;1:74–76.
7. Luscombe CJ, French ME, Liu S, et al. Prostate cancer risk: associations with ultraviolet radiation, tyrosinase and melanocortin-1 receptor genotypes. Br J Cancer 2001;85:1504–1509.
8. Perneger TV. What's wrong with Bonferroni adjustments. Br Med J 1998;316:1236–1238.
9. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci USA 1996;93:7861–7864.
10. Luscombe CJ, Fryer AA, French ME, et al. Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. Lancet 2001;358:641–642.