Letters to the Editor

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Modes of Action of HLA-DR Susceptibility Specificities in Multiple Sclerosis

To the Editor:

In a recent analysis of genotypes associated with multiple sclerosis (MS [MIM 126200]) in a large data set of American families, Barcellos et al. (2003) demonstrated a dose effect of the HLA class II haplotype DR2 (DRB1*1501 [MIM 142857], DQB1*0602) on the risk of MS. In a total data set of 808 affected and 1,574 unaffected individuals, from 549 multicase or single-case kinships, carriage of one or two copies of DR2 conferred, respectively, 2.7-fold and 6.7-fold risks, as compared to genotypes lacking the haplotype.

We have performed a similar analysis in a data set of 937 Swedish sporadic MS cases and 739 Swedish controls. All patients were diagnosed with MS in accordance with the recommendations of McDonald et al. (2001); the mean age at onset was 31.4 years, 52 patients (5.5%) suffered from primary-progressive MS, and the female-to-male ratio was 2.6:1. HLA-DRB1 genotyping was performed by PCR amplification with sequence-specific primers and with a resolution corresponding to standard serological typing (Olerup and Zetterquist 1992). Genotypes for 786 patients had been used in a previous study (Masterman et al. 2000). Permission to perform the study was granted by the ethics committee of Karolinska Institutet, and all patients and controls gave their informed consent. Probability values for Hardy-Weinberg-equilibrium (HWE) testing were calculated by the χ^2 test; probability values for comparisons of genotype counts in patients and controls were calculated by the Fisher exact test, and 95% CIs of odds ratios were determined by Woolf's method and the use of Instat (GraphPad Software 1993).

As we have reported elsewhere (Masterman et al. 2000), both the DR2 split DR15 and the DR3 split DR17 are associated with MS in the Swedish population. Thus, we tested the modes of action of both these specificities, designating as reference "DRX" any specificity other than DR15 or DR17. Control genotypes were in HWE ($\chi^2 = 9.6$, 5 df, $P = .09$), but patient genotypes were not ($\chi^2 = 52.3$, 5 df, $P < .0001$), the largest deviations being an excess of DR17 homozygotes (26 observed vs. 13.6 expected, $\chi^2 = 11.3$) and a paucity of DR17 heterozygotes (92 observed vs. 119.8 expected, $\chi^2 = 6.5$).

The risks conferred by DR15/DR15 and DR15/DRX in the present study (table 1, rows 1–3) are virtually identical to those reported by Barcellos et al. (2003) for DR2-bearing genotypes in a subset of 362 singleton families, confirming the dominant, dose-dependent effect of the DR2 haplotype (and DR15 specificity) on MS susceptibility. By contrast, the mode of action of DR17, though also dose-dependent, is clearly recessive (table 1, rows 4–6), with two copies of DR17 conferring a 6.1-fold risk of MS and one copy conferring no risk at all. Indeed, a comparison of the risks conferred by DR15/DRX and DR15/DR17 further illustrates that a single copy of DR17 has no effect on the risk of MS (table 1, rows 7–9).

Although the mechanism by which alleles of classical or nonclassical HLA genes may predispose carriers to MS is still unknown, Oksenberg et al. (1996) have proposed six models. The first three involve presentation of encephalitogenic peptides by HLA molecules (the determinant model, the thymic-selection model, and the molecular-mimicry model), and the second three involve either regulation of the expression levels of immune molecules (the cytokine-regulation model and the aberrant-expression model) or proximity of associated HLA genes

Table 1

HLA-DR Genotypes in 937 Swedish MS Patients and 739 Swedish Controls

Specificities				
Genotype	Reference[a]	Odds Ratio	95% CI	P[b]
DR15/DR15	DRX/DRX	8.3	4.8–14.5	<.0001
DR15/DRX	DRX/DRX	3.0	2.4–3.9	<.0001
DR15/DR15	DR15/DRX	2.7	1.6–4.8	.0002
DR17/DR17	DRX/DRX	6.1	2.5–15.2	<.0001
DR17/DRX	DRX/DRX	.93	.68–1.3	.64
DR17/DR17	DR17/DRX	6.6	2.6–16.8	<.0001
DR15/DR17	DRX/DRX	3.9	2.5–6.1	<.0001
DR15/DR17	DR15/DRX	1.3	.81–2.0	.32
DR15/DR17	DR17/DRX	4.2	2.6–6.9	<.0001

[a] DRX indicates not DR15 or DR17.
[b] Uncorrected two-sided probability values.

to disease-predisposing non-HLA genes (the linkage disequilibrium [LD] model).

Although the association of two HLA-DR specificities to MS is not inconsistent with any of the single-mechanism models mentioned above—it could be explained, for example, by allelic heterogeneity (or even locus heterogeneity, given the extensive LD of the HLA region)—at the same time, the contrasting modes of action of DR15 and DR17 support an alternative, two-mechanism model. Indeed, to explain the multiple HLA class II associations in rheumatoid arthritis, Zanelli et al. (2000) have proposed such a model, involving both recessive loss of immune protection and dominant exacerbation of ongoing inflammation.

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Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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Nuclear Factor TDP-43 Binds to the Polymorphic TG Repeats in CFTR Intron 8 and Causes Skipping of Exon 9: A Functional Link with Disease Penetrance

To the Editor:

In the January 2004 issue of AJHG, Groman et al. published a collaborative study on the disease penetrance of a common abbreviated tract of five thymidines (T5) in intron 8 of the *CFTR* gene (MIM 602421). By analyzing a large number of affected individuals presenting with male infertility or nonclassical cystic fibrosis (CF [MIM 219700]), they found that the pathogenic or benign effect of T5 correlated with variations in a TG repeat sequence just upstream of the polypyrymidines. In particular, longer TG repeats (12 or 13) are associated with disease phenotypes, and 91% of affected individuals, as opposed to 22% of unaffected ones, have repetitions of 12 or 13 TGs. Although the modifier effect of the TG repeats on the T5 variant has been proposed elsewhere (Cuppens et al. 1998), the principal novelty of the Groman et al. study lies in the high number of patients analyzed from different regions. As the T5 allele is found in ~10% of the general population, the results of this study strongly indicate that determination of the TG repeats may have a significant diagnostic value.

The pathologies studied by Groman et al. are in line with the growing number of human diseases resulting from pre-mRNA splicing alterations and, specifically, those diseases caused by mutations in *cis*-acting elements (the TG and T repeats) that disrupt the use of alternative splice sites, as recently reviewed by Faustino and Cooper (2003). In this specific case, the pathogenetic role of these repeats is owing to their effects on *CFTR* exon 9 at the level of pre-mRNA splicing, with longer UG repeats (and short U tracts) increasing exon 9 skipping and leading to the production of a nonfunctional protein (Delaney et al. 1993; Strong et al. 1993).

An obvious question, not taken into consideration in the study by Groman et al., is what factor(s) or mech-