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LETTER

Ultraconserved regions in multiple sclerosis

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In their recent publication, Bejerano *et al*[1] identified 481 'ultraconserved elements' in the human genome. They defined these elements as genomic segments longer than 200 bp, showing 100% sequence homology with rat and mouse, but excluding ribosomal RNA regions. Many of these elements also show extremely high levels of homology with the chicken and dog genomes. Overall, these elements are more highly conserved than protein coding regions. The mechanisms responsible for maintaining these sequences through evolution are unclear but seem likely to include profound negative selection, suggesting that these segments have important, if not vital, functions. Whether these functions will necessarily be relevant to the pathogenesis of multiple sclerosis (MS) is speculative.

The high levels of homology are reflected in correspondingly low levels of variation within the general human population. Bejerano *et al*[1] identified just six validated single-nucleotide polymorphisms (SNPs) lying within these ultraconserved elements (rs1538101, rs1861100, rs2056116, rs7092999, rs7143938 and rs9572903), a total of 106 767 bp of sequence.

While there remains limited data on the functional effects of SNPs within ultraconserved regions, several pathogenic mutations in the region that regulates sonic hedgehog (*SHH*) expression are associated with preaxial polydactyly, a congential limb malformation.[2] Given the likely biological importance of these ultraconserved regions, it is reasonable to infer that these six variants have a high prior probability of conferring functional significance; however, the nature of this altered function is currently unpredictable. We tested these six SNPs from ultraconserved elements for evidence of association with MS, in which genetic factors are implicated,[3] but their nature and identity are largely unknown. Association was sought by typing each variant in 938 trio families (an affected individual and both parents). All patients satisfied Poser diagnostic criteria[4] with the majority of patients having relapsing-remitting disease (64.8%), 28.7% were secondary progressive and 6.5% were primary progressive.

These assays were performed using TaqMan methodology[5] (the primers used are listed in Supplementary Table S1) on a 7900HT Sequence Detection System, according to the manufacturer's standard conditions. Marker rs9572903 was found to be monomorphic. Results for the other five SNPs, analysed using the TRANSMIT program[6] to search for evidence of transmission distortion, are shown in Table 1.

In each case, 163 samples were typed in duplicate to ensure genotyping accuracy; no inconsistency was observed for any marker. Only one Mendelian inconsistency was observed across all five markers, indicating an approximate genotyping error rate of 0.02%. None of the markers showed deviation from Hardy–Weinberg equilibrium. After appropriate Bonferroni correction, none of the SNPs show any statistically significant evidence for association. SNP rs7143938 showed reduced transmission of the minor allele, which just reached nominal significance and therefore could reflect a modest effect. This SNP lies in the second intron of the Mirror Image Polydactyly Gene 1 (MIPOL1) on chromosome 14q13. This is a developmental gene, and it is not inconceivable that the gene product influences the immune or nervous systems. The observed under-transmission could indicate negative selection of the minor allele but, if so, it is unlikely that this allele would have reached a population frequency of

Table 1 SNPs from ultraconserved regions in multiple sclerosis

Marker	*Gene name*	*Chromosome location*	*Genotyping rate*[a] *(%)*	*Heterozygosity (%)*	*Minor allele frequency (%)*	*Uncorrected*[b] *P-value*[b]
rs1538101	BNC2	9p22.2	92.7	14.0	7.2	0.98
rs1861100	Intergenic	2p16.1	94.2	40.6	27.9	0.12
rs2056116	Intergenic	4p15.33	96.7	48.3	39.4	0.79
rs7092999	Intergenic	10q24.31	98.7	49.5	41.3	0.69
rs7143938	MIPOL1	14q13.3	92.6	43.1	31.8	0.035

[a]The proportion of potential genotypes successfully recorded.
[b]After Bonferroni correction none of these results are statistically significant.

>30% in the face of such negative selection. It is more likely that the evidence for under-transmission is due to sampling as suggested by the lack of significance after Bonferroni correction.

In summary, we found no evidence that variation in these ultraconserved regions influences susceptibility to MS.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)