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SHORT COMMUNICATION

HAPLOTYPES OF THE ENDOGENOUS RETROVIRUS HRES-1 IN MULTIPLE SCLEROSIS PATIENTS AND HEALTHY CONTROL SUBJECTS OF SHANGHAI CHINESE ORIGIN

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Endogenous retroviruses are normal constituents in the genome of vertebrates (Coffin 1984; Stoye and Coffin 1985). Some endogenous retroviruses possess immune regulatory activities (Chernukhin *et al.*, 1995; Scheeren *et al.*, 1992; Simpson *et al.*, 1993; for review see Krieg *et al.*, 1992 and Rasmussen and Clausen, 1997), and in mice they have even been associated with autoimmune diseases (Krieg and Steinberg 1990; Wu *et al.*, 1993). There is also evidence to suggest that a class of endogenous leukemia viruses, present in multiple copies in certain mouse strains, interacts with a normally harmless togavirus, known as lactate dehydrogenase-elevating virus, resulting in the development of a type of poliomyelitis (Anderson *et al.*, 1995). Perhaps similar activities can be assigned to endogenous retroviruses in the human genome. In support of this, a recent study reported the detection of a human endogenous retroviral gene product acting as a superantigen in insulin-dependent diabetes mellitus (Conrad *et al.*, 1997).

Multiple sclerosis (MS) is a demyelinating disease of presumed autoimmune etiology. To a large extent the genetic factors implicated in susceptibility to this disease are not known (Compston *et al.*, 1995; Oksenberg *et al.*, 1996). So far, a certain HLA class II haplotype has been associated with MS in Caucasians from Northern Europe and North America (Oksenberg *et al.*, 1996), but other susceptibility genes are most certainly involved. This assumption is supported by the apparent absence of an association between MS and HLA alleles in some geographical areas such as Asia (Kelly *et al.*, 1995a,b). Other potential susceptibility determinants of MS include endogenous retroviral genes. Recently, an ERV9-like novel retroviral sequence of possible endogenous origin was found in individuals with MS (Perron *et al.*, 1997).

Studies in our laboratory have focused attention upon a possible association between the human T cell leukemia virus-related endogenous sequence, HRES-1, and MS (Rasmussen *et al.*, 1996, Rasmussen and Clausen, submitted). These studies have

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revealed existence of four HRES-1 haplotypes. Interestingly, some HRES-1 haplotypes were more frequent in MS patients than healthy individuals from Denmark. Here we report the distribution of these haplotypes in MS patients and control subjects from China.

Samples of DNA were derived from 42 Chinese MS patients born and resident in Shanghai or the neighbouring regions of Zhejiang and Jiangsu. They all fulfilled the clinical criteria of probable or definite MS (Poser *et al.*, 1983). Also included in the study were 88 healthy control subjects from the same geographical area.

The samples of DNA were subjected to enzymatic amplification by the polymerase chain reaction (PCR) using three HRES-1 primer sets. These primer sets amplified fragments containing polymorphisms affecting the recognition sites of *Hin*dIII, *Eco*57I and *Nci*I, respectively (Rasmussen *et al.*, 1996; Rasmussen and Clausen, submitted). Determination of HRES-1 haplotype was carried out by digestion of the amplified fragments with the appropriate enzyme.

The value D, serving as a measure of the amount of linkage disequilibrium between two polymorphic sites, was obtained by use of the iteration method (Hedrich, 1983). This value was compared with D_{max}, i.e. the maximum obtainable value of D with the given allele frequencies at the loci under examination. Subsequently, we tested the hypothesis that D=0 by calculation of the likelihood ratio statistic Q which is approximately distributed as a chi-square variate with one degree of freedom (Hedrick, 1983). Statistical evaluations of haplotype distributions were carried out by chi-square test.

Genetic variation at the *Eco*57I locus was not detected in any of the Shanghai Chinese. Estimation of the linkage disequilibrium between the *Hin*dIII and *Nci*I loci revealed a D value equal to D_{max} in the group of MS patients ($Q = 30.07$, $P < 0.0005$) as well as the control group ($Q = 59.24$, $P < 0.0005$). The linkage disequilibrium relationship between the alleles at the *Hin*dIII and *Nci*I loci did not differ from that of European Caucasians (Rasmussen and Clausen, submitted).

Due to the lack of genetic variation at the *Eco*57I locus in the Shanghai Chinese, only three haplotypes were detected, namely the *Eco*57I-positive haplotypes 1, 2 and 4 (Table 1). There was no significant difference in the distribution of these haplotypes between the MS patients and control subjects. Consequently, HRES-1 does not seem to be associated with MS in Asians. By contrast, investigation of Europeans revealed a higher frequency of haplotypes 2 and 3 in MS patients compared with healthy control subjects (Rasmussen *et al.*, 1996). An explanation for the difference between the two ethnic groups could be that there is linkage disequilibrium between alleles at the HRES-1 locus and an unidentified susceptibility locus in Caucasians but not in Asians. Alternatively, MS is etiologically heterogenous with different susceptibility genes in different ethnic groups. In support of this are findings suggesting existence of an Asian type of MS which differs clinically as well as immunogenetically from the Western type (Kira *et al.*, 1996).

The frequencies of HRES-1 haplotypes in the MS patients and healthy control subjects from China differed significantly from those of European MS patients and healthy control subjects (Table 2). This was primarily due to a higher frequency of haplotype 2 combined with an absence of haplotype 3 and a low frequency of haplotype 4 in the Chinese individuals. Consequently, these findings provide evidence of a relatively significant degree of difference between Asians and Europeans at the HRES-1 locus. Whether this also applies to other endogenous retroviruses in the human genome remains to be determined. A recent study did not find evidence of distinct loci of a

Table 1. Haplotypes of HRES-1 and their distribution in MS patients and healthy control subjects of Shanghai Chinese origin.

Haplotype[a]	*Hin*dIII	*Eco*57I	*Nci*I	MS patients[b] (n=84)	Control subjects[b] (n=176)
1	-	+	-	38	67
2	+	+	+	39	92
3	-	-	-	0	0
4	-	+	+	7	17

MS patients *vs.* control subjects: test statistic $X^2 = 1.22$; d.f. = 2; $P > 0.50$.

[a] The HRES-1 haplotypes are defined on the basis of the presence or absence of three restriction enzyme polymorphisms as indicated by + or -, respectively (Rasmussen & Clausen, submitted).
[b] Numbers of each haplotype; n = number of chromosomes.

Table 2. Frequencies of HRES-1 haplotypes in MS patients and healthy control subjects of European Caucasian and Shanghai Chinese origin.

Haplotype[a]	Frequency[b]			
	European Caucasians		Shanghai Chinese	
	MS patients (n=174)	Control subjects (n=316)	MS patients (n=84)	Control subjects (n=176)
1	0.30	0.38	0.45	0.38
2	0.38	0.28	0.47	0.52
3	0.11	0.06	0.00	0.00
4	0.21	0.28	0.08	0.10

European control subjects *vs.* Chinese control subjects: test statistic $X^2 = 40.60$; d.f. = 3; $P < 0.0005$.
European MS patients *vs.* Chinese MS patients: test statistic $X^2 = 19.05$; d.f. = 3; $P < 0.0005$.

[a] For definition of the HRES-1 haplotypes, see above.
[b] Haplotype frequencies of the Europeans were determined in a previous study (Rasmussen and Clausen, submitted); the frequencies of the Chinese were calculated on the basis of data from the present study; n = number of chromosomes.

multicopy family of endogenous retroviruses in individuals of different ethnic origin suggesting that these endogenous proviral sequences were incorporated into the human genome prior to racial divergence (Yeh *et al.,* 1995).

In conclusion, the present study focusing upon HRES-1 does not provide new information with regard to non-HLA encoded susceptibility to MS. Instead, the study emphasizes previously recognized problems associated with the identification of MS susceptibility genes in populations of different ethnic background.

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