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A genome screen for linkage disequilibrium in Turkish multiple sclerosis

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Abstract

In order to screen the Turkish population for evidence of association with multiple sclerosis, we typed 6000 microsatellite markers in separately pooled DNA samples from 197 cases and 199 controls following the Genetic Analysis of Multiple sclerosis in EuropeanS (GAMES) protocol. Twelve markers showing evidence for association were identified. One of these markers lying directly in a region which is also implicated in the Turkish linkage screen (chromosome 5p15) and thus shows evidence for both linkage and association in independent data sets.

Keywords: Multiple sclerosis; Turkey; Linkage disequilibrium; Genome screen

1. Introduction

Available epidemiological evidence indicates that several genes determine susceptibility to multiple sclerosis with each contributing only modest effect individually (Compston, 2000). In this complex situation, tests for association are significantly more powerful than those based on linkage and a genome-wide effort to find association by testing all possible variants systematically would be the ideal experiment (Collins et al., 1997; Risch and Merikangas, 1996). Direct screening for association is at present impractical, as identification of all the relevant polymorphisms and cost effective genotyping methods are both required but not likely to be available for some time. Indirect whole genome screening for association reliant on linkage disequilibrium (the non-random association of alleles at linked loci) has been proposed as a compromise. An indirect screen uses a dense set of markers typed on the assumption that some at least will have alleles in sufficiently tight linkage disequilibrium to enable the detection of susceptibility alleles (Jorde, 2000).

Genetic factors determining susceptibility to multiple sclerosis have been extensively studied in northern Europeans, whereas the Turkish population remains relatively under explored. The high frequency of consanguineous marriage in Turkey (Simsek et al., 1999) suggests that genes exerting recessive effects on susceptibility are likely to be over-represented, and therefore that the study of this population favours their identification.

Previous genetic studies of multiple sclerosis in Turkey have confirmed association with alleles of the MHC (Saruhan-Direskeneli et al., 1997) and a recently completed linkage screen in 43 Turkish multiplex families revealed 24 regions of potential linkage (Eraksoy et al., 2003).

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We here present an indirect screen of the genome for association performed in the Turkish population as part of the GAMES collaborative, employing separately pooled DNA from 197 cases and 199 controls using the shared set of 6000 microsatellite markers.

2. Samples and methods

2.1. Individuals

All individuals included in our study were recruited in Turkey and are of Turkish descent. A total of 197 unrelated patients with multiple sclerosis were included. All the patients in this study fulfilled criteria for clinically definite multiple sclerosis (Poser et al., 1983). Demographic details of the patients are consistent with previous epidemiological surveys, with a 1.7:1 F/M ratio, a mean age of 39.3 years and a mean disease duration of 10.9 years. Eighty-four percent of patients had relapsing–remitting and 16% secondary progressive disease. We specifically excluded patients with the primary progressive phenotype. A total of 199 individuals were used as controls. All patients and controls gave written informed consent for genetic analysis and ethical approval was obtained from the local ethics committees and the Turkish Ministry of Health.

2.2. Markers

Linkage disequilibrium screening was performed using the 6000 microsatellite markers provided through the *G*enetic *A*nalysis of *M*ultiple sclerosis in *E*uropean*S* (GAMES) collaborative. Details of these markers are available on the GAMES website (www-gene.cimr.cam.ac.uk/Msgenetics/GAMES).

2.3. DNA pooling

DNA was extracted from a sample of venous blood using standard methods and concentrations measured using a 1:20 dilution in a 1-ml cuvette on an Ultraspec 3000 spectrophotometer (Pharmacia Biotech). Based on these measurements, samples were diluted to convenient working concentrations depending on the yield of DNA. Accurate measurement of DNA concentrations was then established using co-amplification of each sample with a known quantity of a specifically engineered DNA fragment (Corraddu, 2002). The relative yields of the resulting PCR products were then compared in order to calculate the concentration of DNA. This process was repeated three times for each sample in order to establish an average. Pools were constructed by adding 2 μg of DNA from each sample. The process was repeated in order to create two separate DNA pools from the cases and two from the controls. The four separate DNA pools are hereafter referred to as Cases 1, Cases 2, Controls 1 and Controls 2. Each pool was extensively vortexed and mixed, then diluted to a concentration of 20 ng μl^{-1}.

2.4. Genotyping

All PCR amplifications were performed using True Allele PCR premix (Applied Biosystems) in 15 μl reactions l reactions under the manufacturer's recommended conditions. The products from each polymerase chain reaction were subsequently electrophoresed twice on a 3700 DNA Analyser (Applied Biosystems). Genotyping was performed using GENESCAN/GENOTYPER software system. In the refining analysis, each pool was amplified by PCR twice and each of the resulting PCR products was electrophoresed twice, thereby generating up to four new AIPs from each pool.

2.5. Statistical methods

Typing a microsatellite in pooled DNA generates an allele image pattern (AIP) consisting of a series of product fragments, with the strength of the signal from each fragment reflecting the frequency of corresponding alleles. Up to two replicate AIPs were generated from each pool for each marker. Weighted average AIPs for cases and controls were then calculated across the replicates obtained (using the AIPs from both the first and the repeat pool constructions). After normalising according to the total number of alleles in the respective pools, these profiles were compared statistically using a χ^2 test. Statistical significance was established empirically using the observed distribution of results. Alleles with a frequency of $<5\%$ were considered together and only those peaks with a signal of $<10{,}000$ in at least one replicate were included in the analysis. Full details of the statistical methods employed can be found in the accompanying paper by Setakis (2003). Details of the adapting factors, used in the refining analysis, can be found in the accompanying paper from Yeo et al. (2003).

3. Results

The 6000 microsatellite markers provided through the GAMES collaboration were genotyped in each of the four Turkish DNA pools (Cases 1, Cases 2, Controls 1 and Controls 2). Data of sufficient quality for statistical analysis were generated from 4359 of these markers, which were thereby ranked according to their evidence for association. The raw AIPs from the most extreme 150 markers (3%) were reviewed in order to exclude markers showing extreme results on the basis of genotyping errors or poor quality data. Thirty-eight markers survived this process. In order to refine the ranking of these most promising 38 markers, additional AIPs were generated from each pool for each of these markers. These new replicate AIPs were combined with the original data and analysed using the adapting

Table 1
Markers showing strongest evidence for association in the linkage disequilibrium screen

Marker	Empirical p value
D11S4207[a]	0.0251
D1S2781	0.0174
D2S149	0.0035
D3S4018	0.0046
D5S1453[a]	0.0275
D5S1505	0.0031
D5S676[a]	0.0093
D6S460[a]	0.0155
D8S1837[a]	0.0140
D9S1847	0.0126
D12S2075[a]	0.0379
D19S902[a]	0.0246

[a] These markers were included amongst the 529 considered by Yeo et al. (2003).

factors suggested by Yeo et al. (2003). This analysis revealed 12 markers which retained empirical p-values $<5\%$ (see Table 1). Applying the crude correction for length-dependant amplification (LDA) suggested by Yeo et al. (2003) produced no major change in the results (data not shown). Generating extra replicate AIPs for the TNFa marker and analysing this new data using the adapting factors and correction for LDA suggested by Yeo et al. (2003) revealed that this marker was also significant at the 5% level although poor data quality had resulted in it being missed in the initial screen.

4. Discussion

We performed a systematic screen for linkage disequilibrium (LD) of the genome in Turkish patients with multiple sclerosis using separately pooled DNA from 197 cases and 199 controls typed for 4359 microsatellite markers. Twelve novel associations are identified, one of which (D5S676) is located in an area of interest also identified in the recently completed Turkish linkage genome screen (Eraksoy et al., 2003). The identification of overlapping regions by employment of two different methods of screening in different cohorts provides a powerful way of identifying genomic regions which may encode susceptibility factors that are particularly relevant for the Turkish population. Three other markers (D1S2781, D5S1505, D5S1453) which showed association in our screen are located in regions of interest implicated by whole genome linkage analysis of multiple sclerosis from northern European populations (The Transatlantic Multiple Sclerosis Genetics Cooperative, 2001). Two of these are co-located at 5q22 and 5q23 in close proximity, further increasing the likelihood that this region harbours susceptibility genes for multiple sclerosis.

Even though this study is not sufficiently powered to detect all the relevant susceptibility genes, it has modest power to detect at least some genes. As the power of any linkage disequilibrium genome screen is dependent upon the frequency of susceptibility alleles in the population, which varies between countries, systematic screens are worth pursuing in naive populations where the allele frequency may, by chance, be more favourable than in existing surveys. Since Turkey is an intermediate risk population for multiple sclerosis, it is conceivable that the frequency of some alleles may here be more informative than in Northern Europeans where polymorphisms determining susceptibility are relatively over-represented, perhaps exceeding the crucial frequency needed to demonstrate associations. The consanguineous nature of the Turkish population is expected to enhance the power to identify regions containing susceptibility genes acting in a recessive manner.

The most relevant finding of our study is the identification of an associated marker which overlaps with a region implicated in the recently conducted linkage study of Turkish multiple sclerosis multiplex families (Eraksoy et al., 2003). D5S676 is located on chromosome 5p15, close to the region syntenic for a murine susceptibility locus in experimental allergic encephalomyelitis, which has also been implicated in Finnish multiplex families with multiple sclerosis (Kuokkanen et al., 1996). The historic links between Turks and Finns, which still manifest in similarities between the two languages, imply that these two populations share a common ancestry (Comrie et al., 1997; Haywood et al., 1997). Further screening for positional candidates on chromosome 5p in Finland and Turkey would therefore seem a promising strategy in the search for relevant susceptibility factors that may be especially influential in these populations of common ancestry.

Taken together, these results implicate chromosome 5p15 as the best supported region in Turkish multiple sclerosis populations; this should now be considered for positional analysis of candidate genes. The study lends further support to 5q22–23 and 1p34, which have been implicated in other screens, as potentially relevant susceptibility regions.

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