SHORT REPORT

The role of the PTPRC (CD45) mutation in the development of multiple sclerosis in the North West region of the United Kingdom

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Background: A point mutation in protein tyrosine phosphatase receptor, type c polypeptide (PTPRC) has been associated with familial multiple sclerosis. This CG mutation at position 77 of exon 4 results in altered expression of CD45 isoforms on immune cells.
Objective: To study the incidence of PTPRC mutations in subjects with multiple sclerosis in the North West region of the United Kingdom.
Methods: Affected and unaffected subjects from five pedigrees with familial multiple sclerosis, 330 non-familial cases of multiple sclerosis, and 197 controls were studied. Genomic DNA was amplified using CD45IE34 and CD45IE44 primers, digested with MspI, and run on an agarose gel. Polymerase chain reaction products were sequenced to exclude any other mutations.
Results: No PTPRC exon 4 genomic mutations were seen in any of the five families. In the non-familial cases the incidence of mutation was 4.1% in 197 controls and 5.1% in 330 multiple sclerosis patients. No significant association was found in this study with this mutation and disease susceptibility, sex, or an extended disability scale score of < 5.5.
Conclusions: This candidate does not appear to influence the development of familial multiple sclerosis in this population. The negative result could arise from a type II error owing to the number of families and non-familial cases screened. Alternatively it might suggest that the contribution of the PTPRC mutation depends upon the genetic background.

Classical family studies have confirmed the genetic susceptibility to multiple sclerosis. However, the candidate gene approach and more recently genome screening have produced inconsistent findings, apart from an association with the human leucocyte antigen located on chromosome 6p21.[1–3] To dissect the pathogenesis of this complex genetic disorder one approach has been to extend genome screening[4] to compensate for some of its perceived failings—low power, random variation, or population specific effects.[2] With this aim, further analysis is currently under way in 18 sites in Europe.[4]

An alternative approach is to study multiplex families, which, though rare, may identify genes of strong to moderate effect that inform pathogenesis. CD45 is a protein tyrosine phosphatase receptor, type c polypeptide (PTPRC) which is expressed on all haematopoietic cells. Several isoforms of the receptor exist, designated CD45RA, RB, RC, and RO. Each has specific biological activities and patterns of expression.[5] Using multiplex families, a CG mutation at position 77 of exon 4 in PTPRC, the gene coding for CD45 (producing an increased expression of CD45RA isoforms on activated and memory T cells[6]) has been associated with familial multiple sclerosis.[7] The association was replicated in three of five case–control studies on German patients[7 8] but not in a case–control and multiplex study of American patients with multiple sclerosis[9] or in patients with other autoimmune diseases.[10]

Using this second approach it is important to survey the association of any potential disease modifying mutation in independent populations from both related and different regions in order to understand its role. Our aim was to determine the incidence of CG mutations at position 77 of exon 4 in PTPRC in both multiplex families and single cases and controls in another northern European population.

METHODS

Subjects

The population association analysis was done on 330 unrelated white patients from the North West region of the United Kingdom with clinically definite multiple sclerosis, and on 197 healthy controls. The mean (SD) age of onset of disease in the multiple sclerosis group was 31 (9) years. There was no difference in the age of onset between women (30 (9) years) and men (32 (9) years). The mean age of the controls was 43 (15) years at the time blood was taken. In the multiple sclerosis group 74% were female, compared with 77% in the control group, and 57% had an extended disability scale score (EDSS) of < 6. The linkage analysis was carried out in five pedigrees, each of which had at least three cases of clinically definite multiple sclerosis.

Polymorphism analysis

Genomic DNA was derived from affected and unaffected subjects by standard methods. DNA was amplified using PTPRC exon 4 specific primers (forward, 5′-ATTTATTTTGTCCTT CTCCCA-3′, and reverse, 5′-GTTAACAACTTTTGTGTGCC-3′),[3] digested with MspI and run on an agarose gel. The presence of the mutation produces a novel Msp1 restriction site. Digestion of the polymerase chain reaction (PCR) product by Msp1 reduces the size of the fragment from 260 base pairs (bp) to 155 bp. In the wild type, a single product of 155 bp is produced, whereas in the presence of mutant genomic DNA additional fragments of 84 bp and 71 bp are produced. DNA sequencing of the products was carried out to confirm the content of the sequence to exclude the CG mutation at position 77 and any other exon 4 mutations.

Statistical analysis

Patients were categorised as positive if they were heterozygous for the CD45 mutation. Homozygotes have not been described.[6] Pearson 2×2 χ^2 tables were calculated for CD45 status and the presence or absence of disease in the whole group and by sex; 2×2 tables were also calculated for CD45 status and EDSS ≤ 5.5 or ≥ 6. Odds ratios and 95% confidence intervals (CI) were calculated using logistic regression analysis to estimate the relation between CD45 status and disease

Table 1 The incidence of CG mutations at position 77 of exon 4 in PTPRC in the North West region of the United Kingdom

	No mutation	CD45 exon 4 mutation
Controls (n (%))	189 (95.9)	8 (4.1)
MS patients (n (%))	313 (94.9)	17 (5.1)

MS, multiple sclerosis; PTPRC, protein tyrosine phosphatase receptor, type c polypeptide.

susceptibility, correcting for sex as a confounding variable; and to estimate the relation between CD45 status and EDSS $\leqslant 5.5$ or $\geqslant 6$ and for disease duration $\leqslant 10$ or > 10 years correcting for sex, disease duration, and age of onset as confounding variables. A linkage analysis was planned in the family study.

RESULTS

No significant association was found in this study between this mutation and disease susceptibility (table 1, $\chi^2 = 0.32$, $p = 0.569$). In subjects with multiple sclerosis no association was found between the mutation and sex (women, $\chi^2 = 1.53$, $p = 0.217$; men, $\chi^2 = 0.27$, $p = 0.603$), or between the mutation and an EDSS score of $\leqslant 5.5$ ($\chi^2 = 0.01$, $p = 0.909$). Logistic regression analysis showed no significance for the following: disease susceptibility and the mutation correcting for sex ($p = 0.40$); disease susceptibility and the mutation correcting for an EDSS $\leqslant 5.5$ or $\geqslant 6$; disease susceptibility and the mutation correcting for sex, disease duration, and onset age ($p = 0.44$); or disease susceptibility and the mutation correcting for those with multiple sclerosis for more than 10 years, sex, disease duration, and onset age ($p = 0.845$).

No PTPRC exon 4 genomic mutations were seen in any of the five families on agarose gel analysis and this was confirmed by sequencing the PCR products of affected family members.

DISCUSSION

Using both a case–control and a family based approach we have not found any association with the CG mutation at position 77 of exon 4 in PTPRC and multiple sclerosis in the North West region of the United Kingdom. These results suggest that this candidate gene does not influence the development of multiple sclerosis in this population. This negative result could arise from a type II error. In the case–control study the allele frequency for patients with non-familial multiple sclerosis was higher than in the controls (table 1). The increased allele frequency in non-familial multiple sclerosis seen here is significantly less than that seen between cases and controls in the German study,[7] but there is a possibility that an association could be found if larger numbers were studied. A type II error could also occur in the family study owing to the small number of families screened, although we have studied more families than were originally screened in the German population. In one of the original families screened in that study,[7] evidence of enhanced expression of high molecular weight CD45 isoforms was seen on flow cytometric analysis of peripheral blood lymphocytes and no CD45RA exon 4 mutations were seen. Neither in the USA[9] nor in the present study was flow cytometric analysis done, so there is a possibility that there could still be abnormal CD45 expression in some of these patients.

Since the 1950s, evidence has emerged implicating genetic factors in the development of multiple sclerosis.[1] Though significant advances have been made, the genetic basis of the disease remains elusive, apart from consistent linkage and association with chromosome 6p21. The failure to replicate Jacobsen's original work[7] in a population of American patients and the patients reported in this paper illustrates the classic problem with analysing a complex genetic disease using the candidate gene approach, in that the basis of the discordant results may lie in disease heterogeneity, which has been recognised pathologically.[11] Recent progress in the study of neurodegenerative diseases has illustrated the importance of results from single families and isolated populations in exploring complex non-mendelian genetic diseases.[12] In tandem with advances in genome screening based approaches,[4] multiplex families such as those identified for this study are potentially a valuable resource in the future for elucidating the genetic basis of diseases such as multiple sclerosis.

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