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Evidence for an X-linked genetic component in familial typical migraine

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Migraine is a common complex disorder that shows strong familial aggregation. There is a general increased prevalence of migraine in females compared with males, with recent studies indicating that migraine affects 18% of females compared with 6% of males. This preponderance of females among migraine sufferers coupled with evidence of an increased risk of migraine in first degree relatives of male probands but not in relatives of female probands suggests the possibility of an X-linked dominant gene. We report here the localization of a typical migraine susceptibility locus to the X chromosome. Of three large multigenerational migraine pedigrees two families showed significant excess allele sharing to Xq markers ($P = 0.031$ and $P = 0.012$). Overall analysis of data from all three pedigrees gave significant evidence in support of linkage and heterogeneity (HLOD = 3.1). These findings provide conclusive evidence that familial typical migraine is a heterogeneous disorder. We suggest that the localization of a migraine susceptibility locus to the X chromosome could in part explain the increased risk of migraine in relatives of male probands and may be involved in the increased female prevalence of this disorder.

INTRODUCTION

The aetiology of migraine remains unknown, although population, family and twin studies implicate genetic susceptibility in the pathogenesis of the disorder. Migraine shows strong familial aggregation, with ~50% of first degree relatives also affected with the disorder (1). The mode of inheritance, however, remains unclear. A recent review of twin, spouse and family studies strongly suggested that the two major types of migraine, migraine with aura (MA) and migraine without aura (MO), are genetically determined, with the mode of inheritance most likely multifactorial (2). Segregation analyses by Mochi *et al*. suggested that there may be a common genetic background for the two types of migraine (3). This is supported by similarities in medication response in the two types of migraine (4) and also by the fact that the two types can occur in the same family and even the same individual (5,6).

Migraine family studies, aimed at identifying the mode of inheritance of typical migraine, have produced conflicting results. However, a number of consistent factors have been noted regarding both MA and MO which point in the direction of polygenic inheritance and the possibility of an X-linked susceptibility gene. For both MO and MA, there is an unequal sex distribution (female preponderance of 3:1) (7–10) and for MO, probands of the least affected sex (males) have a higher proportion of affected first degree relatives (1,2). An X-linked dominant pattern of inheritance in typical migraine may explain an increased female prevalence. In families where such an inheritance pattern occurred there would be no male-to-male transmission but affected males would transmit to all daughters. Therefore, in these families there would be an excess of affected females.

At present the number of genes involved in common forms of migraine is unknown and not identified, although a gene for a rare sub-type of migraine, familial hemiplegic migraine (FHM), has been mapped to chromosome 19p13 (11). Subsequent studies have indicated heterogeneity of FHM, with ~50% of tested families showing linkage to this genomic region (11–14). More recently mutations in a brain-specific P/Q-type Ca^{2+} channel α1-subunit gene, *CACNA1A*, located on chromosome 19p13, have been shown to be involved in some FHM pedigrees (15).

In view of the unequal sex distribution of the disorder and the possible involvement of X chromosomal inheritance, we performed a genetic linkage scan of the entire X chromosome utilizing 28 microsatellite markers and three large migraine pedigrees. DNA samples from 105 individuals (41 affected) from these families were analysed. All family members were interviewed by a clinical neurologist and classified by two neurologists experienced in headache diagnosis using International Headache Society (IHS) guidelines (16). The study indicated that typical migraine is a heterogeneous disorder and that in some affected pedigrees there is an X chromosomal component.

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RESULTS

Families and diagnoses

Three large multigenerational families collected in Australia were used in this study. These included 105 individuals for whom DNA was available and 103 with neurological evaluations. For this report 43 individuals (41 with DNA available) were classified as affected, after being clinically diagnosed as either MA or MO. Affected individuals within the three pedigrees showed a variation in migraine phenotype. This included differences in age of onset, frequency and severity of attacks, triggering factors and response to medication. However, all individuals classified as affected satisfied the criteria for diagnosis of MA (IHS criteria 1.2.1) or MO (IHS criteria 1.1). In these three families a total of 28 microsatellite markers spanning the entire X chromosome, with an average separation of 6.9 cM, were tested for linkage to migraine.

Linkage analysis

Two approaches were taken in the analysis of linkage results. Using the GENEHUNTER program (17) we performed both traditional parametric (LOD score) analysis and also non-parametric (NPL Z score) analysis. NPL analysis, which tests for excess allele sharing among affected relatives, was used in order to overcome problems associated with mis-specification of transmission model parameters, as is common in the analysis of a complex disorder such as migraine. Multipoint parametric and non-parametric results for all three families are summarized in Table 1

Parametric analysis showed probable linkage of telomeric Xq markers in one of the three tested pedigrees (maximum LOD = 1.48 for MF14 at the DXS1123 locus). A homogeneity test on the complete data set from all three pedigrees produced a LOD score of 3.09, in support of linkage and heterogeneity. Traditionally a LOD score >2 indicates significant X-linkage, hence these parametric analyses indicate the probable presence of a migraine susceptibility locus on the X chromosome.

Non-parametric NPL analysis indicated significant excess allele sharing among affecteds in two of the tested families (MF7 and MF14), providing additional evidence for a migraine susceptibility locus on the X chromosome. Excess allele sharing was indicated with two markers towards the telomere of Xq for MF7 and five markers in the same chromosomal region for MF14. Maximum NPL Z scores of 2.57 (P = 0.031) at the DXS1001 locus and 2.74 (P = 0.012) at the DXS1123 locus were found in the MF7 and MF14 pedigrees, respectively. The remaining pedigree (MF1) showed no significant excess allele sharing with any of the X chromosome markers tested, producing mostly negative NPL Z scores, although a few positive, but non-significant ($P \geq 0.1$) scores were also obtained. This MF1 result is important, particularly since we have previously demonstrated linkage of this pedigree to chromosome 19p13 (Nyholt *et al.*, in press).

Information from the two families showing Xq excess allele sharing (MF7 and MF14) was then combined and used in a multipoint GENEHUNTER analysis. This analysis indicated excess allele sharing across a 30 cM region towards the telomere of Xq and is summarized in Figure 1. The combined analysis produced both a maximum parametric LOD score of 1.76 and a maximum NPL Z score of 2.866 (P = 0.011) at the DXS1123 locus, which has previously been localized to Xq28 (18).

Table 1. X Chromosome multipoint results

Marker	LOD scores			NPL Z scores		
	MF1	MF7	MF14	MF1	MF7	MF14
DXS996	–0.183	–0.145	–0.085	–0.855	–0.680	–0.166
DXS85	–0.510	–0.452	0.101	–1.003	–0.419	–0.673
DXS999	–0.481	–0.689	–0.508	–1.098	–0.352	–0.899
DXS1683	–0.511	–0.689	–0.804	–1.114	–0.358	–1.168
DXS989	–0.488	–0.683	–0.802	–1.122	–0.369	–1.042
DXS997	–0.021	–0.519	–0.797	–0.484	–0.386	–0.986
DMD	0.288	–0.347	–0.670	0.102	0.929	–0.898
MAOA	0.284	–0.212	–0.795	0.038	0.929	–0.845
MAOB	0.284	–0.212	–0.798	0.037	0.929	–0.845
DXS1003	0.287	–0.212	–0.944	–0.066	0.929	–0.939
DXS991	0.288	–0.212	–1.524	–0.593	0.929	–1.490
PGK1P1	0.288	–0.212	–1.522	–0.592	0.929	–1.489
DXS981	0.288	–0.029	–1.521	–0.592	1.366	–1.489
PGK1	0.288	–0.096	–1.521	–1.119	1.803	–1.489
DXS986	0.288	–0.096	–1.523	–1.118	1.803	–1.489
DXS1002	0.038	–0.096	–1.524	–1.119	1.803	–1.490
DXS995	–0.615	–0.096	–1.523	–1.118	1.803	–1.178
DXYS1X	–0.609	–0.091	–1.526	–0.845	1.806	–1.166
DXS990	–0.613	–0.088	–1.526	–0.847	1.803	–1.163
DXS454	–0.609	–0.080	–1.505	–0.842	1.803	–1.161
DXS1120	–0.608	–0.096	–1.508	–0.855	1.803	–1.165
DXS1001	–0.284	0.145	–0.012	0.051	**2.572**	0.150
HPRT	–0.346	–0.949	1.233	0.669	0.042	2.133
DXS984	–0.298	–0.948	0.844	0.621	0.052	1.642
DXS297	–0.257	0.295	0.835	–0.254	1.340	2.292
DXS1123	–0.419	0.277	1.482	–0.519	1.314	**2.739**
DXS15	–0.325	0.251	1.336	0.414	1.307	2.420
DXS1108	–0.323	0.357	1.123	1.413	1.366	1.823

Parametric LOD and non-parametric NPL Z scores for migraine pedigrees MF1, MF7 and MF14. Significant excess allele sharing was found in migraine pedigrees MF7 and MF14, producing maximum NPL Z scores of 2.572 (P = 0.031) and 2.739 (P = 0.012) respectively.

Although independent allele sharing analysis of MF7 and MF14 produced different peak regions, the combined analysis of the two pedigrees indicated allele sharing more towards the MF14 peak region. The difference in peak regions may result from the complexity of MF7, with the presence of four non-familial affected spouses.

Co-segregation of migraine with Xq haplotype in the MF7 and MF14 pedigrees can be seen in Figure 2. Although a predisposing haplotype has been inherited with migraine in all affected individuals in both pedigrees, incomplete penetrance is evident, with some unaffected individuals also inheriting this haplotype. This could be explained by considering the complex nature of this disorder, including the variable age of onset and the effect of environmental factors and/or interacting genes. It should also be noted that there is potential for male-to-male transmission of the

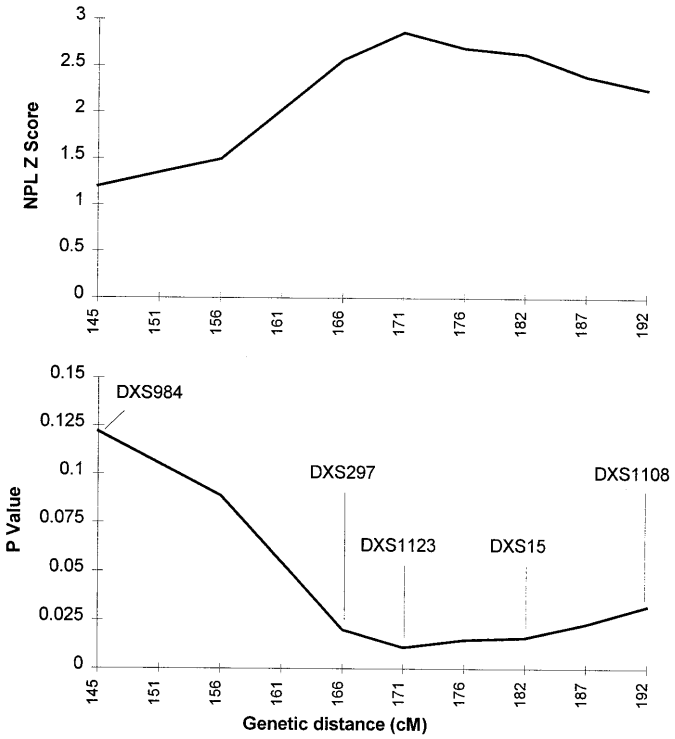

Figure 1. Results of GENEHUNTER non-parametric linkage analysis for migraine families MF7 and MF14 for the telomeric region of Xq. Genetic distance is represented on the *x*-axis in cM; *P* values and multipoint NPL Z scores are represented on the *y*-axis.

disorder in both of these pedigrees. However, examination of haplotype data indicates that in MF7 the predisposing haplotype is transmitted to the affected male offspring from the maternal side. In MF14, haplotype data does not negate the potential for male-to-male transmission, but because the disorder is so common, is heterogeneous and shows incomplete penetrance it would be possible for apparent male-to-male transmission to be a result of maternal incomplete penetrance or the contribution of another causative gene.

DISCUSSION

The results of this investigation provide strong evidence for the location of a migraine susceptibility locus on chromosome Xq and support a model of locus heterogeneity. This is the second migraine susceptibility locus that has been localized. Previous studies by ourselves and others (19) have indicated that the 19p13 region is involved not only in the rare familial hemiplegic migraine, but also in much more common forms of familial typical migraine. Results from the present study indicate that there are at least two genes involved in this common complex disorder.

There is a well-documented increased prevalence of females with migraine compared with male sufferers of the disorder. The prevalence of migraine has generally been shown to be equal in boys and girls before puberty, but increases at a greater rate in girls as adolescence approaches, so that by adulthood the female to male ratio has increased to 3:1 (20,21). It has been postulated that this age-dependent increase in female prevalence could be the result of hormonal fluctuations triggering a primary predisposition (22,23). A number of findings provide support for a relationship between hormonal variations and migraine. These include evidence indicating that many women with migraine report worsening of their headaches around the time of menstruation (24–26). Also, the oral contraceptive pill may precipitate a first attack of migraine or it may worsen or improve the frequency and severity of existing attacks (27–33). In addition, many women with migraine have fewer attacks during pregnancy (34–36), although the condition may be exacerbated in the post-partum period (37). Finally, some women have fewer attacks or cease to have attacks after the menopause (38).

Another possibility to explain the increase in female prevalence is involvement of an X chromosomal component in migraine susceptibility. This could be in the form of a causal X chromosome defect or an interacting X chromosomal component. An excess of affected females in migraine pedigrees could be explained by an X chromsomal susceptibility gene. In such families male-to-male transmission would not be apparent, but it should be noted that in the light of heterogeneity evidence, male-to-male transmission may occur in non-X-linked pedigrees. Migraine is obviously a complex disorder in which both genetic and environmental heterogeneity exist. The identification of an X-linked component in this disorder should stimulate further interest involving candidate genes from this part of the genome and hopefully lead to a better eventual understanding of migraine aetiology.

MATERIALS AND METHODS

Families and diagnoses

Prior to commencement all research was approved by Griffith University's Ethics Committee for Experimentation on Humans. All individuals donating blood samples gave informed consent and all were of Caucasian origin. Diagnosis of migraine was performed following the criteria specified by the International Headache Society (IHS) (16). All pedigree members were personally interviewed by a neurologist to establish diagnosis, following criteria specified by the IHS, with affected individuals classified as MA or MO as previously described (39).

DNA analysis

DNA was extracted from blood samples by a standard SDS/proteinase K method incorporating a salting-out procedure. Twenty eight microsatellite markers spanning the X chromosome were analysed. Primer sequences for the microsatellite markers were obtained from both published reports (40) and the Genome Database (41). Genotypes were determined by polymerase chain reaction (PCR) amplification, as described previously (42).

Linkage analyses

Genotypes for the pedigree members were assessed and analysed for linkage using parametric and non-parametric techniques. For all 28 markers allele frequencies were estimated from pedigree data, using the USERM13 program (43) of the MENDEL package of programs (44). Reliability of USERM13-calculated allele frequencies was compared with allele frequencies calculated directly from a genotyped control population consisting of

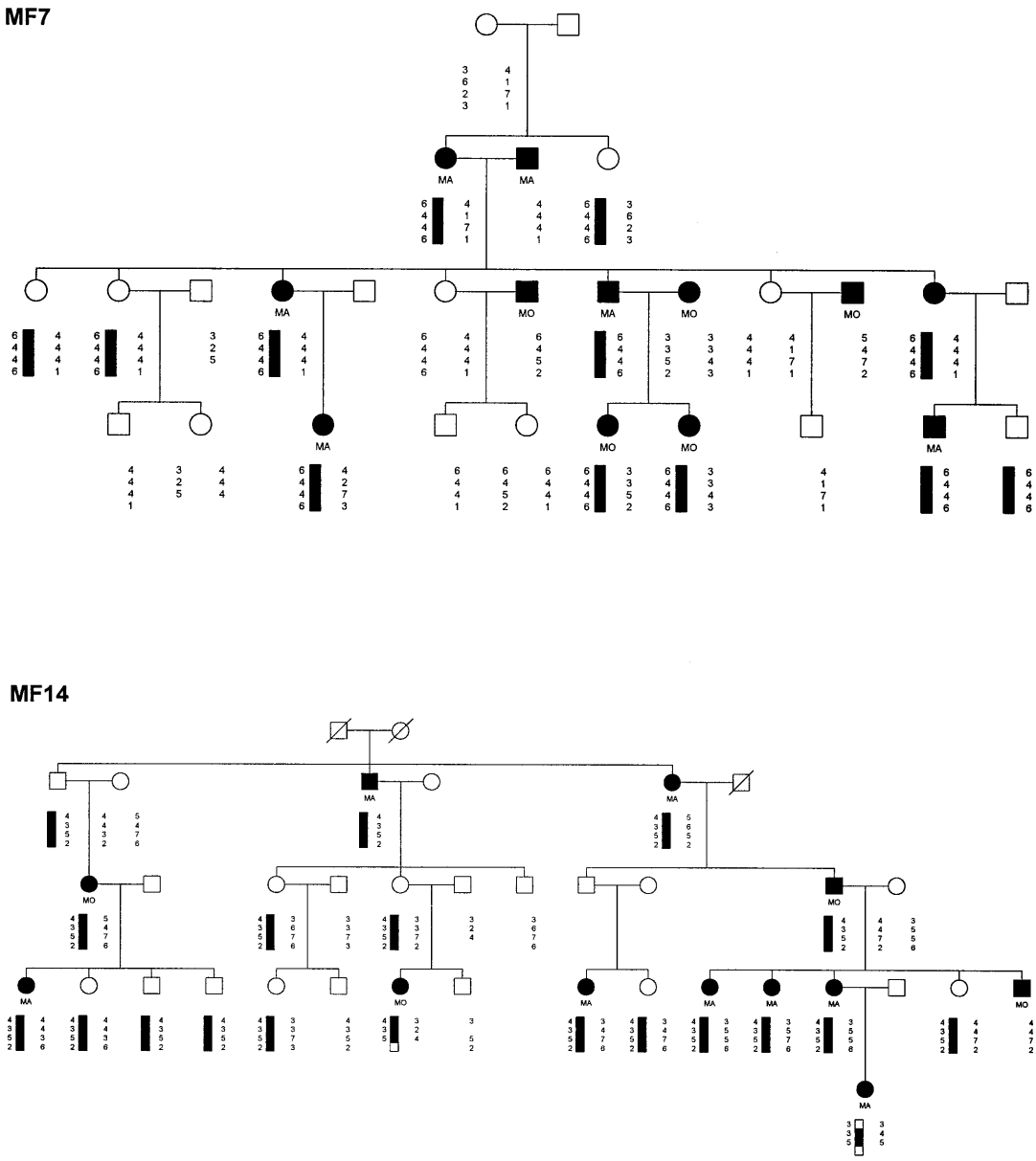

Figure 2. Migraine families MF7 and MF14, showing chromosome Xq haplotypes. Marker order from centromere to telomere is DXS297, DXS1123, DXS15 and DXS1108. Solid symbols, affected individuals; open symbols, unaffected individuals. Individuals diagnosed as migraine with aura (MA), migraine without aura (MO) or unaffected (blank) are given directly under each symbol. A black bar indicates haplotypes shared by affected individuals.

90 unaffected individuals for the MAOA, MAOB and DXS1003 microsatellite markers. Allele frequencies obtained by both methods did not differ significantly. Complete multipoint parametric LOD scores were calculated using the GENEHUNTER (XGH) program (17). For these parametric LOD score calculations the mode of transmission of the disease needs to be specified. A conservative model, assuming an X-linked dominant mode of inheritance with 70% penetrance and a phenocopy rate of 0.7%, was used (39,45). The frequency of the disease was set at 12% (7). The resulting multipoint LOD scores contained information across the entire X chromosome and were subsequently used in a test for heterogeneity using the HOMOG program (46). GENEHUNTER was also used to perform non-parametric analysis. The GENEHUNTER multipoint non-parametric lod (NPL) Z score calculations utilized information from all 28 markers. The X-linkage-specific XGH program was used, with 'max bits' set at 20 and the 'skip large' option switched off, thereby allowing 'trimming' of the pedigrees. This was made necessary due to constraints on the size of families able to be processed by GENEHUNTER. The genetic map order used, based on Genethon (40) data, with map distances (Kosambi cM) given in parentheses, was: DXS996-(13.2)-DXS85-(8.48)-DXS999- (6.03)-DXS1683-(6.53)-DXS989-(6.44)-DXS997-(9.31)-DMD-(13.09)-MAOA-(0.1)-MAOB-(8.07)-DXS1003-(10.03)-DXS991-(0.1)-

PGK1P1-(0.1)-DXS981-(0.1)-PGK1-(1.5)-DXS986-(0.1)-DXS1002-(0.1)-DXS995-(2.9)-DXYS1X-(4.41)-DXS990-(7.25)-DXS454-(8.18)-DXS1120-(19.07)-DXS1001-(12.66)-HPRT-(7.66)-DXS984-(20.48)-DXS297-(4.81)-DXS1123-(11.29)-DXS15-(10.14)-DXS1108.

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