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ORIGINAL INVESTIGATION

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Familial typical migraine: significant linkage and localization of a gene to Xq24–28

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Abstract In a previous study we found evidence for an X-linked genetic component for familial typical migraine in two large Australian white pedigrees, designated MF7 and MF14. Significant excess allele sharing was indicated by nonparametric linkage (NPL) analysis using GENEHUNTER (P=0.031 and P=0.012, respectively), with a combined analysis of the two pedigrees showing further increased evidence for linkage, producing a maximum NPL score of 2.87 (P=0.011) at DXS1123 on Xq27. The present study was aimed at refining the localization of the migraine X-chromosomal component by typing additional markers, performing haplotype analysis and applying a more powerful technique in the analysis of linkage data from these two pedigrees. Results from the haplotype analyses, coupled with linkage analyses that produced a peak GENEHUNTER-PLUS LOD* score of 2.388 (P=0.0005), provide compelling evidence for the presence of a migraine susceptibility locus on chromosome Xq24–28.

Introduction

Familial typical migraine (MIM 300125), consisting of migraine with aura (MA) and migraine without aura (MO), is a common debilitating disorder affecting approximately 12% of the Western population (Stewart et al. 1992). A study by Stewart et al. (1992) indicated that 18% of women, 6% of men and 4% of children suffer from the disorder, although results from Rozen et al. (1999) indicate that the incidence of migraine, particularly in women may be increasing. This study of a population in Minnesota in the United States showed that there was an increased incidence in the number of medically recognized female migraineurs in the periods 1979–1981 and 1989–1990, for all ages, whilst incidence rates in men appeared more stable over time (Rozen et al. 1999). A recent large study in the Netherlands also confirmed the higher prevalence of migraine in women than in men (Launer et al. 1999). The Dutch study of 6491 adults gave uncorrected 1-year prevalence estimates of 25% in women and 7.5% in men and life-time prevalence estimates of 33% in women and 13.3% in men. Migraine is clearly a disorder that affects a significant proportion of the population.

Studies have also shown that migraine has a large genetic component, with heritability estimates varying between 40% and 60% (Honkasalo et al. 1995; Larsson et al. 1995), depending on the population studied. Migraine shows strong familial aggregation, with approximately 50% of migraine sufferers having an affected first-degree relative (Goadsby et al. 1991). Although family studies, aimed at identifying the mode of inheritance of typical migraine, have produced conflicting results, a number of consistent factors have been noted regarding both MA and MO, which point in the direction of an X-chromosomal inheritance factor. For both MA and MO there is an unequal sex distribution (female preponderance of 3:1; Launer et al. 1999; Rasmussen and Olesen 1992; Rozen et al. 1999; Stewart et al. 1991, 1992), and for MO probands of the less affected sex (males) have a higher proportion of affected first-degree relatives (Russell and Olesen 1993; Stewart et al. 1997). These results suggest that an X-linked dominant form of inheritance is involved in the disorder.

Recent results from our laboratory support this possibility and also provide strong evidence that the disorder is heterogeneous, with linkage results implicating genomic regions on both chromosome 19 (Nyholt et al. 1998a) and the X chromosome (Nyholt et al. 1998b). Utilizing two large multigenerational pedigrees, designated MF7 and MF14, and 28 markers spanning chromosome X, we recently reported evidence for an X-linked genetic component in familial typical migraine. In this study multipoint

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nonparametric linkage (NPL) analysis using the GENEHUNTER program (X-linkage version 1.1; Kruglyak et al. 1996) produced positive NPL scores across a large region on Xq, with significant excess allele sharing indicated with two markers in MF7 and five markers in MF14. However, although the combined analysis of the two pedigrees indicated excess allele sharing more towards the MF14 peak (total NPL_{max} of 2.87 at DXS1123, P=0.011), the relatively low significance achieved and the fact that the different peak regions for MF7 and MF14 were approximately 46 cM apart (Nyholt et al. 1998b) clearly indicated the need for finer mapping in these pedigrees.

Therefore to further localize and increase the inheritance (linkage) information content across the implicated regions on chromosome Xq the present study utilized six additional markers in MF7 and MF14, including one within the serotonin receptor candidate gene (*HTR2C*; Milatovich et al. 1992), and performed haplotype analysis in addition to a more powerful technique in the analysis of linkage data.

Materials and methods

All pedigree members were personally interviewed by a clinical neurologist to establish diagnosis following criteria specified by the International Headache Society (Headache Classification Committee 1988). Individuals in migraine pedigrees MF7 and MF14 were classified as affected, after being clinically diagnosed as either MA or MO, as described previously (Nyholt et al. 1998a, 1998b). Affected individuals within the two pedigrees showed a variation in migraine phenotype. This included differences in age at onset, frequency and severity of attacks, triggering factors and response to medication. However, all individuals classified as affected satisfied the criteria specified by the International Headache Society for diagnosis of MA (criterion 1.2.1) or MO (criterion 1.1; Headache Classification Committee 1988). In these two families a total of 16 microsatellite markers spanning approximately 105 cM on chromosome Xq21-qter, with an average separation of 6.6 cM, were tested for linkage to migraine.

To overcome problems arising from misspecification of transmission model parameters due to unknowns regarding the inheritance model (i.e. penetrance) of migraine, nonparametric multipoint linkage analyses were performed using the S_{all} scoring function in the GENEHUNTER-PLUS (X-linkage version 1.2) modification (Kong and Cox 1997) of the GENEHUNTER (X-linkage version 1.3) package (Kruglyak et al. 1996). The S_{all} statistic of GENEHUNTER measures identity-by-descent (IBD) allele sharing between *all* individuals within a pedigree simultaneously and assigns a higher score when more of them share the same allele by descent. Therefore the S_{all} statistic should provide a more accurate localization compared to the alternative S_{pairs} scoring function, which measures IBD allele sharing between *pairs* of affected relatives within a pedigree. GENEHUNTER-PLUS (LOD*) was used over GENEHUNTER (NPL) as it makes more efficient use of incomplete inheritance information and permits the calculation of a nonparametric LOD score (LOD*) based on allele sharing. The LOD* scores were calculated using the newly implemented 'exponential model' which provides a better fit to data consisting of a small number of pedigrees with extreme IBD sharing than the original 'linear model' (Kong and Cox 1997). Reconstruction of the most likely haplotypes and determination of crossover events were calculated using the exact-likelihood method implemented in GENEHUNTER. Marker information and genetic map order were obtained using Genethon (Gyapay et al. 1994; http://www.genethon.fr/genethon_en.html), Genome Database (http://gdbwww.gdb.org) and GeneMap'99 data (http://www.ncbi.nlm.nih.gov/genemap99/). The genetic map, with distances (recombination fraction, θ) given in parentheses was: DXS990 – (0.085) – DXS454 – (0.095) – DXS1120 – (0.040) – *HTR2C* – (0.049) – DXS8064 – (0.076) – DXS1001 – (0.084) – DXS1206 – (0.041) – *HPRT* – (0.076) – DXS984 – (0.141) – DXS8106 – (0.041) – DXS297 – (0.045) – DXS1123 – (0.041) – DXS8091 – (0.075) – DXS8061 – (0.049) – DXS15 – (0.028) – DXS1108.

Results

In complex diseases such as migraine, which are thought to result from a combination of different genes with varying penetrances interacting with environmental factors, many unaffected individuals may possess the disease-predisposing genotype at one loci but lack the required second disease locus genotype, or environmental influences, necessary for development of the disease. It is therefore advisable to consider all unaffected individuals to have 'unknown' phenotype when performing linkage analyses. In this way the linkage analysis is based solely on the marker status of the affected individuals in the pedigree (Terwilliger and Ott 1994). For these reasons we report nonparametric linkage results based on IBD sharing and

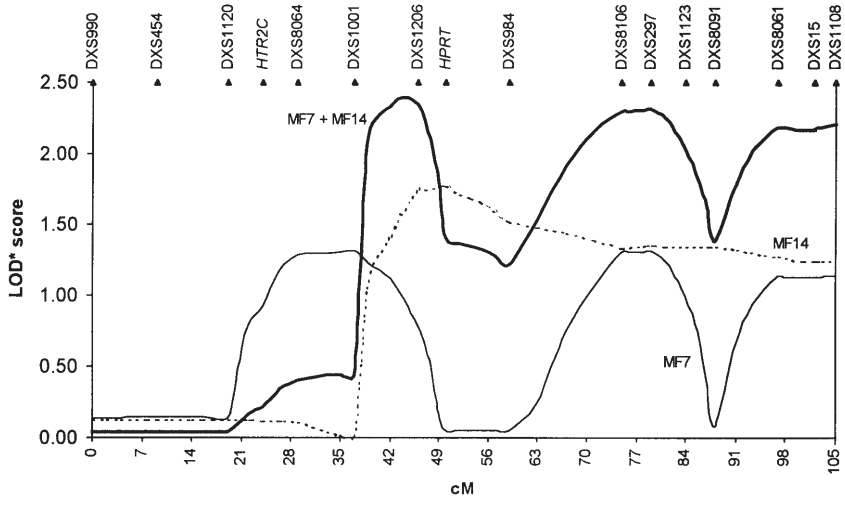


Fig. 1 Results of multipoint nonparametric linkage analysis using the S_{all} scoring function in the GENEHUNTER-PLUS modification (Kong and Cox 1997) of the GENEHUNTER package (Kruglyak et al. 1996), for migraine pedigrees MF7 and MF14, plotted as the GENEHUNTER-PLUS LOD* score (*Y*-axis) against the genetic distance (in Kosambi centimorgans) from the first microsatellite locus (*X*-axis)

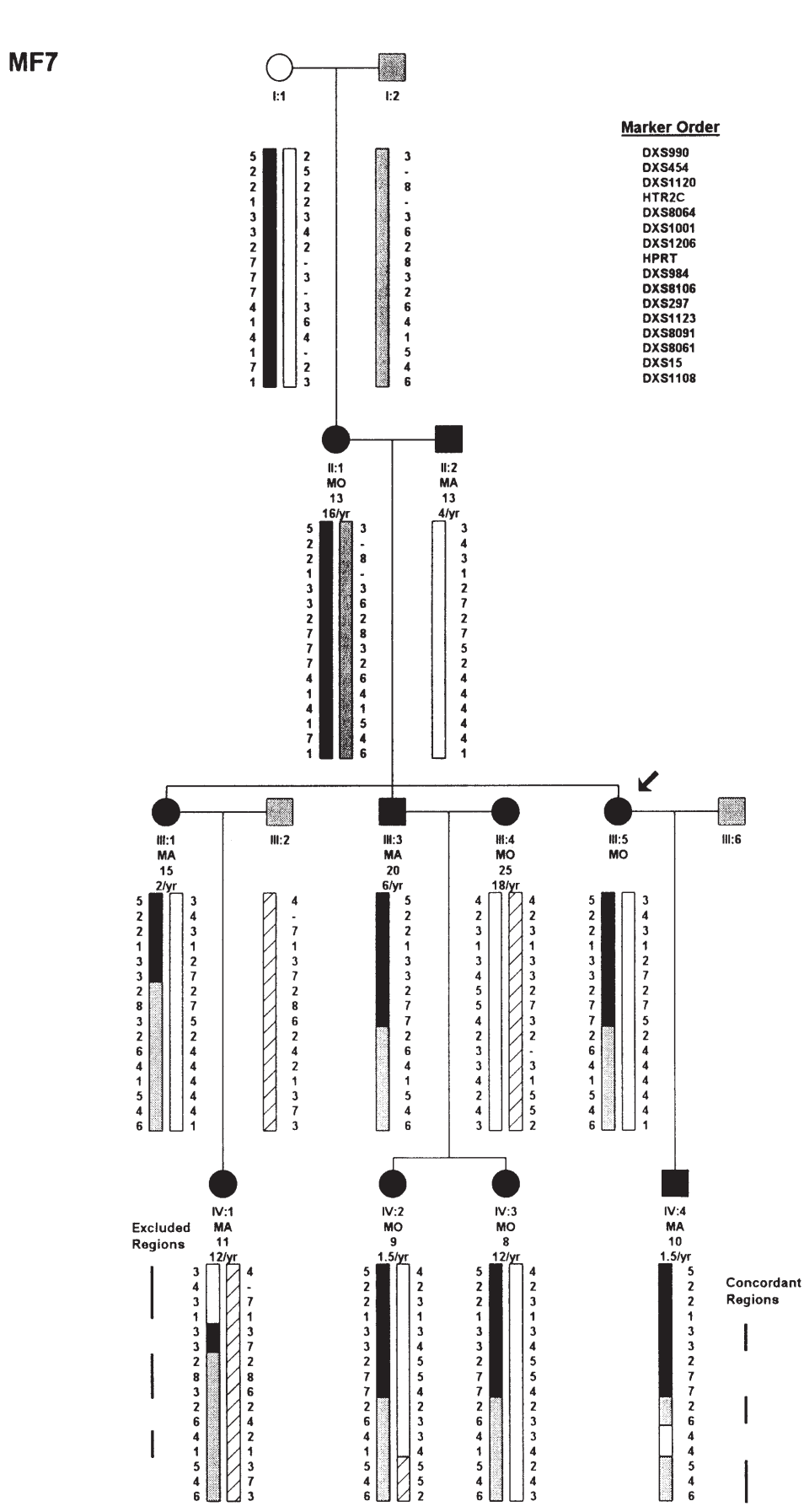
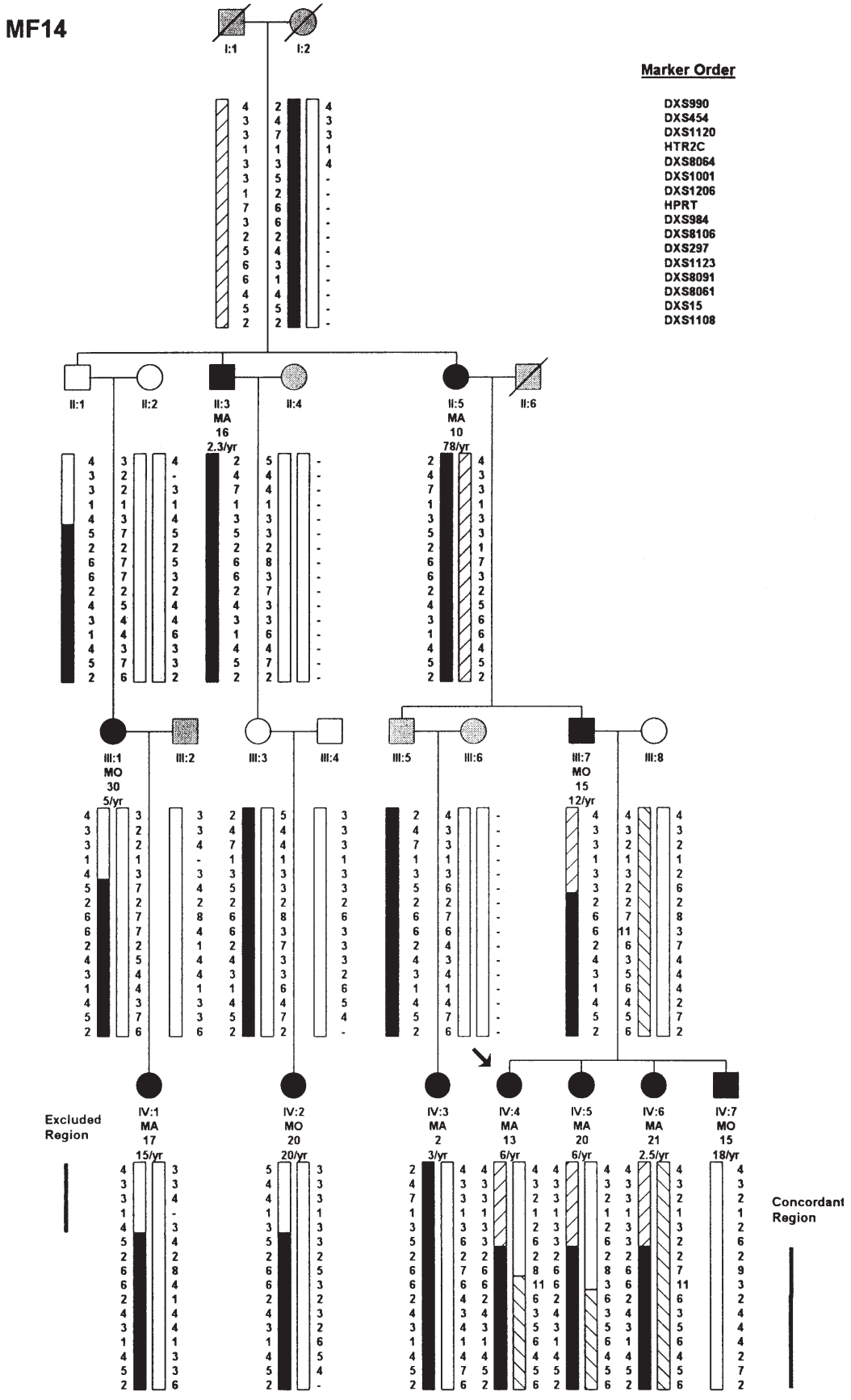


Fig. 2 Pedigrees of X-linked migraine families MF7 and MF14, showing disease-linked haplotypes. Haplotypes were constructed using the GENEHUNTER package (Kruglyak et al. 1996). *Blackened circles* Affected females; *blackened squares* affected males; *MA* migraine with aura; *MO* migraine without aura; *1st line under migraine type* age at migraine onset in years; *2nd line under migraine type* frequency of migraine attacks per year; *grey symbols* unknown diagnosis; *white symbols* unaffected diagnosis; *arrow* proband; *dash within a marker order* untyped marker allele (deemed not critical to the identification of recombination events), which could not be inferred by the haplotype analysis. Haplotypes cosegregating with the affected status are indicated by *black and grey bars* in MF7 and a *black bar* MF14. The critical crossovers defining the proximal and distal boundaries of the familial migraine candidate regions are shown in MF7 individuals III:1, III:5, IV:1 and IV:4, and MF14 individual III:7, placing the disease locus between the markers DXS1001-DXS1206, DXS984-DXS1123 or DXS8091-qter. *White and patterned bars* non-disease-associated haplotypes

b


MF14
I:1
I:2
Marker Order
DXS990
DXS454
DXS1120
HTR2C
DXS8064
DXS1001
DXS1206
HPRT
DXS984
DXS8106
DXS297
DXS1123
DXS8091
DXS8061
DXS15
DXS1108
II:1
II:2
II:3
MA
16
2.3/yr
II:4
II:5
MA
10
78/yr
II:6
III:1
MO
30
5/yr
III:2
III:3
III:4
III:5
III:6
III:7
MO
15
12/yr
III:8
Excluded
Region
IV:1
MA
17
15/yr
IV:2
MO
20
20/yr
IV:3
MA
2
3/yr
IV:4
MA
13
6/yr
IV:5
MA
20
6/yr
IV:6
MA
21
2.5/yr
IV:7
MO
15
18/yr
Concordant
Region

haplotype data from meioses involving affected individuals only. However, it should be noted that the construction of haplotypes and determination of IBD status for marker genotypes utilized information from all individuals.

The results of multipoint nonparametric linkage analyses using the GENEHUNTER-PLUS program (Kong and Cox 1997) are shown in Fig. 1. Peak LOD* scores were obtained for MF7 and MF14, at the DXS1001 (LOD*= 1.319, *P*=0.0069) and *HPRT* (LOD*=1.771, *P*=0.0021) loci, respectively. In addition to these two markers being only 13 cM apart, examination of the LOD* score curves in Fig. 1 shows good correlation of peak regions between these two families. Furthermore, combined analysis of MF7 and MF14 produced *significant* evidence for X-linkage, with a peak LOD* score of 2.388 (*P*=0.0005) equidistant between DXS1001 and *HPRT*.

Cosegregation of haplotype with migraine status in both MF7 and MF14 is shown in Fig. 2. Critical recombination events in MF7 affected individuals III:1 (DXS1001×DXS1206), III:5 (DXS984×DXS8106), IV:1 (*HTR2C*×DXS8064) and IV:4 (DXS297×DXS1123, DXS8091×DXS8061) localize the disease locus to between markers *HTR2C*-DXS1206, DXS984-DXS1123 or DXS8091-qter. Furthermore, a critical crossover in MF14 affected individual III:7 (DXS1001×DXS1206), made it possible to reduce the *HTR2C*-DXS1206 interval to DXS1001-DXS1206, which, assuming both pedigrees are segregating the same gene and the GeneMap'99 *HTR2C* mapping data to be accurate, apparently excludes involvement of the *HTR2C* candidate gene. These implicated regions, DXS1001-DXS1206, DXS984-DXS1123, and DXS8091-qter, translate to a physical distance of approximately 5 Mb (Nagaraja et al. 1998; Pilia et al. 1996), 11 Mb (Zucchi et al. 1996) and 6.5 Mb (Palmieri et al. 1993; Nagaraja et al. 1998) on chromosome Xq24, Xq27 and Xq28, respectively.

Discussion

Migraine is a complex neurological disorder that is particularly prevalent in women. We chose a linkage approach to investigate this disorder and utilized large multigenerational pedigrees to try to pinpoint the location of migraine genes. Using such an approach, we have been able to provide evidence for the heterogeneity of the disorder and also the location of two migraine susceptibility loci. Results using a large Australian pedigree, termed MF1, indicated significant excess allele sharing (maximum NPL score of 6.64, *P*=0.0026) across a 12.6-cM region on chromosome 19p13 containing the calcium channel gene, *CACNA1A*, implicated in the rare and severe familial hemiplegic migraine (Nyholt et al. 1998a). Mutations within this gene have been shown to be the cause of this severe migraine sub-type in some familial hemiplegic migraine pedigrees (Ophoff et al. 1996). The MF1 pedigree shows excess allele sharing for chromosome 19 markers located near this locus and gives a maximum parametric lod score for the $(CAG)_n$ trinucleotide repeat located within the 3′ end of this 47 exon gene. This triplet repeat, although not showing migraine associated expansion in MF1, does show expansion in spinocerebellar ataxia type 6 (Zhuchenko et al. 1997). In our study the MF1 pedigree gave evidence implicating chromosome 19; however, three other migraine pedigrees, termed MF7, MF14 and MF15, did not show linkage to the tested chromosome 19 markers, and provided significant evidence for the heterogeneous nature of the disorder (Nyholt et al. 1998a).

Considering the higher prevalence of migraine in women than in men and the increased risk of migraine in female relatives of male probands, we investigated the possibility of an X-linked dominant gene. Utilizing MF1, MF7 and MF14, we tested 28 markers spanning the entire X chromosome and showed independent significant excess allele sharing in MF7 (NPL_{max} of 2.57 at DXS1001, *P*=0.031) and MF14 (NPL_{max} of 2.74 at DXS1123, *P*=0.012; Nyholt et al. 1998b). Importantly, MF1 did not show linkage to any X chromosome marker. Furthermore, analysis of all three families using the HOMOG program (Ott 1986) indicated evidence in support of linkage to the X chromosome and genetic heterogeneity of the disorder in general (*P*=0.0065).

The present study builds upon these previous X-chromosomal results, utilizing the MF7 and MF14 Australian white pedigrees. In addition to using a higher resolution map of markers, the present study also tested a marker within the serotonin receptor candidate gene, *HTR2C*, for involvement in these migraine pedigrees. The *HTR2C* gene was of particular interest since it has been localized to Xq24 (Milatovich et al. 1992), within the X-chromosomal region implicated in our families, and because abnormal serotonin metabolism has been observed in migraine sufferers (Ferrari and Saxena 1993), and migraine medications are known to interact with serotonin receptors (Peroutka 1990; Silberstein 1994; Silberstein and Lipton 1994).

In addition to obtaining stronger and *significant* X-linkage and providing evidence which seemingly excludes involvement of the *HTR2C* candidate gene, the present study was also able to significantly reduce the potential location of a migraine susceptibility gene to three distinct regions towards the telomere of Xq. Furthermore, coupling the haplotype data with the peak LOD* score of 2.388 occurring within the DXS1001-DXS1206 region, approximately 2 cM from DXS1206, indicates that a disease locus most likely lies within the 5-Mb region between DXS1001 and DXS1206 on Xq24; however, regions on Xq27 and Xq28 cannot at present be ruled out. It should also be noted that there is potential for male-to-male transmission of the disorder from individual III:7 father to IV:7 son; however, apparent male-to-male transmissions may arise due to the genetic heterogeneity and high population prevalence of migraine (12%), resulting in matings between persons who are affected (i.e., admixture), further complicating the inheritance pattern of the disorder.

Apart from *HTR2C*, a scan of publicly available databases reveals no other strong candidate genes within these implicated regions and, although further fine mapping is

planned in these two families, it is pertinent to note that genetic mapping designed to narrow the disease-gene region from the three intervals implicated in this study may rely on the availability of further informative families. Therefore our laboratory is currently involved in studies aimed at the identification and subsequent molecular genetic investigation of candidate EST loci.

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