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A typical migraine susceptibility region localizes to chromosome 1q31

Abstract Migraine (with and without aura) is a prevalent neurovascular disease that shows strong familial aggregation, although the number of genes involved and the mode of inheritance is not clear. Some insight into the disease has been gained from genetic studies into a rare and very severe migraine subtype known as familial hemiplegic migraine (FHM). In this study, we took a family-based linkage and association approach to investigate the FHM susceptibility region on chromosome 1q31 for involvement in typical migraine susceptibility in affected Australian pedigrees. Initial multipoint ALLEGRO analysis provided strong evidence for linkage of Chr1q31 markers to typical migraine in a large multigenerational pedigree. The 1-LOD* unit support interval for suggestive linkage spanned approximately 18 cM with a maximum allele sharing LOD* score of 3.36 obtained for marker D1S2782 (P=0.00004). Subsequent analysis of an independent sample of 82 affected pedigrees added support to the initial findings with a maximum LOD* of 1.24 (P=0.008). Utilising the independent sample of 82 pedigrees, we also performed a family-based association test. Results of this analysis indicated distortion of allele transmission at marker D1S249 [global $\chi^2_{(5)}$ of 15.00, P=0.010] in these pedigrees. These positive linkage and association results will need further confirmation by independent researchers. However, overall they provide good evidence for the existence of a typical migraine locus near these markers on Chr1q31, and reinforce the idea that an FHM gene in this genomic region may also contribute to susceptibility to the more common forms of migraine.

Keywords Migraine · Gene · Linkage · Association

Introduction

Typical migraine, comprised of migraine with aura (MA) and migraine without aura (MO), is a chronic, painful and debilitating neurovascular disease that is generally characterised by recurrent attacks of severe headache usually accompanied by nausea, vomiting, photo- and phonophobia [1]. Migraine has been shown to affect a large proportion of Caucasian populations, with a recent comprehensive study indicating that around 25% of women and 8% of men suffer from the disease [2].

Strong familial aggregation of typical migraine and an increased concordance for the disease in monozygotic twins over dizygotic twins suggests that it has a significant genetic component. Heritability estimates are calculated to be between 40% and 60%, indicating that disease variation, in part, is explained by environmental determinants [3,4]. The mode of transmission of typical migraine is not clear, but is most likely multifactorial [5]. Although the MA and MO subtypes exhibit some clinical heterogeneity, segregation analysis by Mochi et al. [6] suggested that there may be a common genetic aetiology for MA and MO, and a major gene contributing to typical migraine pathogenesis. This idea is substantiated by the fact that both subtypes of migraine can occur within the same family and even within the same individual, with up to 33% of sufferers experiencing both types of the disease [7,8]. In addition, migraine pro-

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phylactics have been shown to result in similar effects in patients treated for both types of migraine [9].

At present, the type and number of genes involved in typical migraine is not known. Despite this, several studies into familial hemiplegic migraine (FHM), a very severe subtype of MA, have led to the discovery that mutations in a brain-specific calcium channel subunit gene (*CACNA1A*), located on chromosome 19, cause FHM in about 50% of affected families [10]. FHM is a rare disease and is distinguished from typical migraine by its association with hemiparesis and a clear autosomal dominant mode of inheritance. However, certain clinical features are common to both FHM and typical migraine, including similarities in headache characteristics and triggers [11]. Hence, FHM genetic studies provide a valuable model for investigating the genes involved in the more prevalent types of migraine with and without aura. For this reason we have been conducting linkage studies utilising large Australian migraine pedigrees with a focus on the known FHM (*CACNA1A*) gene region on chromosome 19p13. Our results to date show suggestive linkage to the FHM region on 19p13 in a large multigenerational pedigree affected with typical migraine with a maximum parametric LOD score of 1.92 (*P*=0.001) obtained for a triplet repeat polymorphism situated in exon 47 of the *CACNA1A* gene [12]. Expansion of this repeat was not observed, but it is possible that mutations elsewhere in the *CACNA1A* gene may be responsible for migraine in this pedigree. Other family studies performed in our laboratory have also indicated that typical migraine is genetically heterogeneous, with a recent study showing significant linkage of a second gene on chromosome Xq24–28 to the disease [13,14].

Family linkage studies conducted by Gardner et al. [11] have implicated an additional FHM susceptibility locus within a broad region (44 cM) on chromosome 1q31. Furthermore, independent research carried out by Ducros et al. [15] has indicated a second FHM locus at 1q21–23, which is approximately 30 cM centromeric to the region reported by Gardner et al. [11]. At this stage it is not clear whether there is a single locus, or two distinct loci, on the chromosome 1q region. Of particular interest, however, is the report of another neuronal calcium channel α-1 subunit gene, *CACNA1E*, in the region of 1q25–31 [16]. This gene, thought to be associated with R (resistant) or T (transient) type calcium channels has high sequence identity to *CACNA1A* (approximately 85%), and is therefore an excellent candidate for FHM, and possibly also for typical migraine involvement. Considering the hypothesis that FHM and typical migraine may be caused by a common defective gene(s), we decided to test the FHM susceptibility region on chromosome 1q31 for involvement in the more prevalent and genetically complex typical migraine disorder. Hence, this study employed a family-based linkage and association approach to test pedigrees affected with typical migraine (MO and MA), specifically utilising markers located within the FHM genomic region on chromosome 1q31.

Table 1. Affected relative pairs of a sample of 82 typical migraine pedigrees[a]

Category	No. of individuals with typical migraine
Total families	82
Affected relative pairs	
Sibling pairs	97
Half-sibling pairs	4
Uncle (aunt)-nephew (niece) pairs	90
Grandparent-grandchild pairs	24
Great grandparents-grandchild pairs	3
Cousin pairs (first+second)	19
Great uncle (great aunt)-nephew (niece) pairs	15
Subtotal	252

[a]All possible combinations of pairs formed by all affecteds within each family (excluding parent-child pairs)

Materials and methods

Patients and families

The study protocol was approved by the Griffith University Ethics Committee for experimentation on humans. All individuals were of Caucasian origin and gave informed consent before participating in the research. Sufferers of typical migraine were shown to exhibit phenotypic variation, which included differences in age of onset, frequency and severity of attacks, environmental triggers and medication response. However, all affected individuals were diagnosed as having either MA or MO, based on criteria specified by the International Headache Society (IHS) (MA=criteria 1.2.1 and MO=criteria 1.1) and through interview by a clinical neurologist (Dr. Peter Brimage)(Headache Classification Committee of the International Headache Society 1988). Under the hypothesis of a common genetic aetiology, all individuals with MA and MO were grouped together and phenotyped as being affected with typical migraine, as well as being analysed separately as affected with MA only.

Our initial investigations focused on three previously published typical migraine pedigrees (MF1, MF7, MF14) [12,13]. These large multigenerational families consisted of 123 members in total (105 DNA available), 60 of which were affected with either MA, MO or both. In addition to the three large families, DNA from 296 subjects (263 migraineurs) from an independent sample of 82 additional families affected with typical migraine was ascertained for genotyping. These pedigrees were comprised of 252 affected relative pairs, excluding parent-child pairs. All families had at least two affected individuals and at least one parent was available for genotyping in all but 10 pedigrees (Table 1).

Markers and genotyping

Genomic DNA was extracted from blood samples using a standard SDS-proteinase K method [17], incorporating a salting out procedure [18]. In total, eight dinucleotide repeat markers were selected for testing in this study. Microsatellite information including primer sequences, marker spacing (cM) and order was obtained from Genethon, Genome Database and NCBI. The entire map had an average marker spacing of 4 cM and including recombination fraction distances (in parentheses) was; D1S2757-(0.06)-D1S306-(0.059)-D1S249-(0.022)-D1S2782-(0.06)-D1S205-(0.039)-D1S419-(0.044)-D1S229-(0.046)-D1S213. The forward primer for each marker was labelled with FAM, TET or HEX fluorescent dyes. Amplification of all markers was performed using standard PCR conditions as follows; a total volume of 15 µl was prepared con-

taining 30 ng of DNA, 1 unit of *Taq* polymerase, 1.75 mM MgCl$_2$, 5 mM dNTPs and buffer. Samples were then subjected to thermal cycling conditions of 1 cycle at 94°C for 4 min, 35 cycles of 94°C for 1 min, 60°C for 1 min, and 1 cycle of 72°C for 2 min. All PCR products were pooled, where possible, then fractionated by capillary electrophoresis and genotyped using an ABI 310 GENETIC ANALYSER and GENOTYPER software, respectively (Perkin Elmer).

Data analysis

This research employed the complementary strategies of family-based linkage and association analyses using Australian pedigrees affected with typical migraine. Non-mendelian inheritance errors in pedigrees were checked and allele frequencies for all microsatellite markers calculated using the PEDMANAGER program. Individuals that suffered from either MA or MO were analysed collectively as being affected with typical migraine. Given the high prevalence of MA in our affected pedigrees (approximately 70%), we also chose to consider individuals who suffered from MA as a separate phenotype, treating individuals with MO as unaffected for this analysis. For all multipoint linkage analysis the ALLEGRO computer program was used [19], which is an extension of the well-known GENEHUNTER programs [20,21]. In addition to providing improvements in computational algorithms, ALLEGRO also allows the user to test multiple hypotheses, incorporating various allele sharing and disease models, simultaneously [19]. The model-free analyses performed by ALLEGRO incorporated the S$_{pairs}$ scoring function. The S$_{pairs}$ statistic measures identity-by-descent (IBD) allele sharing between all pairs of affected relatives and reportedly performs well over all disease models [22]. The exponential allele sharing model was also used. This model is suited to datasets with small numbers of pedigrees, or where pedigrees are very different in size [21]. ALLEGRO reports an allele sharing LOD score (LOD*), which is interpreted in the same way as the LOD scores obtained in a traditional parametric analysis [21]. Significance (α) levels for the linkage analysis LOD*scores were specified a priori according to the genome-wide guidelines suggested by Lander and Kruglyak [23]. Like GENEHUNTER, ALLEGRO is restricted by the size of the pedigree to be analysed and therefore our large pedigrees required some trimming, starting with unaffected members at the base. Reconstruction of the most-likely haplotypes and recombination points was calculated approximately by the method applied in ALLEGRO.

The family-based association test (FBAT) was performed for the independent sample of 82 migraine-affected pedigrees. The FBAT is a unified approach for assessing association between marker and disease alleles. Unlike the classic transmission disequilibrium test (TDT), which was designed for specific pedigree structures (triads), the FBAT utilises data from nuclear families, sibships or a combination of the two to test for linkage and linkage disequilibrium. The test for linkage is valid when multiple affected members in each pedigree are used, and the power to detect linkage in this situation is increased when there is association. The test for association is valid if at least one affected member from each pedigree is used, or if the empirical variance is used to account for any correlation between transmissions in families when linkage is present [24]. Since four markers were tested for association under two different phenotypes (MA/MO and MA), the significance level for the FBAT analysis was set at 0.05/8 ≈ 0.005, and 0.05 set as nominal evidence for association.

Results

Three multigenerational pedigrees affected with typical migraine were initially utilised for investigating the FHM susceptibility region on chromosome 1q31. Multipoint analysis, incorporating all eight markers spanning 33 cM
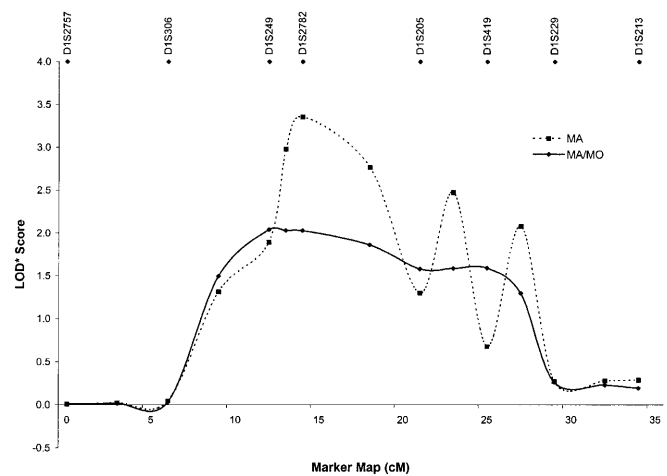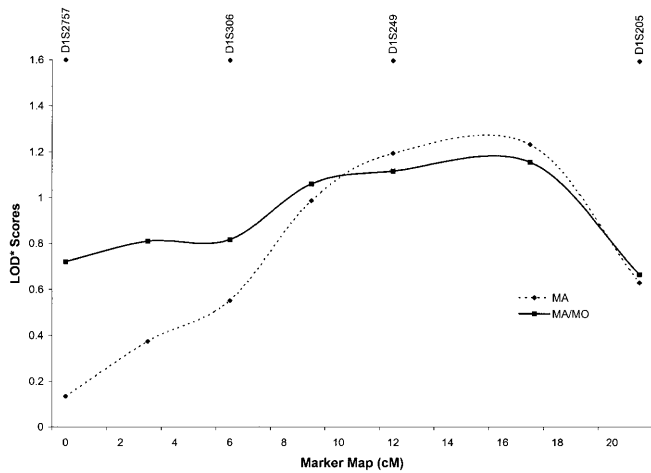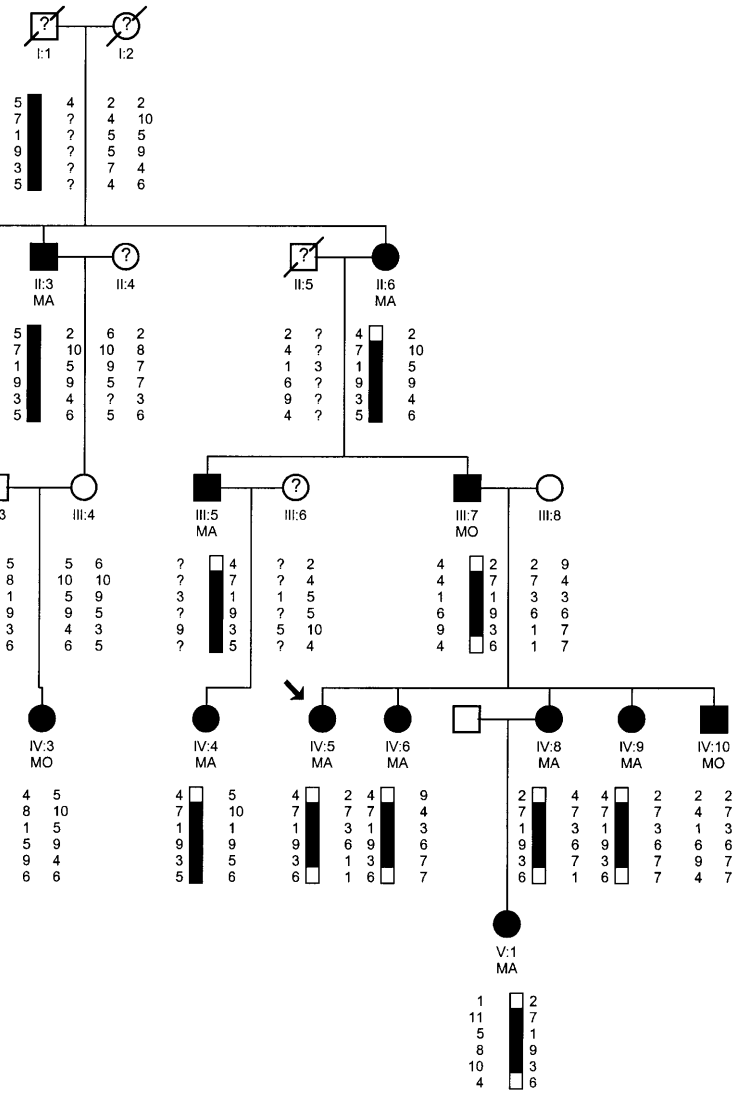

Fig. 1 Results of multipoint model-free ALLEGRO allele-sharing analysis for eight markers on chromosome 1q31, for MF14 only. *MA* is migraine with aura and *MO* is migraine without aura. The *y*-axis shows the allele-sharing LOD* scores resulting from analyses of the typical migraine (MA/MO, *unbroken line*) and MA (*dotted line*) phenotypes. The *x*-axis shows the genetic distance in Kosambi cM across these markers

on chromosome 1q31, was performed on affected individuals from these pedigrees using the ALLEGRO program. The results of the model-free analysis showed significant excess allele sharing in one of the large pedigrees (MF14), with a maximum allele-sharing LOD* score of 3.36 (P=0.00004) for the MA phenotype, and 2.04 (P=0.001) for MA/MO at marker D1S2782 (Fig. 1). The other two pedigrees (MF1 and MF7) produced non-significant LOD scores (P>0.05) for all markers tested. Given the evidence for linkage of these markers to the disease in MF14, haplotypes were then reconstructed. Cosegregation of marker haplotypes with the disease in this pedigree are displayed in Fig. 2. The complex nature of typical migraine is illustrated in MF14 with cases of non (or incomplete) penetrance and phenocopy evident in individuals II:1 and IV:3, IV:10, respectively. Importantly, haplotyping shows key recombination events in affected individuals II:6 (D1S306×D1S249) and IV:1 (D1S2782×D1S205) that narrow a critical genomic region to a 14.1 cM distance between markers D1S306 and D1S205 in this pedigree.

In an attempt to substantiate the involvement of this region on Chr1q31 in typical migraine, we also tested four highly polymorphic and potentially implicated microsatellite markers in a large independent sample of 82 additional families affected with the disease. These markers (D1S2757, D1S306, D1S249, D1S205) were from our initial map set. They were evenly spaced, and separated by an average genetic distance of approximately 7 cM. Multipoint ALLEGRO results produced nominal allele-sharing LOD* scores of 1.24 (P=0.008) for MA and 1.16 (P=0.01) for MA/MO, peaking between markers D1S249 and D1S205 (Fig. 3).

The complementary mapping strategy of allelic association testing using the family-based approach was also carried out for the 82 additional families using the FBAT

Fig. 2 MF14 showing segregation of typical migraine susceptibility haplotypes estimated by ALLEGRO for chromosome 1q31 microsatellite markers (D1S306, D1S249, D1S2782, D1S205, D1S419, and D1S229 from top to bottom). *Blackened symbols* indicate affected individuals, with migraine diagnosis determined as MA or MO. Key recombination events occur at individuals II:6 and IV:1 between markers (D1S306×D1S249) and (D1S2782×D1S205). This limits the disease locus to the 14.1-cM region between D1S306 and D1S205

Fig. 3 Results of multipoint model-free ALLEGRO allele-sharing analysis for four markers on chromosome 1q31, for 82 typical migraine families. The *y*-axis shows the allele-sharing LOD* scores resulting from analyses of the typical migraine (MA/MO, *unbroken line*) and MA (*dotted line*) phenotypes. The *x*-axis shows the genetic distance in Kosambi cM across these markers

program. The test statistic for the FBAT analysis was marginally positive at marker D1S249 for this large sample of typical migraine families under both phenotypes. A global χ^2 of 15.00 for 5*df* (*P*=0.010) for MA and χ^2 of 17.98 for 7 *df* (*P*=0.012) was obtained for MA/MO. As either linkage or association in the data may explain these small *P* values, we also tested for association using the empirical variance to account for correlation between transmissions in families when linkage is present. In this analysis, the evidence for association of alleles at the D1S249 locus decreased slightly for both phenotypes to *P*=0.048 and *P*=0.033 for MA and MA/MO, respectively. Specifically, alleles 9–12 contributed largely to the global allele transmission distortion by showing slight over-transmission compared with that expected (*P*<0.05).

Discussion

Migraine is a multifactorial condition influenced by genetic and lifestyle characteristics. At present the mode(s)

of inheritance is unclear and the type and number of genes involved in the disease is not known. The *CACNA1A* gene, which causes some cases of the rare migraine subtype FHM, encodes the α1A subunit of the P/Q-type voltage-gated calcium channel that is predominantly expressed in the brain [10]. These calcium channels control a number of fundamental neuronal processes, including the mediation and release of neurotransmitters such as serotonin [25]. Since both FHM and typical migraine display some clinical overlap, it has been postulated that the more-prevalent typical migraine (with and without aura) may also be a channelopathy. We have recently reported evidence for linkage to this same *CACNA1A* region on chromosome 19 in one of our large Australian typical migraine pedigrees, and are now screening this and other adjacent genes for mutations in affected family members from this pedigree [12].

Linkage to *CACNA1A* occurs in approximately 50% of families affected with FHM, indicating at least one other gene is involved in this disease [10]. In 1997 Gardner et al. [11] reported results of a linkage study investigating a large pedigree clearly affected with autosomal dominant FHM. These researchers excluded the 19p13.1 *CACNA1A* gene region for involvement in this pedigree and therefore tested 12 microsatellite markers spanning 44 cM on chromosome 1q31 [11]. This region (1q25–31) reportedly contains another voltage-gated neuronal calcium channel subunit gene, *CACNA1E* [16]. Model-based analyses of the markers utilised by Gardner et al. [11] indicated significant linkage to FHM to a 44-cM region, with a maximum LOD score peaking slightly between markers D1S249 and D1S2782 (Z_{max}=3.328) [11]. Therefore, *CACNA1E* is an excellent candidate gene for FHM in this linked pedigree. To investigate the 1q31 region for involvement in the more prevalent forms of typical migraine (with and without aura), we conducted both linkage and association studies in Australian Caucasian families affected with this disease.

Utilising three large multigenerational pedigrees, we initially tested eight chromosome 1q31 markers for linkage to typical migraine. The results of the model-free analysis showed good evidence for linkage in one of the pedigrees tested (MF14). Haplotype analysis of this pedigree showed key recombination events limiting this region to 14.1 cM. Interpretation of this haplotype information, combined with the peak linkage results, suggests that the most-likely location of a disease susceptibility gene is within an 8.2-cM region between markers D1S205 and D1S249. Follow-up linkage analysis of an independent population of 82 typical migraine pedigrees was then performed. Four markers spanning this region were than tested and multipoint analysis also provided nominal evidence for linkage across these loci. These additional results offer good support to our initial findings in MF14.

The maximum allele-sharing LOD* score of 3.36 (*P*=0.00004) obtained for the MA phenotype in pedigree MF14 is strongly suggestive of linkage in this pedigree and thus warrants further investigation. It is also interesting to note that all our linkage peaks were maximised near the very same loci (D1S249 and D1S2782) as the FHM results reported by Gardner et al. [11]. The results of the present study interpreted together with those reported by Gardner et al. [11] strongly support the idea that a common defective gene may be influencing both FHM and typical migraine, specifically MA.

Linkage analysis employing allele-sharing methods, whilst being a useful first-step strategy for implicating regions of interest in multifactorial traits, may not be useful for pinpointing disease genes, since true peaks resulting from these tests often span genetically broad regions [26]. Fortunately, by measuring allelic association between marker and disease alleles, there is the potential to localise a susceptibility gene to a more narrow region [27]. Using the FBAT program, we incorporated allelic association into our analysis and found distortion of allele transmission at marker D1S249, thus providing evidence for linkage disequilibrium at this marker locus. The positive association results reported here for D1S249 will also require further independent study for confirmation, given the nominal *P* value for this marker. If corroboration is established then a gene affecting typical migraine at the population level may localise to the immediate vicinity of the D1S249 marker.

This is the first study to provide evidence for the localization of a typical migraine susceptibility region on chromosome 1. Overall, our linkage results indicate that the most plausible location of a disease gene is within the 8.2-cM region between markers D1S249 and D1S205. Also, the positive allelic association results obtained for the 82 independent migraine pedigrees support the location of a susceptibility gene residing near the D1S249 locus. Consequently, we are now searching for candidate genes, located within this vicinity with the intention of testing single nucleotide polymorphisms for association to the disease. We are also testing marker loci around chromosome 1q21 specifically to determine whether the FHM susceptibility region implicated by Ducros et al. [15] is also involved in typical migraine in our populations.

In conclusion, it is interesting and important to note that we have previously reported linkage of chromosome Xq markers to migraine in pedigree MF14, and indicated a concordant haplotype shared among affected individuals in this migraine family [13,14]. The significant excess allele sharing demonstrated in this pedigree with both Xq and 1q31 markers raises the possibility that "within-family" locus heterogeneity exists, with genes on both chromosome Xq24–28 and 1q31 potentially contributing to the disease either independently or interacting epistatically. The identification of the specific genes involved in this pedigree could greatly aid in the understanding of this polygenic neurovascular disorder.

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