Migraine association and linkage analyses of the human 5-hydroxytryptamine (5HT$_{2A}$) receptor gene

DR Nyholt$^1$, RP Curtain$^1$, PT Gaffney$^2$, P Brimage$^2$, PJ Goadsby$^3$, LR Griffiths$^1$

Molecular Genetics, School of Health Sciences$^1$, NHS, Griffith University, Gold Coast, Queensland, Australia; Institute of Neurological Sciences$^2$, Prince of Wales Hospital, Sydney, Australia; Institute of Neurology$^3$, The National Hospital for Neurology and Neurosurgery, London, UK


Cephalalgia

5-Hydroxytryptamine (5HT), commonly known as serotonin, which predominantly serves as an inhibitory neurotransmitter in the brain, has long been implicated in migraine pathophysiology. A hypothesized role of 5HT in headpain involves 5HT in the available pool, exerting an inhibitory effect on the dorsal raphe nucleus, preventing the dorsal raphe from releasing its own 5HT, which would initiate the cervico-trigemino-vascular pathway, causing headpain (6). Abnormalities in the serotonergic system have been observed before and during migraine attacks and have been the target of therapeutic developments (7). The major medication groups used currently for prophylactic migraine treatment include beta blockers, antidepressants, calcium channel blockers, 5HT antagonists, and anticonvulsants. Many of these agents interact with serotonergic neural systems, by binding to 5HT$_{2A}$ or 5HT$_{XC}$ receptor sites, by “down-regulating” the 5HT$_{2A}$ receptor, or by modulating the discharge of the serotonergic neurons (8-10). Since 5HT metabolism is abnormal in migraine sufferers, and migraine medications are known to interact with 5HT receptors, 5HT receptor genes may be considered candidates in migraine aetiology.

This is the first report of a genetic study which tests a 5HT receptor gene for involvement in migraine. In this study, anMspI polymorphism in the human 5HT receptor gene (5HT$_{2A}$), which has been localized to chromosome 13q14-q21, and a closely linked microsatellite marker (D13S126) were tested for linkage and association with migraine (11).
Materials and methods

Blood samples collected from 96 affected and 91 unaffected individuals were used for the association studies. 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. For the linkage analyses, blood samples were collected from members of 3 large multi-generational families containing 36 affected members. Diagnosis of migraine was performed by a clinical neurologist following the criteria specified by the International Headache Society (IHS). Affected individuals were classified as migraine with aura (MA) or migraine without aura (MO) (12). DNA was extracted from blood samples by a standard SDS-Proteinase K method (13) incorporating a salting out procedure (14).

PCR reactions for the MspI polymorphism were performed using 200 ng of genomic DNA, 150 nM of each primer and 1.75 mM MgCl₂ in a final volume of 100 µl. Samples were subjected to 3 cycles of 3 min at 94°C, 45 sec at 60°C, 1.5 min at 72°C; then 35 cycles of 1 min at 94°C, 45 sec at 60°C, and 1.5 min at 72°C (11); 30 µl of PCR products were then digested with 40 U of MspI at 37°C for 5 h and fragment sizes determined after electrophoresis in 2% agarose gels.

PCR reactions for the D13S126 microsatellite marker were performed using 25 ng of genomic DNA, 200 nM of each primer and 1.75 mM MgCl₂ in a final volume of 20 µl. Samples were subjected to an initial denature of 5 min at 94°C, then 35 cycles of 1 min at 94°C and 1 min at 60°C, with a final extension period of 5 min at 72°C. PCR products were fractionated by capillary electrophoresis through a 2.8% denaturing polymer using an ABI Prism 310 Genetic Analyser with alleles determined using GENESCAN software.

For the association studies, genotypes for the MspI polymorphism and the D13S126 microsatellite marker were determined in migraine sufferers and allele frequencies compared with those found in non-sufferers. Results were tested for significant difference by chi-square (χ²) analysis. For the linkage studies, genotypes for the pedigree members were assessed and analysed for linkage using the FASTLINK (15–17), Model-Free Linkage (MFLINK) (18) and the Affected Pedigree Member (APM) (19) linkage analysis programs. For (FASTLINK) parametric lod score calculations, the exact mode of transmission of the disease needs to be specified. For the FASTLINK analysis, a conservative model, assuming an autosomal dominant mode of inheritance with 70% penetrance with a phenocopy rate of 0.7% was used (4, 20). The frequency of the disease was set at 12% (21). To overcome problems arising from misspecification of transmission model parameters, two non-parametric linkage analyses, MFLINK and APM, were carried out. MFLINK analysis, which requires no specification of transmission model parameters, apart from an approximate estimate of the population prevalence, detects a genetic effect at a particular locus, rather than attempting to estimate the map position of a locus specified effect (18). The APM method evaluates the sharing of alleles by state among the migraine cases in each family but is sensitive to misspecification of allele frequencies (19). This problem was overcome by using the allele frequencies from the tested control population in our association studies.

Results

For the HTR2A MspI polymorphic marker, genotypes were determined for 96 sufferers and 91 non-sufferers of migraine. Digestion of the 372 bp PCR product with MspI yielded a 372 bp product for allele a and 156 bp and 216 bp products for allele A. For the D13S126 microsatellite marker, genotypes were determined for 82 sufferers and 85 non-sufferers of migraine with amplification products ranging from 96 to 110 bp. Allele frequencies were determined and the results analysed by χ² analysis, as shown in Tables 1 and 2. Statistical analysis of the total allele counts for both the MspI and D13S126 markers indicated that there was no significant difference between the migraine and non-migraine populations, χ²=0.34, p=0.560 and χ²=6.71, p=0.460, respectively.

Genotypes for the MspI and D13S126 markers were also determined for affected and unaffected members of three migraine families as shown in Fig. 1. Linkage results were analysed by FASTLINK (Table 3), MFLINK and APM. All three families were relatively uninformative for the MspI marker. All three families were, however, informative for the D13S126 marker. No family showed significant linkage (lod score≥3) of migraine to HTR2A for either marker. However, significant linkage was found between the MspI polymorphism and the D13S126 microsatellite marker with a combined maximum lod score over the three families of 7.15 at a recombination fraction (θ) of zero. Also, combined lod scores from the linkage analysis between the D13S126 marker and migraine found significant non-linkage (lod<-2) up to a recombination fraction (θ) of 0.028. MFLINK analysis for the three families produced combined maximum “model-free” lod scores of 0.434 and 0.077 for the MspI and D13S126 markers respectively, correlating well with the FASTLINK results. APM analysis produced negative statistics for both the MspI and D13S126 markers, with combined p-values over the three families of 0.825 and 0.696, respectively, also indicating absence of involvement with migraine.
Analysing the contribution of genetic factors to complex diseases such as migraine by conventional parametric lod score analysis undoubtedly results in misspecification of the transmission model. Further complication of linkage analysis arises from conflicting data regarding differences in the genetic aetiology between migraine with and without aura. Migraine in itself is a dynamic disease with sufferers known to show symptoms correlating to differing clinical criteria throughout their life. Coupled with differing clinical criteria occurring in the same family, a common underlying genetic effect may contribute to all forms of migraine. This study therefore analysed sufferers of both migraine with aura and migraine without aura under the same parameters. To overcome problems arising from misspecification of transmission model parameters, two non-parametric linkage analyses which require no assumptions regarding mode of inheritance, MFLINK and APM, were also performed.

The FASTLINK linkage results for the three migraine pedigrees exclude close linkage of HTR2A to migraine. The total lod score of -2.00 at \( \theta = 0.028 \) obtained from all three tested migraine families excludes involvement up to 2.8 cm either side of the D13S126 locus. Non-involvement of this locus was also correlated by the MFLINK and APM results.

During the early preparation of this article, Pardo et al. (1995) reported association of an Esterase D (ESD) red cell enzyme marker with migraine (22). The ESD gene has been localized to 13q14.11, which is close to the 5HT2A receptor gene (13q14-q21). Pardo suggested that the two genes may be in linkage disequilibrium and suggested a causal role of 5HT2A in migraine. This study, similar to the study of Buchwalder et al. (23), found no evidence of involvement of the 5HT2A receptor gene in migraine.

**Discussion**

Chi-square analysis indicated that there were no significant differences between the migraine and non-migraine populations for the MspI and D13S126 markers. Hence, both the HTR2A MspI polymorphic marker and the D13S126 microsatellite marker did not show a significant association with migraine occurrence. Association studies only test point markers, and those regions of the genome in linkage disequilibrium with the tested marker; linkage studies test the actual marker plus areas linked to the marker. In order to confirm the negative association results, linkage studies were also performed using the MspI and D13S126 markers.
Fig. 1. Segregation of the MspI and D13S126 marker alleles in migraine families 1, 7 and 14. For neurological diagnosis: MA=migraine with aura and MO=migraine without aura.
excluded a region of 2.8 cM either side of its locus. Although no involvement of the 5HT\textsubscript{2A} receptor gene in migraine was indicated, it is possible that other 5HT receptor and related genes such as 5HT transporters and metabolizers may be involved in migraine aetiology.

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References

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