Neurogenetics
DOI 10.1007/s10048-011-0312-7

SHORT COMMUNICATION

Confirmation that Xq27 and Xq28 are susceptibility loci for migraine in independent pedigrees and a case-control cohort

B. H. Maher · M. Kerr · H. C. Cox · J. C. MacMillan · P. J. Brimage · T. Esposito · F. Gianfrancesco · L. M. Haupt · D. R. Nyholt · R. A. Lea · L. R. Griffiths

Received: 24 August 2011 / Accepted: 19 December 2011

Abstract Investigations into migraine genetics have suggested that susceptibility loci exist on the X chromosome. These reports are supported by evidence that demonstrates male probands as having a higher proportion of affected first-degree relatives as well as the female preponderance of 3:1 that the disorder displays. We have previously implicated the Xq24-28 locus in migraine using two independent multigenerational Australian pedigrees that demonstrated excess allele sharing at the Xq24, Xq27 and Xq28 loci. Here, we expand this work to investigate a further six independent migraine pedigrees using 11 microsatellite markers spanning the Xq27–28 region. Furthermore, 11 candidate genes are investigated in an Australian case-control cohort consisting of 500 cases and 500 controls. Microsatellite analysis showed evidence of excess allele sharing to the Xq27 marker DXS8043 (LOD* 1.38 P=0.005) in MF879 whilst a second independent pedigree showed excess allele sharing to DXS8061 at Xq28 (LOD* 1.5 P=0.004). Furthermore, analysis of these key markers in a case control cohort showed significant association to migraine in females at the DXS8043 marker (T1 P=0.009) and association with MO at DXS8061 (T1 P=0.05). Further analysis of 11 key genes across these regions showed significant association of a three-marker risk haplotype in the *NSDHL* gene at Xq28 (P=0.0082). The results of this study add further support to the presence of migraine susceptibility loci on chromosome Xq27 and Xq28 as well as point to potential candidate genes in the regions that warrant further investigation.

Keywords Migraine · MA · MO · X-linked

Electronic supplementary material The online version of this article (doi:10.1007/s10048-011-0312-7) contains supplementary material, which is available to authorized users.

B. H. Maher · M. Kerr · H. C. Cox · L. M. Haupt · R. A. Lea · L. R. Griffiths (✉)
Genomics Research Centre, School of Medical Science, Griffith Health Institute, Griffith University, Gold Coast, Queensland 4222, Australia
e-mail: l.griffiths@griffith.edu.au

J. C. MacMillan
Queensland Clinical Genetics Service, Royal Children's Hospital, Herston, Brisbane, Australia

P. J. Brimage
Institute of Neurological Sciences, Prince of Wales Hospital, Randwick, New South Wales, Australia

T. Esposito · F. Gianfrancesco
Institute of Genetics and Biophysics, Italian National Research Council, Naples, Italy

D. R. Nyholt
Neurogenetics Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia

Introduction

Migraine is a common episodic disorder displaying a complex aetiology which is often inherited in families. Numerous studies have demonstrated the genetic contribution to migraine through pedigree and twin studies with heritability estimates ranging between 34% and 57% [1]. Molecular studies have been used to identify a number of migraine susceptibility loci on various chromosomes. However, due to the genetic heterogeneity, the mode of transmission of migraine remains largely unclear. The biased preponderance of migraine in women [2, 3], as well as studies that demonstrate that a high proportion of

Published online: 01 February 2012

affected males have a greater number of affected first-degree relatives [4] suggests an X-linked dominant form of migraine inheritance. Families transmitting migraine through this mode of inheritance would show father-to-daughter transmission and an absence of father-to-son transmission as well as a possible excess of affected females.

The X chromosome has been previously identified by our laboratory as harbouring a susceptibility locus at Xq24–28 [5, 6]. In particular, studies of two Australian pedigrees identified significant excess allele sharing indicated by non-parametric linkage analysis producing maximum global LOD* scores of 2.31 between DXS8106 and DXS091 (Xq27), and LOD* 2.18 at DXS8061 (Xq28). In this study, we sought to further investigate the Xq27 and Xq28 locus through studies of six new independent Australian migraine pedigrees as well as candidate gene studies in an Australian case-control cohort.

Materials and methods

Sample Collection and Phenotyping

This study examined eight Australian Caucasian pedigrees, six new pedigrees designated MF6, MF47, MF55, MF541, MF878 and MF879, as well as the previously reported pedigrees MF7 and MF14 [5, 6]. These pedigrees include 161 individuals for whom DNA was available. This study also examined a case-control study cohort comprised of 500 migraineurs (360 MA/140 MO; 393:107 female/male) and 500 unrelated control individuals. To minimise potential bias from population stratification, the control group was matched for sex, age (±5 years) and ethnicity to the migraineurs.

Ethical clearance was granted prior to the commencement of the study by the Griffith University Human Research Ethics Committee. All participants signed informed consent statements prior to inclusion in the study and were interviewed by a clinical neurologist. Individuals were diagnosed for migraine (MA or MO) according to International Headache Society (IHS) criteria [7]. Venous blood was collected from participants, and DNA was isolated from lymphocytes using a standard salting out procedure [8].

Genotyping of microsatellites

Eleven dinucleotide microsatellite markers were examined in the pedigree analysis of which two were selected for further analysis in the case-control cohort. Marker order and positions were acquired from Duffy's interpolated Rutgers Map [9] with markers spaced approximately 5.5 cM apart spanning a 66 cM region. Marker names and cumulative positions in Kosambi centimorgans (cM) are: DXS1206 (0.0)–DXS984 (13.8)–DXS8106 (23.39)–DXS8043 (28.6)–DXS297 (32.8)–DXS8091 (35.54)–DXS1123 (37.22)–DXS8061 (47.68)–DXS15 (48.24)–DXS1073 (51.09)–DXS1108 (51.57).

Microsatellites were amplified using standard polymerase chain reaction (PCR) with 5′ labelled forward primers. PCR products were multiplexed and sized by capillary electrophoresis using the Applied Biosystems ABI 3130 Genetic Analyser. Genotypes were called using the Applied Biosystems GENEMAPPER® software version 4.0. Primer sequences are given in Online resource 1, and PCR conditions are available on request.

Statistical analysis of pedigrees

Prior to statistical analysis genotype data was screened for Mendelian and relationship inconsistencies using Pedcheck [10]. Error checking was also performed in the MINX program and unlikely genotypes were resolved or removed. The Kong and Cox GENEHUNTER PLUS (X-linkage version 1.2) statistical software [11] was used to carry out non-parametric multipoint linkage analysis as described previously [5]. Previously reported pedigrees MF7 and MF14 were reanalysed with additional makers and an updated marker map. Linkage peaks yielding LOD* scores ≥1 are suggestive of linkage for X chromosome markers whilst LOD* scores ≥2 indicate significant linkage. LOD scores were converted to *P* values using the ConvertingLODtoP-value spreadsheet [12]. Haplotypes were determined from genotype and pedigree information using the X-linked version of Merlin, Minx [13].

Markers and genotyping in case-control cohort

Gene maps of the Xq27 and Xq28 regions were obtained from NCBI (*Homo sapiens* Genome Build 37.2) and public databases (e.g. Pubmed) and were interrogated for functional gene information in order to select a final list of two genes in the Xq27 locus and nine genes in the Xq28 locus for further examination (Online resource 2).

Hapmap CEU data were downloaded for each candidate gene, and the tagger algorithm in Haploview [14] was used to identify 30 SNPs across ten of the 11 genes that captured the maximum degree of variation attainable while being constrained by multiplexing genotyping techniques. The 30 SNPs were genotyped at Australian Genome Research Facility (AGRF) using the Sequenom MassArray on an Autoflex Spectrometer and iPLEX GOLD chemistry. An insertion/deletion polymorphism in the 11th gene—*GPR50* was amplified using standard PCR with 5′ FAM labelled forward primer (Online resource 1). PCR conditions are available on request. Genotypes were determined by capillary electrophoresis using the Applied Biosystems ABI 3130 Genetic Analyser. Genotypes

were called using the Applied Biosystems GENEMAPPER® software Version 4.0.

Statistical analysis of case-control cohort

Microsatellites were tested for association using the CLUMP analysis program [15]. To detect association between the SNP markers and migraine, we performed chi-square (χ^2) analysis to test for significant differences in allele and genotype frequencies (females only for associated SNPs) in case versus control results. Hardy–Weinberg equilibrium (HWE) for females control genotypes were also calculated using PLINK [16]. For the candidate gene study, locus-specific correction set the significance level at 0.008 for Xq27 and 0.001 for Xq28. Haplotype frequency estimates and analysis of the haplotype blocks were performed using Haploview [17].

Results

Pedigree analysis

In this study, we focused on eight migraine pedigrees. Four of these pedigrees (MF7, MF14, MF47 and MF879) showed evidence of excess allele sharing at Xq27 and Xq28. At the Xq27 locus, a maximum LOD* score 1.38 (P=0.0058) was identified for MF879. Similarly, at the Xq28 locus, a maximum LOD* score of 1.50 (P=0.0043) spanned the locus from DXS8061 to the final marker DXS1108 in MF47.

An initial global scan of all migraine families included in the study was suggestive of linkage across Xq27 and Xq28 with maximum LOD* scores peaking at 1.63 (P=0.003) at DXS8043. Previous analysis of MF7 and MF14 has shown evidence to support genetic heterogeneity of the disorder in these families [5] potentially suggesting that two independent loci exist in this region. Analysis of the new migraine families supports this theory as the individual analysis of MF879 only shows excess allele sharing at Xq27, and conversely, MF47 only shows excess allele sharing at Xq28. A global analysis was therefore performed on each region using only those pedigrees that implicated the respective loci in order to refine the linkage regions for further analysis. This analysis revealed a maximum global LOD score of 3.69 ($P=1.88\times10^{-5}$) between markers DXS8043 and DXS297 at Xq27 for the combined analysis of MF7, MF14 and MF879. At Xq28, the combined analysis of MF7, MF14 and MF47 showed a LOD* of 3.65 ($P=2.07\times10^{-5}$) at DXS8061.

Haplotype analysis confirmed a narrowed Xq27 region from the previous study through critical recombination events in the new MF879 pedigree. Individuals IV:4 and IV:5 in MF879 show recombination at DXS8043xDXS297, the markers that bound the Xq27 locus, suggesting that a susceptibility candidate in this region is between these markers. While the new pedigree MF47 was also shown to display excess allele sharing to the Xq28 region, haplotype analysis did not narrow the region that was identified previously [5].

Investigation of key genetic markers in a case-control cohort

In order to test if the signals identified at the Xq27 and Xq28 loci were pedigree-specific or translated to the general population, we examined a microsatellite marker in each region as well as a SNP in close proximity to each marker in an Australian migraine case-control cohort. Both DXS8043 and the SNP rs5920369 (−720 bp) at Xq27 showed evidence of association in the tested female population (P=0.009 and P=0.01, respectively). Similarly, Xq28 DXS8061 and rs6653488 (+2.8 kb) also showed marginal association in the female cohort (P=0.07 and P=0.02 respectively) that was strengthened at DXS8061 in the MO class (P=0.05).

Interrogation of public databases for gene annotations in the ~2.4 Mb Xq27 locus bordered by markers DXS8043 and DXS297 identified very few known genes. Consequently, only two candidate genes were selected for further analysis, *SLITRK2* and *CXorf1*. Four SNPs were identified for analysis across these two genes. All SNPs were in HWE, however, none showed association.

In contrast to the Xq27 locus, the Xq28 region harbours numerous potential candidate genes. In this region, ten candidate genes were selected based on known or implied functional roles in hormonal, vascular or neurological systems .Of the SNPs analysed, one SNP was found to be in HWD (P<0.001) and was not used for further studies. Chi square analysis identified a SNP in *CNGA2* with positive association, P=0.048, which was also stronger in females (genotype P=0.0038) when analysed by gender, however, this did not remain significant after corrections. No other SNPs were associated in the entire cohort or when stratified by gender.

Finally, to further investigate the association to MO observed at the microsatellite marker DXS8061, the SNPs around this marker were also tested for association to this migraine class. This analysis identified that the three SNPs genotyped in closest proximity to the DXS8061 marker independently showed a trend towards association with

Table 1 Xq28 MO risk haplotype rs5970389, rs6653488 and rs2071256

Haplotype	Freq (MO)	Freq (cases)	χ^2	P value	OR	95% CI
TTC	0.689	0.591	6.997	0.0082	1.53	1.11–2.11
CCG	0.148	0.229	6.687	0.0097	0.58	0.38–0.88
CTG	0.112	0.12	0.0105	0.7464	0.92	0.57–1.48
TTG	0.035	0.051	0.867	0.3518	0.69	0.31–1.51

MO ($P \leq 0.07$). Haplotype analysis of this series of SNPs revealed a three-marker risk haplotype ($P=0.0082$, OR= 1.53 CI=1.11–2.11) in the *NSDHL* gene (Table 1).

Discussion

In this study, we have expanded on previous work to confirm known migraine susceptibility regions on the X chromosome and reduce the number of potential candidate genes for testing. In the initial study, the Xq27 locus was identified as spanning an 11 Mb region from markers DXS984 to DXS1123. Through genotyping of an additional marker in this region (DXS8043), we have reduced the locus shared by three pedigrees to a 2.4 Mb region, assuming that all pedigrees are segregating to the same susceptibility gene. In addition, the genotyping of additional migraine pedigrees has identified two previously unreported pedigrees that segregate to the two susceptibility regions strongly confirming a role for the X chromosomes in migraine.

This study also provides further evidence to indicate that the identified regions can be considered two separate loci, independently harbouring susceptibility variants. While MF7 and MF14 show excess allele sharing at both regions, MF879 only associated with the Xq27 locus; similarly, MF47 only associated with the Xq28 locus. The Xq27 region has now been implicated in migraine in three independent studies. Oedegaard and colleagues [18] conducted a genome-wide linkage study of bi-polar disorder and comorbid migraine using pedigrees derived from the National Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder. They identified a linkage peak with a LOD score of 1.6 ($P=0.003$) in the Xq27 region (marker DXS9908 approx 1 Mb from DXS8043) that segregated with only the migraine and not the bipolar phenotype. A previously published study using Australian Twins also identified association at two SNPs—rs6525667 and rs910618 (both at $P=0.009$) that localise to the Xq27 region identified here. Furthermore, a recent study investigating the X chromosome in migraine identified the Xp22 locus, however, the authors suggest that their data may also provide additional support for association at Xq24–Xq28 [19].

Our finding that the key markers identifying the X chromosome susceptibility regions are also associated in a case-control population provides evidence that these loci are not pedigree-specific and may be contributing to migraine in the general population. This result provides the impetus to identify the causative variants through candidate gene studies. The Xq27 loci appears to be a particularly 'gene poor' region. To date few annotations in this region are available on public databases. Consequently, only two genes (*SLITRK2* and *CXorf1*) were examined in the region with neither showing association. In contrast, the Xq28 region contains numerous potential candidate genes such as the neurotransmitter genes *SLC6A8, GABRE, GABRA3* and *GABRQ,* ion channel genes *CLIC2* and *CNGA2* and the ATPase *ATP2B3*.

While this study did not strongly implicate any of the tested genes in migraine, suggestive evidence of a three-marker haplotype in the MO subtype may be seen in the *NSDHL* gene. This gene is involved in the synthesis of cholesterol, and loss-of-function mutations are typically associated with CHILD syndrome, a severe disorder that is lethal in males. However, numerous studies have linked CVD with migraine, and some studies suggest an influence of cholesterol levels on both disorders [20]. Nonetheless, the results of this study nominally suggest an involvement of *NSDHL* in migraine, therefore, if this gene is involved, significant further research into the functional implications of genetic variants in this gene need to be investigated.

Conclusion

The unequal sex distribution of migraine is a unique facet of this disorder that may be explained by hormones and/or underlying genetic variations on the X chromosome that potentially cause migraine predisposition. The polygenic nature of migraine suggests that an interaction of both these mechanisms is also a possibility. The results of this study suggest that two distinct susceptibility variants may exist at Xq27 (marked by DXS8043—DXS297) and Xq28 (DXS8061—XqTer) and that these susceptibility loci may extend to association in the general population. Further studies are warranted to identify the pathological relevance of these loci to migraine aetiology.

Acknowledgments This research was supported by funding an Australian DEST International Science Linkages grant. Bridget Maher was supported by an Australian Postgraduate Award Scholarship, and Rod Lea is partially supported by a Corbett Research Fellowship.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All experiments comply with the current laws of Australia.

References

1. Mulder EJ, Van Baal C, Gaist D, Kallela M, Kaprio J, Svensson DA, Nyholt DR, Martin NG, MacGregor AJ, Cherkas LF, Boomsma DI, Palotie A (2003) Genetic and environmental influences on migraine: a twin study across six countries. Twin Res 6 (5):422–431
2. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF (2007) Migraine prevalence, disease burden, and the need for preventive therapy. Neurology 68(5):343–349

3. Stovner LJ, Zwart JA, Hagen K, Terwindt GM, Pascual J (2006) Epidemiology of headache in Europe. Eur J Neurol 13 (4):333–345
4. Stewart WF, Bigal ME, Kolodner K, Dowson A, Liberman JN, Lipton RB (2006) Familial risk of migraine: variation by proband age at onset and headache severity. Neurology 66(3):344–348
5. Nyholt DR, Curtain RP, Griffiths LR (2000) Familial typical migraine: significant linkage and localization of a gene to Xq24-28. Hum Genet 107(1):18–23
6. Nyholt DR, Dawkins JL, Brimage PJ, Goadsby PJ, Nicholson GA, Griffiths LR (1998) Evidence for an X-linked genetic component in familial typical migraine. Hum Mol Genet 7(3):459–463
7. IHS (2004) The international classification of the headache disorders 2nd ed. Cephalalgia 24(Suppl 1):1–150
8. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16(3):1215
9. Duffy DL (2006) An integrated genetic map for linkage analysis. Behav Genet 36(1):4–6
10. O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63(1):259–266
11. Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61(5):1179–1188
12. Nyholt DR (2000) All LODs are not created equal. Am J Hum Genet 67(2):282–288
13. Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30(1):97–101
14. Barrett JC (2009) Haploview: visualization and analysis of SNP genotype data. Cold Spring Harb Protoc 2009 (10):pdb ip71
15. Sham PC, Curtis D (1995) Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 59(Pt 1):97–105
16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575
17. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21 (2):263–265
18. Odegaard KJ, Greenwood TA, Lunde A, Fasmer OB, Akiskal HS, Kelsoe JR, Consortium NGIBD (2010) A genome-wide linkage study of bipolar disorder and co-morbid migraine: Replication of migraine linkage on chromosome 4q24, and suggestion of an overlapping susceptibility region for both disorders on chromosome 20p11. J Affect Disord 122(1–2):14–26
19. Wieser T, Pascual J, Oterino A, Soso M, Barmada M, Gardner KL (2010) A novel locus for familial migraine on Xp22. Headache 50 (6):955–962
20. Silberstein SD (2005) Cardiovascular risk factors associated with migraine. Lancet Neurol 4(7):391–392