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A genome-wide analysis of 'Bounty' descendants implicates several novel variants in migraine susceptibility

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ABSTRACT

Migraine is a common neurological disease with a complex genetic aetiology. The disease affects ~12% of the Caucasian population and females are 3 times more likely than males to be diagnosed. In an effort to identify loci involved in migraine susceptibility we performed a pedigree-based genome-wide association study (pGWAS) of the isolated population of Norfolk Island. This unique population originates from a small number of British and Polynesian founders who are descendents of the Bounty mutiny and forms a very large multigenerational pedigree [1]. These population genetic features may facilitate disease gene mapping strategies [2].

In this study we identified a high prevalence (25.5%) and heritability of migraine in the Norfolk Island population. We performed a pedigree-based GWAS and utilised a statistical and pathological prioritisation approach to implicate a number of variants in migraine. A SNP located in the zinc finger protein 555 (*ZNF555*) gene (rs4807347) showed evidence of statistical association in our Norfolk Island pedigree ($P=9.6 \times 10^{-6}$) as well as replication in a large independent and unrelated cohort with >500 migraineurs. In addition, we utilised a biological prioritisation to implicate 4 SNPs, in within the *ADARB2* gene, 2 SNPs within the *GRM7* gene and a single SNP in close proximity to a *HTR7* gene. Association of SNPs within these neurotransmitter-related genes suggests a disrupted serotonergic system that is perhaps specific to the Norfolk Island pedigree, but that might provide clues to understanding migraine more generally.

INTRODUCTION

Migraine is a chronic and debilitating neurological disease characterised by recurrent attacks of severe headache that is usually accompanied by nausea, vomiting, photophobia and phonophobia. Clinical diagnosis is established by fulfilment of symptom-based criteria defined by the International Headache Society (IHS) [3]. Migraine prevalence is ~12% in Caucasian populations and females are ~3 times more likely than males to be affected. Ethnic, geographic, lifestyle and socioeconomic factors are also associated with variable risk of migraine [4].

The disorder displays strong familial aggregation with first degree relatives of migraine probands having a 2 to 4-fold increased risk of developing the disorder compared to the general population [5, 6]. Population based twin studies report heritability estimates that range from 0.34 to 0.57 [7, 8]. The complex molecular genetic nature of migraine is evident from the large number of different loci discovered so far via linkage and candidate gene association studies.

Ion channel and ion transport genes are implicated in the rare, autosomal dominant MA subtype, FHM [9-11] and more recently in an extended pedigree with autosomal dominant MA [12]. Functional analyses indicate mutations in these genes alter normal neural activity and promote cortical hyperexcitability [12, 13]. In addition to these rare, familial genetic variants, a population level migraine risk variant has recently been discovered on chromosome 8 [14]. The minor allele frequency (MAF=0.206) by meta-analysis indicates the variant is extremely common and conveys mild genetic risk (OR=1.18). At this stage there does not seem to be a single major locus that confers an effect on migraine risk across all affected pedigrees and populations.

One method for addressing complex genetic models is to study isolated, founder populations. The geographic isolation of such population's means there is limited opportunity for intermarriage, which leads to elevated levels of endogamy and consanguineous unions. The resulting founder effect may increase the frequency of genetically influenced diseases compared to "outbred" populations with the reduction in genetic diversity possibly serving to decrease the overall number of disease susceptibility genes [2]. Extreme isolation also exposes individuals to a similar environment and promotes a more homogenous lifestyle, which minimises non-genetic variation. Overall, it is expected that the genetic models will be simplified improving the likelihood of detecting susceptibility genes.

Norfolk Island is a small volcanic island in the South Pacific located almost 1,600 kilometres northeast of Sydney, Australia. The Norfolk Island population is descendent from 11 British 'Bounty' Mutineers and 6 Tahitian women, who colonised nearby Pitcairn Island in 1790 [15]. In 1856, the small community of 193 relocated to Norfolk Island (then uninhabited) when population growth became unsustainable on Pitcairn [16]. The present day Norfolk Islanders have maintained a relatively homogeneous lifestyle due to geographic isolation, strict quarantine and immigration laws, and community centred culture. Detailed genealogical databases on Norfolk Island allow family histories to be traced back to the original founders. The Island's current population is approximately 2,000 (excluding visitors and tourists) [17].

We have previously used genealogical information to estimate the structure of the entire Norfolk Island pedigree which is comprised of ~6500 individuals spanning 12 generations back to the 17 founders [1]. Subsequent pedigree reconstruction analysis incorporating

genetic data has identified a 377-member pedigree that has the statistical power for gene mapping studies [1, 15] and was available for phenotyping. We have also quantified Polynesian admixture and inbreeding in the Norfolk pedigree and shown these effects to be associated to several cardiovascular disease traits [15]. Analysis of a 10cM density microsatellite scan revealed moderate evidence of linkage to regions on chromosome 9 and 13 [18]. These loci were nominally replicated in unrelated Norfolk Island Cohort members and also provided support and replication of known migraine and epilepsy loci [19, 20]. These findings may benefit from re-evaluation with high-density SNP panels as traditional genome-wide microsatellite maps leave broad areas of the genome uncovered [21, 22]. On this premise, genome wide SNP genotyping at a mean density of 4.7kb was undertaken in core-members of the Norfolk Island pedigree.

The aim of the current study was to map genes associated with migraine risk in the Norfolk Island isolate, especially given migraine prevalence estimates for Norfolk Island (25.5%) are approximately twice as high as the established prevalence of 12% in outbred Caucasian populations [18, 23]. We estimated the prevalence and heritability of migraine in the Norfolk Island population and then perform a pedigree-based genome-wide association study (pGWAS) of the core pedigree using the Illumina 610-quad genotyping BeadChip and a linkage-based association testing algorithm implemented in the SOLAR program [24].

RESULTS

In total, we analysed migraine phenotype information from a 377-member pedigree previously described [1, 15]. Of this pedigree, 96 individuals are positive for migraine according to the IHS criteria. This strong familial clustering (Relative Risk = 2.1) is consistent with the notion that inherited factors play a role in disease risk and establishes the Norfolk Island population as “high risk” for migraine. The remaining 281 individuals were not affected with migraine at the time of recruitment. Heritability of the migraine phenotype was estimated by SOLAR using an age and sex adjusted model assuming additive genetic factors. This analysis produced an h^2 of 0.53 ($P=0.016$), which is consistent with other studies and warrants a pGWAS to map susceptibility genes.

Illumina 610-quad genotype data was collected for $n=285$ individuals who were selected from the core 377-member pedigree as being highly informative individuals in terms of linkage. A high proportion of affected females were observed (74%), which is consistent with the female-male ratio of approximately 3 to 1 ($P=0.0012$). Migraineurs were slightly younger (46 yrs) on average compared to non-migraineurs (50yrs) ($P=0.035$). Admixture and inbreeding coefficients were not associated with migraine ($P>0.2$). A pGWAS was performed by testing SNPs for association within a linkage-based probit regression model adjusted for sex, age, admixture and inbreeding (ie population structure). A Manhattan plot of P-values is depicted in Supplementary Figure 1.

Given the uniqueness of this pedigree-based population we used a combination of statistical and functional prioritisation to investigate our results. Focussing on the top 0.05% of SNPs yielding the lowest P-value from the pGWAS, we also prioritised SNPs based on their functional plausibility in terms of disease pathology. To do this we implemented a similar

approach to Igl et al (2010), which prioritised SNPs based on P-value as well as plausibility for a functional role in disease pathology [25].

Results indicated 172 SNPs fell within the top 0.05% region of the probability distribution (see Supplementary Table 1). The most strongly associated SNP occurred in the intronic region of the ADAMTSL1 gene (MIM 609198) on chromosome 9p22.2-p22.1 (rs4977338; $P=1.96 \times 10^{-6}$). Given many of the 172 top ranking SNPs were not associated with migraine candidate genes we then assessed the SNP panel according to whether they were physically near genes with known annotation placing more value on genes with a putative role in migraine neuropathology ie. genes that are known to a) be expressed in the brain or central nervous system (CNS) b) regulate neurological pathways (eg. neurotransmitters) c) be plausibly related to migraine neuropathology (eg. cellular hyperexcitability, ion channel disruption).

Using this strategy to assess only the top 172 SNPs we prioritised 13 SNPs in 9 genes (Table 1). There were four SNPs within *ADARB2* that made the top 0.05% cut-off and that were statistically significant at the M_{eff} -adjusted *gene-wide* level ($P < 1 \times 10^{-4}$). HAPLOVIEW analysis showed that the 4 SNPs were in strong linkage disequilibrium and formed a single haplotype block spanning 22kb within *ADARB2*. Interestingly, one of the *ADARB2* SNPs (rs2271275) confers an amino acid change (Thr-Ala) providing a compelling candidate variant for involvement in disease causation. In addition, two SNPs in a glutamate receptor gene, *GRM7* ($P=2.7 \times 10^{-5}$ and 7.26×10^{-5}) and a single SNP in close proximity to a serotonin receptor gene, *HTR7* ($P=1.67 \times 10^{-4}$) were also implicated in disease risk using our approach. The relationship between the 3 key genes - *ADARB2*, *GRM7*, *HTR7* - was explored *in silico* using the online Gene Multiple Association Network Integration Algorithm software

(GeneMANIA) [26]. Results supported co-localisation and co-expression of these genes via intermediates (not shown).

The uniqueness of the study population and design may prohibit conventional replication in independent cohorts [27]. Despite this limitation, the 13 biologically prioritised SNPs (Table 1) along with the top 10 statistically significant SNPs originally detected (Supplementary Table 2) were assessed in an independent replication cohorts - the Women's Genome Health Study (WGHS). The WGHS cohort includes 23,294 unrelated women of European ancestry who are derived from the approximately 72% of Women's Health Study (WHS) participants providing samples and consent for blood-based analysis. Greater than 5000 individuals from the WGHS were diagnosed with migraine [28-30]. SNP rs2800143 was unavailable in the WGHS cohort. Of the top SNPs ranked based on P-value and/or biological significance, evidence of replication was detected for rs4807347, intronically located in the zinc finger protein 555 (*ZNF555*) gene ($P=0.019$; $\text{Beta}=-0.074$; $\text{SE}=0.03$) in the WGHS cohort [31].

DISCUSSION

Despite the restriction our unique study design placed on the ability to validate these associations we did find some evidence of replication for an intronic SNP (rs4807347) in ZNF555 and for rs2800143. Zinc finger proteins are highly abundant in eukaryotic genomes and possess diverse functions including, but not restricted to DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly and lipid binding [32]. Interestingly, a study of spreading depression (SP) in rat cerebral cortex detected significant fold changes (>2.0 or <-2) in gene expression for a number of zinc-finger proteins 3 hours after SP, suggestive of a role in stress response and DNA repair after a migraine attack [33]. Estrogen treatment of rat trigeminal ganglia in vitro to assess hormonal effects on migraine has revealed down-regulation of zinc finger protein 36 (ZKSCAN1) gene expression [34], ZKSCAN1 was postulated to have an anti-inflammatory function achieved by binding mRNAs encoding tumour necrosis factor (TNF), which accelerated mRNA degradation [34]. Although ZNF555 is yet to be functionally characterized, it may be of potential interest in terms of post migraine attack recovery, given functional evidence for other zinc family protein members.

This study also showed several neurotransmitter-related genes (ADARB2, GRM7 and HTR7) to be associated with the migraine phenotype in the Norfolk Island pedigree. A pGWAS utilising a combination of statistical significance and biological prioritisation of SNPs suggested in particular, that a non-synonymous variant of the ADARB2 gene might be involved in disease susceptibility in this unique population. RNA-editing genes have been suggested as candidates for complex neurological disorders such as epilepsy, depression and schizophrenia [35]. The ADARB2 SNP rs2271275 has previously been associated with early-onset obsessive-compulsive disorder in some American families [36]. The ADARB2 locus on

chromosome 10p15.3 has not previously been implicated in migraine susceptibility. However, a recent migraine GWAS conducted in European populations did provide evidence supporting a link between a locus on 8q22.1 (rs1835740) and glutamate regulation [14]. We did not find any trend toward a statistical association of rs1835740 ($P = 0.54$), which is more likely to be explained by differences in the unique Norfolk Island isolate.

We also implicated SNPs in two serotonergic genes (HTR7 and GRM7). These genes are widely and predominantly expressed throughout the brain [37] and function by positively activating adenylate cyclase via g-protein coupling and may have roles in circadian rhythm function, neuroendocrine function and affective behaviour disorders [38]. These genes are strong biologically plausible candidates, especially given the amounting evidence of altered serotonergic neurotransmission during and between migraine attacks [39]. A role for serotonergic system disruption during migraine attacks is further supported by the effect of triptans, a class of serotonin receptor agonist used to treat migraine. Triptans modulate trigeminovascular responses in neurons in the ventroposteromedial (VPM) nucleus, which are likely involved in the transmission of pain [40]. Interestingly evidence of association is reported for HTR7 variant, rs1298056 (genotypic P -Value=0.0058) in a study of 122 SNPs of the serotonergic system in a Spanish population of 528 migraine and 528 control individuals [41].

There was no evidence of replication of loci between the previous linkage investigation and the current association method in the Norfolk pedigree. This result is not unexpected, due to the different genotyping platforms, marker densities, statistical methods and cohort sizes. Overall the current study had limited power, which could be aided by on-going recruitment of pedigree members. Future studies of migraine using the Norfolk population isolate may also

consider identifying migraine probands and recruiting complete, individual sub-pedigrees for genetic studies.

This study identified a high prevalence and heritability of migraine in the genetically isolated population of Norfolk Island. A pGWAS utilising a combination of statistical significance and biological prioritisation implicated a number of SNPs in migraine risk including a SNP located in the zinc finger protein 555 (ZNF555) gene (rs4807347), which showed some evidence of replication in an independent migraine cohort. Association of the SNPs in neurotransmitter genes ADARB2, GRM7 and HTR7 suggests a common neurological pathway perhaps peculiar to Norfolk island and may help explain the long hypothesised of serotonergic system disruption in migraine pathophysiology in some populations.

SUPPLEMENTAL DATA DESCRIPTION

Supplementary Document 1. Methodology

Supplementary Figure 1. Manhattan Plot of autosomal genome-wide associations for migraine in the Norfolk Island pedigree.

Supplementary Table 1. Summary of the top 0.05% of SNPs (n=172) detected in the Norfolk study.

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Web Resources

GeneMANIA, <http://genemania.org>

Haploview, <http://www.broadinstitute.org/haploview/haploview>

Pedigree RElationship Statistical Test (PREST),

<http://www.utstat.utoronto.ca/sun/Software/Prest/index.html>

PEDSYS, <http://www.sfbr.org/Departments/genetics>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

Sequential Oligonucleotide Linkage Analysis Routines (SOLAR),

<http://www.sfbr.org/Departments/genetics>

SimWalk2, <http://www.genetics.ucla.edu/software/download?package=2>

Whole Genome Association Study Viewer (WAGViewer),

<http://people.genome.duke.edu/~dg48/WGAViewer/>

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Table 1. pGWAS results for the Norfolk pedigree and WGHS replication cohort

Chr	SNP REF. NO.	P-VALUE	Beta*	POSITION (BP)	FUNCTION	MINOR/ MAJOR ALLELE	MAF	DIST. TO GENE (BP)	GENE SYMBOL	WGHS Cohort	
										Allelic P-value	Beta (SE)
Top 10 GWAS SNPS											
4	rs11930554	2.84E-6	1.068	131787382	INTERGENIC	C/T	0.138	-359452	<i>AC092540.1</i>	0.35	0.026 (0.03)
4	rs11936003	2.84E-06	1.068	131788092	INTERGENIC	G/A	0.138	-360162	<i>AC092540.1</i>	0.35	0.026 (0.03)
4	rs7690766	3.35E-06	1.064	131774208	INTERGENIC	G/A	0.135	-346278	<i>AC092540.1</i>	0.37	0.025 (0.03)
9	rs4977338	1.96E-06	-0.823	18718086	INTRONIC	T/G	0.140	0	<i>ADAMTSL1</i>	0.48	-0.022 (0.03)
9	rs10512405	1.21E-05	-0.542	113236797	INTRONIC	C/T	0.403	0	<i>SVEP1</i>	0.18	-0.032 (0.02)
10	rs883248	3.83E-06	0.666	1250184	INTRONIC	G/A	0.439	0	<i>ADARB2</i>	0.91	0.003 (0.02)
10	rs7079024	3.03E-06	0.630	3445668	INTERGENIC	C/T	0.470	83418	<i>RP11-482E14.1</i>	0.94	-0.002 (0.02)
10	rs10795033	1.72E-05	0.578	3447072	INTERGENIC	C/T	0.470	82014	<i>RP11-482E14.1</i>	0.95	-0.002 (0.02)
17	rs2525570	1.15E-05	0.603	29681245	INTRONIC	G/A	0.469	0	<i>NF1</i>	0.78	0.006 (0.02)
19	rs4807347	9.56E-06	0.941	2857287	3PRIME UTR	A/C	0.144	0	<i>ZNF555</i>	0.019	-0.074 (0.03)
Biologically and statistically prioritised SNPs											
1	rs6425412	0.0002	11.25	177073727	INTRONIC	G/A	0.034	0	<i>ASTN</i>	0.24	0.055 (0.05)
2	rs2600685	5.19E-05	0.52	175627048	INTRONIC	A/G	0.492	0	<i>CHRNA1</i>	0.32	-0.023 (0.02)
3	rs11714003	0.0003	-0.78	54234467	INTRONIC	G/A	0.089	0	<i>CACNA2D3</i>	0.34	0.039 (0.04)
3	rs1391950	2.70E-05	0.55	7058417	INTRONIC	G/A	0.490	0	<i>GRM7</i>	0.77	-0.007 (0.02)
3	rs11713183	7.26E-05	-0.51	7078179	INTRONIC	T/C	0.427	0	<i>GRM7</i>	0.23	0.027 (0.02)
5	rs1561836	0.0002	0.85	22794657	INTRONIC	C/T	0.128	0	<i>CDH12</i>	0.55	-0.023 (0.04)
5	rs210993	0.0002	0.50	161619504	INTERGENIC	A/G	0.344	36959	<i>GABRG2</i>	0.74	0.008 (0.02)
10	rs10903399	7.68E-05	0.64	1227868	DOWNSTREAM	C/T	0.330	205	<i>ADARB2</i>	0.61	0.012 (0.02)
10	rs1046914	3.43E-05	0.67	1228206	3PRIME UTR	G/A	0.328	0	<i>ADARB2</i>	0.60	0.013 (0.02)
10	rs2271275	2.67E-05	0.65	1230968	NON-SYNON	G/A	0.368	0	<i>ADARB2</i>	0.62	0.012 (0.02)
10	rs883248	3.83E-06	0.67	1250184	INTRONIC	G/A	0.439	0	<i>ADARB2</i>	0.91	0.003 (0.02)
10	rs2800143	0.0002	-0.65	92463214	INTERGENIC	A/G	0.128	37366	<i>HTR7</i>	NA	NA
12	rs11615115	4.02E-05	3.79	100802452	INTRONIC	G/A	0.045	0	<i>SLC17A8</i>	0.14	0.068 (0.05)

BP=Base Pairs, Chr=Chromosome, MAF=Minor Allele Frequency, NA=Nota Available, WGHS=Women's Genome Health Study

*The beta coefficient is a measure of risk. A negative beta indicates the minor allele increases migraine risk, a positive beta indicates a decreased risk.