GENOME WIDE ASSOCIATION STUDY IDENTIFIES RISK LOCI FOR CLUSTER HEADACHE

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Abstract

Objective: To identify susceptibility loci for cluster headache and obtain insights into relevant disease pathways.

Methods: We carried out a genome-wide association study, where 852 UK and 591 Swedish cluster headache cases were compared with 5,614 and 1,134 controls, respectively. Following quality control and imputation, single variant association testing was conducted using a logistic mixed model, for each cohort. The two cohorts were subsequently combined in a merged analysis. Downstream analyses, such as gene-set enrichment, functional variant annotation, prediction and pathway analyses, were performed.

Results: Initial independent analysis identified two replicable cluster headache susceptibility loci on chromosome 2. A merged analysis identified an additional locus on chromosome 1 and confirmed a locus significant in the UK analysis on chromosome 6, which overlaps with a previously known migraine locus. The lead single nucleotide polymorphisms were rs113658130 (p = 1.92 x 10^{-17}, OR [95%CI] = 1.51 [1.37-1.66]) and rs4519530 (p = 6.98 x 10^{-17}, OR= 1.47 [1.34-1.61]) on chromosome 2, rs12121134 on chromosome 1 (p = 1.66 x 10^{-8}, OR= 1.36 [1.22-1.52]) and rs11153082 (p = 1.85 x 10^{-8}, OR= 1.30 [1.19-1.42]) on chromosome 6. Downstream analyses implicated immunological processes in the pathogenesis of cluster headache.

Interpretation: We identified and replicated several genome-wide-significant associations supporting a genetic predisposition in cluster headache in a genome-wide association study involving 1,443 cases. Replication in larger independent cohorts combined with comprehensive phenotyping, in relation to e.g. treatment response and cluster headache subtypes, could provide unprecedented insights into genotype-phenotype correlations and the pathophysiological pathways underlying cluster headache.

Introduction

Cluster headache (CH) is a rare, debilitating disorder with an estimated prevalence of 1 in 1000 world-wide with higher rates in northern countries, further from the equator.[1] It presents with unilateral pain distributed along the trigeminal nerve's first branch.[2] It is clearly distinct from other headache disorders based on attack duration, prominent ipsilateral cranial autonomic features, restlessness / agitation and response to specific treatments.[3] Concomitant migraine can occur, but the reported frequency varies.[4,5] Some 85% of CH patients experience attack periods interspersed with attack-free periods of at least three months per year (episodic CH). The remainder have chronic CH with limited remissions. CH exhibits heritability evidenced by familial aggregation and cases of concordance amongst monozygotic twins.[6] In familial cases, segregation analysis predominantly shows an autosomal dominant inheritance model with reduced penetrance.[6]

Attempts to determine the underlying genetic architecture include candidate association studies of genes with a putative pathogenic role in CH. The pathophysiology remains unclear; a neurovascular process involving the trigeminovascular system, trigeminal autonomic reflex, and posterior hypothalamus is hypothesised.[7] Functional imaging studies observed activation of the ipsilateral inferior hypothalamic grey matter in CH attacks.[8] These findings, together with the circadian periodicity of CH, influenced selection of candidate genes. Unfortunately, reported associations with e.g. *HCRTR2*, *CLOCK*, and *ADH4* presently lack replicability.[6]

Genome-wide association studies (GWAS) have provided insight into many neurological disorders including migraine.[9] A large migraine meta-analysis yielded associations suggestive of vascular and neuronal mechanisms.[9] To date, there is one GWAS on CH involving 99 cases and 360 controls[10]. It lacked power to detect loci of genome-wide significance (GWS) and suggested associations were not reproducible.[10,11] This present study aims to identify novel genetic risk variants for CH by performing a GWAS.

Methods

Participant Recruitment and Phenotyping

This is a multicentre study comprising of GWAS of two independent cohorts sourced from specialised headache clinics; one from the United Kingdom (UK) and one from Sweden (Supplementary table 1).

UK Cohort

Recruitment occurred between 2006-2018, starting at the National Hospital for Neurology and Neurosurgery, London, UK. Ethical approval was obtained for four additional UK sites (RAC#2060008 and UCLH: 04/N034). All were specialist-led headache clinics. A diagnosis was made using the International Classification of

Headache Disorders-3beta edition ICHD-3b.[2] Review by two independent neurologists was required if one ICHD-3b criteria was not met. Control genotype data consisted of a cohort of UK individuals without headache (n = 463), the 1958 birth cohort (n = 2,699) from the Wellcome Trust Case Control Consortium (WTCCC) and the National Blood Survey cohort (NBS, n = 2,501).

Swedish Cohort

Recruitment occurred between 2014-2017 at the Karolinska University Hospital neurology clinic, Stockholm, Sweden. Ethical approval was obtained from the Stockholm Regional Ethical Review Board (registration number 2014/656-31). Patients fulfilling the ICHD-3b diagnostic criteria were included.[2] Diagnosis was confirmed by headache specialists and through medical records. Additional information was derived using a diagnostic questionnaire. Control genotype data was obtained from neurologically healthy controls from the Immunomodulation and Multiple Sclerosis Epidemiology study (n=1,299).

DNA extraction and genotyping

Participants provided blood or saliva for DNA extraction. UK cases were genotyped at the Human Genotyping Facility (HuGe-F), Netherlands using the Illumina Infinium 24v1.0 Global Screening Array (GSA). Genotyping for the WTCCC and NBS controls used the custom 1.2M Illumina chip. Swedish cases were genotyped using the SNP&SEQ Technology Platform, Sweden and controls at deCODE, Iceland using the GSA.

Data Processing and Quality Control (QC)

Raw IDAT files were processed using GenomeStudio (Illumina). PLINK and Peddy software was used for data QC.[12,13] The QC procedure was performed according to standard guidelines and details for each study are summarised in supplementary table 2.

Imputation

The HRC/1KG imputation preparation and checking tool was used to identify errors related to strand, reference and alternate allele assignments, and allele frequency differences (> 0.2) relative to the Haplotype Reference Consortium panel v1.1 (HRCv1.1).[14] Estimated haplotypes were phased using Eagle v2.3 and imputation was performed on the Michigan server using HRCv1.1.[15,16] Monomorphic SNPs or those with an imputation quality score R^2 of < 0.3 or MAF < 0.01 were excluded. Because of the smaller sample size, results for the Swedish-only results were filtered for MAF < 0.05.

Single variant association testing

Single variant association testing was performed using Scalable and Accurate Implementation of Generalised mixed model (SAIGE)[17], fitting a null logistic mixed model. Imputed genotype data were used for all markers

and only autosomal SNPs were analysed. In the UK cohort principal component analysis (PCA) axes, generated with PLINK to identify population stratification, were used as covariates.[12] PCA axis 1-6 and sex were used as covariates in the Swedish cohort. In the merged analysis, we used separately imputed data from UK and Sweden which was again subjected to a PCA after merging. The first ten axis were used as covariates for combined SAIGE analysis. In each linkage disequilibrium (LD) cluster of SNPs containing GWS hits, the SNP with the lowest *p*-value in case vs. control comparisons was defined as the lead SNP. The Manhattan and Q-Q plots from association tests were created using R v3.6.2 and regional association plots were created with Locus Zoom.[18,19] Downstream analysis was conducted using R v3.6.2 unless otherwise stated.[18]

Functional variant annotation and prediction

Annotation and functional consequence prediction of disease-associated SNPs were conducted using FUMA v1.3.6a and the Variant Annotation Integrator tool.[20,21] The selected lead SNP from each associated locus was annotated. Utilised metrics included alternate allele frequency by population; Variant Effect Predictor (VEP) annotation, including the Combined Annotation Dependent Depletion (CADD) score; and Genomic Evolutionary Rate Profiling (GERP) score annotation.[22,23] CADD scores over 10 are predicted to be the 10% most deleterious possible substitutions in the human genome, 20 are predicted to be the 1% and 30 are predicted to be the 0.1%. GERP score ranges from -12.3 to 6.17, where higher scores indicate higher evolutionary constraint and a score greater than 2 can be considered constrained.

Gene-based association testing

Gene-based association analysis was conducted using MAGMA through FUMA v1.3.6a.[20,24] To identify candidate genes associated with CH, the mean association of all SNPs within a gene was calculated, accounting for LD. Gene windows were extended 35kb upstream and 10kb downstream of the annotated gene start and end sites to include regulatory regions.

Gene expression and expression quantitative trait loci (eQTL) analysis

Gene expression was determined using GTEx v8, spatiotemporal analysis of gene expression was accessed using Human Brain Transcriptome dataset and a cell type specific Brain RNA-seq atlas.[25–27] Gene mapping and expression quantitative trait loci (eQTL) of interest were determined through FUMA using default settings and eQTL data from the brain, spleen, vascular and immunological tissue based on GTEx v8 and the eQTL catalogue.[20] Only cis-eQTLs with multiple testing correction False Discovery Rate (FDR) < 0.05 were included in downstream analysis.

Pathway analysis

Pathway analysis was conducted using regions surrounding identified lead SNPs. In each case, protein-coding genes within a 1 Mb window on either side of the lead SNP were used as input for the gprofiler2 R package with the default background of annotated genes.[28] Pathways with a multiple testing-adjusted p-value < 0.05 (FDR) underwent further analysis. Sources for predefined pathways and complexes included Gene Ontology, KEGG and Reactome.[29–31]

Genetic colocalisation analysis

Bayesian colocalisation analysis was used to determine shared causal regions or lead variants between CH and the migraine GWAS from the UK Biobank GWAS database, publicly available at http://www.nealelab.is/uk-biobank, using R package coloc.[32] Causal regions were defined as the range of positions of SNPs between two recombination hotspots. Coloc reports posterior probability for each of five hypotheses tested: H0 – neither CH nor migraine had a genetic association within the tested region, H1 – only CH had a genetic association within the tested region, H2 – only migraine had a genetic association within the tested region, H3 – both CH and migraine had a genetic association within the tested region, but did not share causal variants, H4 – both CH and migraine shared a single causal variant with the tested region. High posterior probability for H4 supports colocalization of the signals.

Results

The clinical phenotype of CH cases within each cohort are summarised in supplementary table 1.

UK cohort

852 cases and 5,614 controls were included after QC. 86 SNPs passed the GWS threshold (p <5 x 10^{-8}) and clustered in three independent loci. Two loci were located on chromosome 2, (chr2q13, chr2q33). The lead SNPs in these regions were rs4519530 (p = 2.49 x 10^{-8}, OR = 1.39 [1.24-1.55]) (2q13) and rs113658130 (p =7.39 x 10^{-10}, OR = 1.63 [1.39-1.90]) (2q33). An additional locus on chromosome 6 also reached GWS, with lead SNP rs9386670 (p = 1.41 x 10^{-8}, OR = 1.4 [1.25-1.57]) (Table 1).

Swedish cohort

591 cases and 1,134 controls were included after QC. 53 SNPs reached GWS threshold (p < 5 x 10^{-8}). The two independent loci located on chromosome 2 identified in the UK association analysis (chr2q13, chr2q33) were replicated. The lead SNPs in these regions included rs72825689 (p =1.07 x 10^{-8}, OR = 2.82 [1.98-4.03]) and rs4675692 (p = 1.22x$10^{-8,}$ OR = 1.61 [1.37-1.90]) respectively (Table 1). The third locus on chromosome 6 did not reach genome-wide significance.

UK-Swedish combined results

Following QC, there were 1,443 cases and 6,748 controls of European ancestry. A genomic inflation factor of 1.06 suggested some occurrence of population stratification between Sweden and UK (Fig 1).

The combined association analysis identified four loci with GWS ($p < 5 \times 10^{-8}$) (Fig 2, Table 1). Two loci on chromosome 2 had significant independent association with CH in both cohorts. The locus with with the strongest association, 2q33 with lead SNP (rs113658130, $p = 1.92 \times 10^{-17}$, OR = 1.51 [1.37-1.66]) is located in a long intergenic non-coding RNA *LINC01877.* The closest protein coding gene is SATB homeobox 2 (*SATB2*) (Fig 3A, Table 1). The second locus is represented by lead SNP rs4519530, it is an intronic variant at 2q13, it is in the MER Proto-Oncogene, Tyrosine Kinase (*MERTK)* gene ($p = 6.98 \times 10^{-17}$, OR = 1.47 [1.34-1.61]) (Fig 3B, Table 1). The third locus corresponds to the one identified on 6q16 in the UK cohort, lead SNP rs11153082 ($p = 1.85 \times 10^{-8}$, OR = 1.30 [1.19-1.42] Fig 3C). It is located in the four and a half LIM domains 5 (*FHL5*) gene (table 1). A new locus was identified on chromosome 1q41 with lead SNP rs12121134 ($p = 1.66 \times 10^{-8}$, OR = 1.36 [1.22-1.52]). This locus has a *p*-value below $< 10^{-3}$ in the UK respectively the Swedish cohort ($p = 3.29 \times 10^{-4}$ and $p = 4.90 \times 10^{-5}$). This region does not contain any known genes (Fig 3D, Table 1). The closest gene is *LINC01705,* a long non-coding RNA, located 11 kb upstream from rs12121134, and the nearest coding gene is dual specificity phosphatase 10 (*DUSP10*). All of the lead SNPs were imputed and well above the imputation quality score R^2 threshold.

Functional variant annotation and prediction

Closer investigation of our GWS loci revealed that the 2q13 locus in *MERTK*, is represented by two independent lead SNPs; rs4519530 (reported in Table 1) and rs72825689 ($r^2 = 0.03$ with rs451930), which lies intergenic to *MERTK* ($p = 1.79 \times 10^{-12}$, OR 2.19 [1.76-2.73]) in the merged analysis. All lead SNPs were located in non-coding regions of the genome. Four variants in high LD with the lead SNPs ($r^2 > 0.9$) were exonic variants with moderate impact. Two of these were missense variants in *MERTK* (rs7604639, rs2230515) and two in *FHL5* (rs2273621, rs9373985). Both *FHL5* variants had high CADD scores of 23.6 and 15.5 respectively and all variants showed a high level of mammalian conservation with GERP scores above 2.

Gene-based association testing

Gene-based testing used the mean association signal from all SNPs within each gene, accounting for LD. 18,559 genes were analysed. A multiple testing-corrected *p*-value threshold of 2.69×10^{-6} was applied to identify genes significantly associated with CH. Five candidate genes passed this threshold: Transmembrane Protein 87B

(*TMEM87B*) (p = 1.06 x 10^{-12}), *MERTK* (p = 5.55 x 10^{-11}), Anaphase Promoting Complex Subunit 1 (*ANAPC1*) (p = 3.63 x 10^{-10}), Fibulin 7 (*FBLN7*) (p = 2.57 x 10^{-7}) on chr. 2q13, and *FHL5* (p = 2.03 x 10^{-6}) on chr. 6q16.

Gene expression and expression quantitative trait loci (eQTL) analysis

All of the candidate genes (*MERTK*, *ANAPC1*, *TMEM87B*, *FHL5*, and *FBLN7*) are expressed in the human brain. Cell type analysis showed that *ANAPC1* and *FBLN7* have the highest RNA expression in neurons while *MERTK* and *TMEM87B*, have the highest RNA expression in brain support cells, namely microglia and astrocytes. *FHL5* had minimal expression in brain but highly expressed in brain endothelial cells. Spatiotemporal expression pattern of the genes expressed in the human brain showed that *MERTK*, *TMEM87B*, *ANAPC1* were expressed in all regions of the brain. *MERTK* and *FBLN7* showed to be more highly expressed in the adult brain, the temporal expression in *FBLN7* particularly differed in the neocortex. Using lead SNPs, (rs12121134, rs4519530, rs72825689, rs113658130 and rs11153082) eQTLs were identified in 11 genes, specifically looking at brain, spleen, vascular and immunological tissues through FUMA. We observed overlapping eQTLs significant for all these tissues, eQTLs with the lowest p-values which were mapped by GWS SNPs are represented for each tissue type in Table 2.

Pathway analysis

74 pathways were significantly enriched for 46 genes in candidate regions ($p < 0.05$). The five most significant were cytokine-cytokine receptor interaction (KEGG:04060, p = 0.000397), interleukin-1 (IL-1) family signaling (REAC:R-HSA-446652, p =6.66 x 10^{-5}), growth factor receptor binding (GO:0070851 p = 4.64 x 10^{-5}), interleukin-36 (IL-36) pathway (REAC:R-HSA-9014826, p = 4.21 x 10^{-5}) and interleukin-1 receptor binding (GO:0005149, p = 1.30 x 10^{-10}). Of special interest for the brain were pathways related to neuroglial cells; positive regulation of glial cell proliferation (GO:0060252 p = 0.0297), and regulation of gliogenesis (GO:0014013, p = 0.0407). Moreover, there were a number of pathways relating to differentiation and activation of immune cells, as well as cell adhesion, many of which included the *MERTK* gene.

Migraine GWAS overlap

Based on the significant lead SNPs of the combined analysis, three of the four loci showed no association with migraine. This was confirmed using colocalisation analysis which showed that for the loci on chromosomes 1 and 2, H1 (only CH had a genetic association within the tested region) was most likely. However, the lead SNP (rs11153082) at the chromosome 6 locus showed overlap with migraine. We confirmed this signal resulting from the same causal variant as in the migraine GWAS from the UK Biobank GWAS database (posterior probability for shared causal variant at chr6q16: 97.4%). To exclude concurrent migraine driving this association, 655 UK

patients with known migraine status (n = 195 had co-existing migraine) were reassessed. Fisher's exact tests for the alternate allele frequency across the two groups, CH only compared to CH with concurrent migraine, in the lead SNP showed no significant differences (rs9386670: $p = 0.93$, OR = 1.02 [0.71-1.47]).

Discussion

We conducted a GWAS in CH, identifying four susceptibility loci with large effect sizes, of which one has previously been associated with migraine. The strongest association was for a region on chromosome 2 containing a long intergenic non-coding RNA *LINC01877. LINC01877* is highly expressed in brain, most abundantly in the hippocampus and hypothalamus. The Special AT-rich sequence-binding protein 2 (*SATB2*) gene is 168 kb proximal to the lead SNP. Mutations causing haploinsufficiency in this gene cause *SATB2* associated syndrome (SAS), a disorder characterised by neurodevelopmental delay and craniofacial abnormalities.[33] In the developing brain, *SATB2* is required for cell-type specification of the upper layer pyramidal neurons in the neocortex and formation of the corpus callosum.[33] In adult mice, it is strongly expressed in hypothalamic regions and the A12 cell group of dopaminergic neurons.[34] Conditional knockout mice exhibit abnormalities in structures with a role in nociceptive processing, namely the somatosensory cortex and thalamocortical projection axons.[35] A second independently significant region on chromosome 2 overlies an intronic region of *MERTK* and is in LD with two missense mutations reaching GWS. *MERTK* encodes a TAM receptor (TYRO33, AXL and MERTK), regulators of microglial function and the phagocytosis of apoptotic cells.[36] Homozygous mutations in *MERTK* cause retinitis pigmentosa, a condition sometimes associated with headache.[37,38] Cell-type expression analysis showed *MERTK* is highly expressed in microglia and *Mer* deficient mice exhibit an aggregation of apoptotic cells in neurogenic regions of the CNS.[36] MERTK has a role in neuro-inflammation and has been associated with Multiple Sclerosis.[39] Furthermore, MERTK mediates astrocyte elimination of excess synapses, regulating synapse remodelling through neural circuit refinement.[40]

An association identified at rs11153082 on chromosome 6 correlates with a locus implicated in migraine and headache.[9] This association was stronger in the UK cohort though the subsequent subgroup analysis found this was not dependent on the presence of co-existent migraine, indicating possible pleiotropy at this locus. The lead SNP driving this association is an intronic variant in the *FHL5* gene and overlaps the *UFL1* gene (UFM1 Specific Ligase 1) which was identified as an eQTL in several tissues relevant for CH pathophysiologylogy. In addition, *UFL1* was below GWS ($p = 8.46 \times 10^{-6}$) in the gene-based association testing. *UFL1* encodes a protein constituting part of the ubiquitin-fold modifier 1 (*UFM1*) conjugation system involved in the apoptosis and trafficking of vesicles in the endoplasmatic reticulum.[41]

Cell-type analysis showed enhanced microglial and neuronal expression. Pathways related to neuroglial cells such as positive regulation of glial cell proliferation, and regulation of gliogenesis were also implicated in our results. Neuro-inflammation has been implicated in several pain disorders and microglia mediate the generation of neuropathic pain through amplification of excitatory neuronal currents and attenuation of inhibitory currents.[42] Microglia influence central sensitisation events in chronic pain and are responsible for synaptic pruning in brain development, modulating functional connectivity.[43,44] Connectivity defects have been demonstrated in CH.[45] Although the mechanism remains unclear, microglial dysfunction may dysregulate synaptic elimination and plasticity, causing connectivity impairment in CH. Microglia are also potential therapeutic targets for medications currently used in CH management. For example, Verapamil, the prophylactic agent of choice, exhibits neuroprotective action through inhibition of microglial PHOX activity, mediating generation of reactive oxygen species via binding to its catalytic subunit gp91.[46,47] Similarly, valproic acid, a histone deacetylase inhibitor, triggers microglial apoptosis to inhibit over-activation.[48]

Our results implicate immunological processes in the pathogenesis of CH. Immune eQTLs reaching significance include monocytes, T-cells, and the spleen. Gene set enrichment analysis was significant for pathways involved in cytokine activity, especially the regulation of IL-1 and IL-36. The role of cytokines in the generation of headache has previously been suggested by enhanced nociceptive neuronal responses of the trigeminal nucleus caudalis, and subsequent hyperalgesia, following the injection of recombinant human IL-1β into the cerebrum of rats.[49] Several candidate genes from our analysis appear to influence the cAMP-responsive element binding protein (CREB) pathway and *MERTK* activates CREB.[50] *FHL5* is an activator of CREB, which subsequently is a transcription factor for *UFL1*.[51] The CREB pathway is critical for light entrainment of the circadian clock and also contributes to sensitisation of nociceptive cells and meningeal pain hypersensitivity. It has a role in pain transmission evidenced by CREB activation in the trigeminal ganglion *in vitro* post-stimulation of nociceptive neurons. Triptans, used to treat CH, reduce CREB activity within the trigeminal system.[52]

There are limitations to this study. This is a relatively small cohort due to the rarity of CH. A larger study or meta-analysis is required to derive additional associations with variants with a lower effect size. Genotyping of the UK controls on a different array platform introduces a potential confounder. The independent replication of loci in the Swedish cohort, which we performed on cases and controls genotyped on the same array (GSA), reduces the likelihood of spurious associations. Ensuring a phenotypically homogenous cohort is challenging as CH is reliant upon clinical diagnosis. Our cohorts were carefully phenotyped to minimise possible confounding with migraine which is phenotypically distinct. We have identified replicable genome-wide-significant

associations which contribute to a genetic predisposition in CH. Microglial expression appears predominant amongst candidate genes and pathway analysis implicated cytokine and immune activity as a pathogenic driver. Future targeted sequencing of loci will fine-map these regions to help identify pathogenic variants and facilitate functional and mechanistic studies. In combination with deep phenotyping, this has potential in providing genotype-phenotype correlations such as genotype and treatment response which can lead to future tailor-made treatment options respectively CH subtypes, such as episodic vs. chronic CH, which can improve diagnosis. This could offer unprecedented insights into the pathophysiological pathways underlying CH and novel targets for therapeutic intervention.

Post-script paragraph

Two parallel manuscripts (Harder et al. and O'Connor et al.), submitted to the journal, report the first replicated genomic loci associated with CH. Whereas Harder et al. investigated Dutch CH cases (n = 840) and controls (n = 1,457) and Norwegian CH cases (n = 144) and controls (n = 1,800), O'Connor et al. investigated UK cases (n = 852) and controls (n = 5,614) as well as Swedish cases (n = 591) and controls (n = 1,134). The four loci reported by Harder et al. correspond to four loci reported by O'Connor et al., with the index variants reported in the two studies being in linkage disequilibrium with each other (D' = 0.86 and r^2 = 0.36 for rs12121134 and rs11579212; D' = 0.98 and r^2 = 0.95 for rs4519530 and rs6541998; D' = 0.95 and r^2 = 0.34 for rs113658130 and rs10184573; D' = 0.93 and r^2 = 0.38 for rs11153082 and rs2499799, in the 1000 Genomes data for European populations). The independent discovery of the four loci in the two studies provides additional support that they represent genuine risk loci for cluster headache.

Next, we combined the summary statistics from the four studies (Dutch, Norwegian, UK, Swedish) using inverse-variance weighted meta-analysis as implemented in METAL (with the 'STDERR' option), after harmonizing the datasets using EasyQC.[53,54] In total, 8,039,373 variants were analyzed. The association to CH remained significant for all the eight index variants (in the four loci) reported in the two papers: rs11579212 (effect allele, EA: C), OR 1.31 (95% CI 1.21-1.41), *p*-value 8.98 x 10^{-13}; rs12121134 (EA: T), OR 1.40 (95% CI 1.29-1.53), *p*-value 9.18 x 10^{-15}; rs6541998 (EA: C), OR 1.40 (95% CI 1.30-1.51), *p*-value 2.37 x 10^{-19}; rs4519530 (EA: C), OR 1.41 (95% CI 1.31-1.52), *p*-value 4.18 x 10^{-29}; rs10184573 (EA: T), OR 1.38 (95% CI 1.28-1.50), *p*-value 3.35 x 10^{-16}; rs113658130 (EA: C), OR 1.54 (95% CI 1.41-1.69), *p*-value 1.28 x 10^{-21}; rs2499799 (EA: C), OR 0.77 (95% CI 0.70-0.84), *p*-value 2.73 x 10^{-8}; rs11153082 (EA: G), OR 1.33 (95% CI 1.23-1.43), *p*-value 2.98 x 10^{-14}. The eight index variants in the overlapping loci showed a consistent effect

direction across the two studies. Colocalization analysis identified a high posterior probability for three loci (those on chromosomes 1 and 2) to likely represent the same causal variant.[32] rs12121134 and rs11579212 have a posterior probability that the causal variants are the same (H4) of 80.4%, for rs4519530 and rs6541998 H4 is 87.4% and for rs113658130 and rs10184573 H4 is 96.9%. For the locus on chromosome 6, the colocalization analysis shows a higher probability that the loci in the two studies represent distinct causal variants (H3, 78.7%) rather than the same causal variant (H4, 21.2%).

Finally, the meta-analysis resulted in three additional loci becoming genome-wide significant: (1) a locus on chromosome 7 with 31 significant (p-value < 5 x 10^{-8}) variants with index variant rs6966836 (chr7:117002998, EA:C), OR 1.25 (95% CI 1.16-1.35), p-value 2.46 x 10^{-9}; (2) a locus on chromosome 10 with two significant variants with index variant rs10786156 (chr10:96014622, EA:C), OR 1.24 (95% CI 1.15-1.33), p-value 7.61 x 10^{-9}, and (3) a locus on chromosome 19 with two significant variants with index variant rs60690598 (chr19:55052198, EA:T), OR 1.87 (95% CI 1.51-2.33), p-value 1.70 x 10^{-8}.

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Author Contributions

EOC, CF, CR, LS, GLC, MV, KP, RT, NWW, JV, HH, MM and ACB contributed to the conception and design of the study. EOC, PS, CF, FL, LS, YHY, NG, NS, FA, ICH, BD, MZC, BSS, RS, SE, JA, OQ, CC, GLC, TK, EK, LG, AVEH, BSW, LSV, SL, DD, RT, NWW, IK, AS, CS, EW, CR, JV, HH, MM and ACB contributed to the acquisition and analysis of data. EOC, PS, CF, JV, CR and ACB contributed to drafting a significant portion of the manuscript or figures.

Potential Conflicts of Interest

Nothing to report

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Figure Legends

Figure 1: Quantile-quantile (QQ) plot of combined GWAS showing SNP *p*-values in GWAS analysis versus expected *p*-values. The straight line in the Q-Q plot indicates the distribution of SNPs under the null hypothesis.

Figure 2: Manhattan plot of combined cohort cluster headache (CH) association analysis for 1,443 CH cases and 6,748 controls highlighting genome-wide significant associations at chromosome 1, 2, and 6. The broken grey line is indicative of the threshold for genome wide significance.

Figure 3: Regional association plots using imputed SNP data. SNP positions, recombination rates, and gene boundaries are based on GRCh37/hg19. **A**. Showing the lead SNP rs113658130 (*p*-value = 1.92×10^{-17}) at chromosome 2 which overlies the long intergenic non-coding RNA *LINC01877*. **B.** Showing the lead SNP rs4519530, an intronic variant in *MERTK* (*p*-value = 6.98×10^{-17}). **C.** Showing the lead SNP rs11153082 (*p*-value = 1.85×10^{-8}) at chromosome 6 which overlaps *FHL5*. **D.** Showing chromosome 1q41 locus with the lead SNP rs12121134 (*p*-value = 1.66×10^{-8}).

Table Legends

Table 1: Summary of Lead SNPs at each locus associated with cluster headache.

Table 2: Expression Quantitative Trait Loci (eQTL) for genome wide significant SNPs at cluster headache susceptibility loci.

TABLE 1. Summary of Lead SNPs at each locus associated with cluster headache

Region	***LINC01705/DUSP10***	***MERTK***	***LINC01877 / SATB2***	***FHL5***
Chr	1q41	2q13	2q33	6q16
UK ANALYSIS				
rsID	rs6687758	rs4519530	rs113658130	rs9386670
Variant details	1:222164948: A/G	2:112759182: C/T	2:200504209: C/T	6:97060688: C/A
EA	G	C	C	A
Odds Ratio (95% CI)	1.27 (1.12-1.45)	1.39 (1.24-1.55)	1.63 (1.39-1.90)	1.40 (1.25-1.57)
***p*-value**	3.29×10^{-4}	2.49×10^{-8}	7.39×10^{-10}	1.41×10^{-8}
EAF Cases	0.23	0.67	0.76	0.38
EAF Controls	0.19	0.6	0.7	0.31
SWEDISH ANALYSIS				
rsID	rs6671564 ($r^2 = 0.09$ with rs6687758)	rs72825689 ($r^2 = 0.03$ with rs4519530)	rs4675692 ($r^2 = 0.6$ with rs113658130)	rs4098006 ($r^2 = 0.003$ with rs9386670)
Variant details	1:222014257: G/A	2:112790104: T/C	2:200449911: G/A	6:97084953: G/A
EA	A	C	G	A
Odds Ratio (95% CI)	1.39 (1.19-1.63)	2.82 (1.98-4.03)	1.61 (1.37-1.90)	1.28 (1.09-1.51)
***p*-value**	4.90×10^{-5}	1.07×10^{-8}	1.22×10^{-8}	2.72×10^{-3}
EAF Cases	0.50	0.08	0.67	0.50
EAF Controls	0.43	0.04	0.56	0.46
COMBINED ANALYSIS				
rsID	rs12121134 ($r^2 = 1$ with rs6687758)	rs4519530	rs113658130	rs11153082 ($r^2 = 0.98$ with rs9386670)
Variant details	1:222194880: C/T	2:112759182: C/T	2:200504209: C/T	6:97059666: A/G
EA	T	C	C	G
Odds Ratio (95% CI)	1.36 (1.22-1.52)	1.47 (1.34-1. 61)	1.51 (1.37-1.66)	1.30 (1.19-1.42)
***p*-value**	1.66×10^{-8}	6.98×10^{-17}	1.92×10^{-17}	1.85×10^{-8}
EAF Cases	0.25	0.71	0.75	0.39

EAF Controls	0.19	0.62	0.69	0.33

Table footnote: SNP = Single nucleotide polymorphism, Chr = Chromosome, rsID = reference SNP ID number, EA = Effect allele, EAF = Effect allele frequency, CI = Confidence interval.

TABLE 2. Expression Quantitative Trait Loci (eQTL) for genome wide significant SNPs at cluster headache susceptibility loci

Locus	eQTL	Position	rs number	Tissue	*P* value	FDR
Neurological eQTLs						
1q41	*DUSP10*	222219753	rs35718308	Tibial Nerve	4.89×10^{-6}	0.00496
2q13	*FBLN7*	112868426	rs10180018	Frontal Cortex	2.96×10^{-6}	6.66×10^{-4}
		112888335	rs67246870	Cortex	9.82×10^{-7}	6.11×10^{-7}
				Tibial Nerve	1.35×10^{-12}	3.40×10^{-11}
2q13	*MERTK*	112716930	rs72825612	Anterior cingulate cortex	2.06×10^{-6}	6.26×10^{-3}
				Caudate basal ganglia	8.41×10^{-13}	2.35×10^{-8}
				Cerebellar Hemisphere	4.64×10^{-6}	0.010
				Cortex	3.86×10^{-9}	3.21×10^{-5}
				Frontal Cortex	5.69×10^{-6}	0.019
				Hippocampus	4.30×10^{-8}	4.25×10^{-4}
				Nucleus accumbens basal ganglia	3.88×10^{-9}	3.08×10^{-5}
				Putamen basal ganglia	5.99×10^{-8}	4.15×10^{-4}
				Tibial Nerve	1.32×10^{-42}	8.83×10^{-36}
2q13	*RGPD8*	112715142	rs7569614	Tibial Nerve	2.66×10^{-6}	1.39×10^{-86}
2q13	*TMEM78B*	112775064	rs7422195	Tibial Nerve	1.77×10^{-11}	5.35×10^{-15}
2q13	*ZC3H8*	112715142	rs7569614	Tibial Nerve	3.15×10^{-5}	1.28×10^{-27}
2q13	*ZC3H6*	112743033	rs4441478	Frontal Corte x	4.57×10^{-5}	0.0242
2q33	*FTCDNL1*	199908378	rs994261	Tibial Nerve	2.80×10^{-4}	1.12×10^{-31}
6q16	*UFL1*	97060688	rs9386670	Cerebellum	1.40×10^{-6}	4.22×10^{-7}
Vascular eQTLs						
2q13	*FBLN7*	112888335	rs67246870	Aorta	1.79×10^{-15}	7.83×10^{-20}
				Tibial Artery	2.01×10^{-19}	2.92×10^{-60}
2q13	*MERTK*	112716930	rs72825612	Aorta	8.21×10^{-25}	1.50×10^{-19}
				Coronary Artery	1.06×10^{-9}	1.59×10^{-5}
				Tibial Artery	2.93×10^{-20}	1.93×10^{-15}
2q13	*RGPD8*	112715142	rs7569614	Aorta	1.02×10^{-9}	4.39×10^{-70}
				Tibial Artery	1.92×10^{-8}	1.31×10^{-87}
2q13	*TMEM87B*	112778713	rs4643544	Aorta	5.31×10^{-11}	3.59×10^{-7}
		112779732	rs72825667	Tibial Artery	1.77×10^{-8}	1.40×10^{-13}
2q13	*ZC3H8*	112715142	rs7569614	Tibial Artery	1.1×10^{-4}	1.22×10^{-23}
		112684371	rs4468823	Coronary Artery	2.28×10^{-5}	9.50×10^{-4}
2q33	*SATB2*	199824230	rs6759265	Tibial Artery	2.45×10^{-4}	0.0031
6q16	*UFL1*	97060688	rs9386670	Tibial Artery	8.53×10^{-13}	5.07×10^{-9}
Immune eQTLs						
1q41	*DUSP10*	222191505	rs17011200	Monocyte	2.80×10^{-6}	0.0140
2q13	*CHCHD5*	112784082	rs72825673	Platelet	2.91×10^{-6}	0.0146
2q13	*MERTK*	112781917	rs13419523	Monocyte	1.58×10^{-11}	7.90×10^{-8}
		112788095	rs13406390	Neutrophil	5.31×10^{-8}	2.65×10^{-4}
		112681991	rs72823490	Spleen	8.27×10^{-23}	1.34×10^{-17}

2q13	*RGPD8*	112673147	rs56822761	Monocyte	3.19×10^{-6}	0.020
				Neutrophil	6.69×10^{-8}	3.34×10^{-4}
		112659110	rs869016	Spleen	3.34×10^{-9}	1.90×10^{-35}
2q13	*TMEM87B*	112775064	rs7422195	Monocyte	2.23×10^{-6}	0.011
		112774105	rs56361454	T-cell	3.07×10^{-23}	1.54×10^{-19}
				Neutrophil	1.40×10^{-9}	7.02×10^{-6}
6q16	*UFL*	97060688	rs9386670	Neutrophil	4.07×10^{-16}	2.03×10^{-12}

Table footnote: eQTL= E x pression Quantitative Trait Loci, FDR = False Discovery Rate, Neurological, vascular and spleen eQTLs from the GTE x /v8 database, other Immunological eQTLs from the eQTL catalogue, DUSP10 = dual specificity phosphatase 10, FBLN7 = Fibulin 7, MERTK = MER proto-oncogene tyrosine kinase, RGPD8 = RANBP2 like and GRIP domain containing 8, TMEM87B = transmembrane protein 87B, ZC3H8 = zinc finger CCCH-type containing 8, ZC3H6 = zinc finger CCCH-type containing 3, FTCDNL1 = formiminotransferase cyclodeaminase N-terminal like, SATB2 = SATB homeobo x 2, UFL1 = UFM1 specific ligase 1, CHCHD5 = coiled-coil-heli x -coiled-coil-heli x domain containing 5.

Figure 1

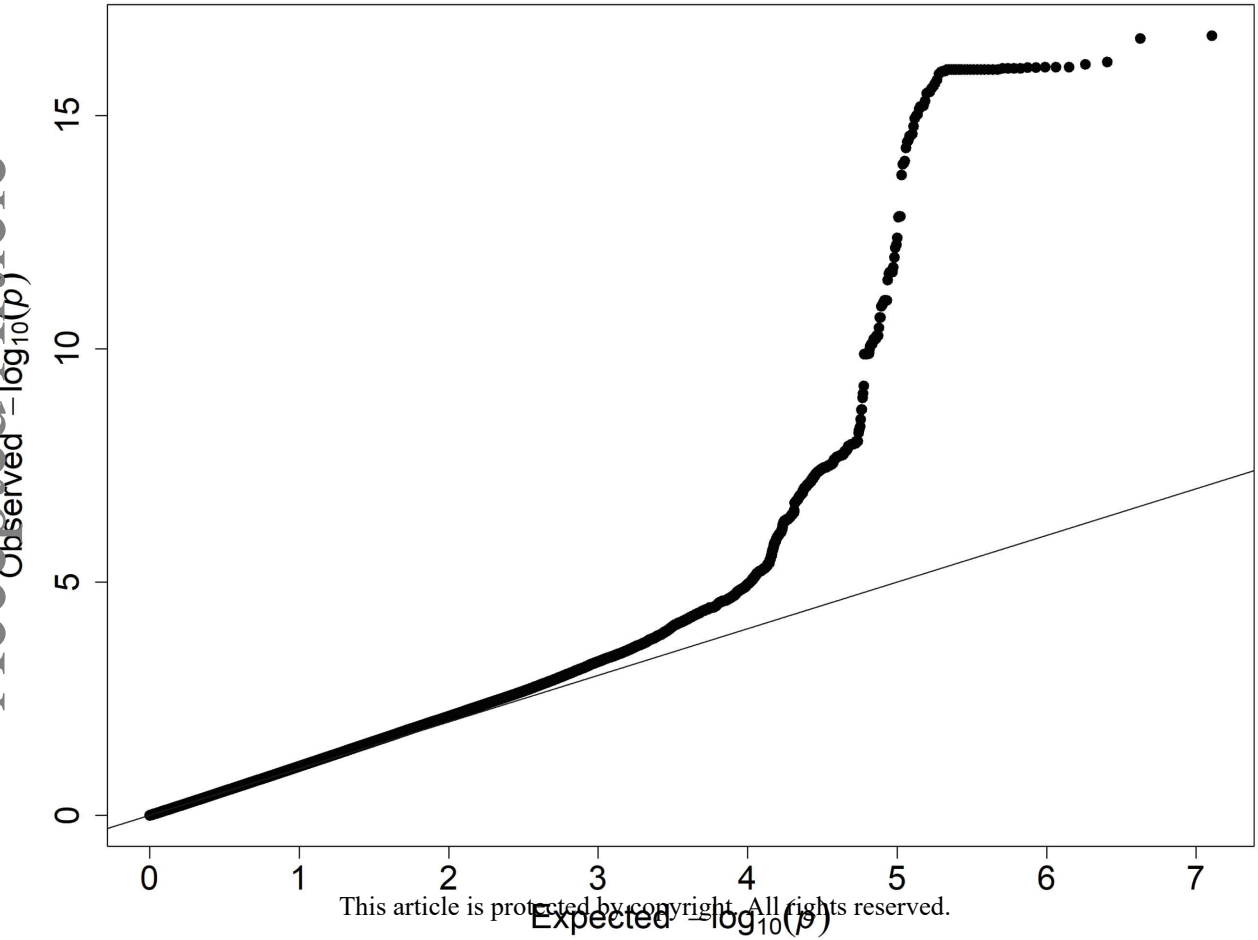

Figure 2

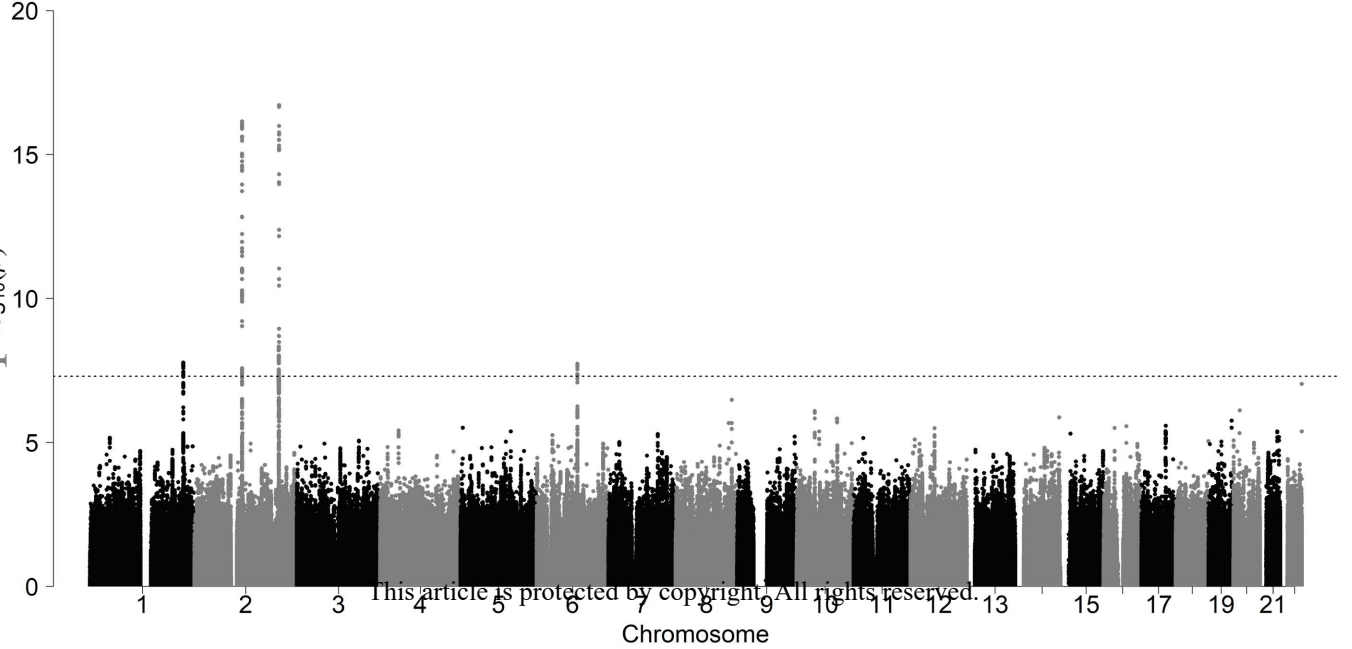

Figure 3