

Validation of genome-wide association susceptibility loci for cluster headache support a role for inflammation in the pathophysiology

Supplementary information

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Supplementary Table 1: Primers for gene expression, Pyro-sequencing and genotyping

Genes of interest	Chr	Forward primer 5'-3'	Reverse primer 3'-5'
<i>DUSP10</i>	1	CGAGTGAATGGGGGCTGAAT	CAGGAGGGTGGCTGTTACTG
<i>FTCDNL1</i>	2	GTCTTCTTCCAGAGTGGGGC	CAGCACCAGATCCTCAGCAA
<i>CAPN2</i>	2	GAGTCGCCCCGACCTTTCTC	GCCTCGTAGTCCTGGTTGAG
<i>FHL5</i>	6	CATTCTGCGTGGACTGCTAC	CGCAACTGAGTTCTGGCTTT
<i>UFL1</i>	6	AGCACGTATCCGTGGACTAT	GAAGCTGCTCCTGAAATCCA
<i>WNT2</i>	7	CTCCCTCTGCTCTTGACCTG	TTATCGCACATCACCCTGGA
<i>PLCE1</i>	10	GAGCTGCAATCGAAGTCTGG	AAGGCCTTCTGTGAGTCCTC
<i>LRP1</i>	12	GCATCCTGATTGAGCACCTG	GTTCACACGGATCACACTCG
House-keeping genes		Forward primer 5'-3'	Reverse primer 3'-5'
<i>TBP</i>	6	AGGCAACACAGGGAACCTC	TTGCAGCTGCGGTACAATCC
<i>IPO8</i>	12	ATTGGAAGAAACCGCGCTTG	TGTGTACACCTCCTGCAGTG
Pyro-sequencing primers		Forward primer 5'-3'	Reverse primer 3'-5'
<i>MERTK</i>	2	GTAGAAATGGGGTTTTATTATG TTGGTTAG Sequencing primer 5'-3' AGTGTTGGGATTATAGG	CAAAAATTAACCTCTATACTCCT TCTTATC
TaqMan		SNP	Assay ID
<i>MERTK</i>	2	rs2230515	C_27487306_10
<i>FHL5</i>	5	rs2273621	C_2810742_10
<i>PLCE1</i>	10	rs2274224	C_3137872_20

Chr = Chromosome, *DUSP-10* = Dual specificity phosphatase 10, *MERTK* = MER proto-oncogene tyrosine kinase, *FTCDNL1* = Formiminotransferase cyclodeaminase N-terminal like, *FHL5* = Four and a half LIM domains 5, *UFL1* = UFM1 specific ligase 1, *WNT2* = Wingless-type MMTV integration site family, member 2, *PLCE1* = Phospholipase C epsilon 1, *LRP1* = Low-density lipoprotein receptor-related protein 1, *CAPN2* = Calpain-2 catalytic subunit, *TBP* = TATA-binding protein, *IPO8* = Importin 8

Supplementary Table 2: Analysis of rs2273621, rs2274224 and rs2230515 in GWAS cohort

Gene	Variant	Allele	Frequency % (n)		OR (95% CI)	Adj P
			CH patients	Controls		
MERTK	rs2230515	G	71.42 (767)	60 (348)	0.6 (0.48–0.76)	0.000077
		A	28.58 (307)	40 (232)		
FHL5	rs2273621	A	61.71 (643)	66.84 (393)	1.28 (1.01–1.63)	0.12
		G	38.29 (399)	33.16 (195)		
PLCE1	rs2274224	G	65.13 (693)	61.19 (361)	0.92 (0.73–1.16)	< 1
		C	34.87 (371)	38.81 (229)		

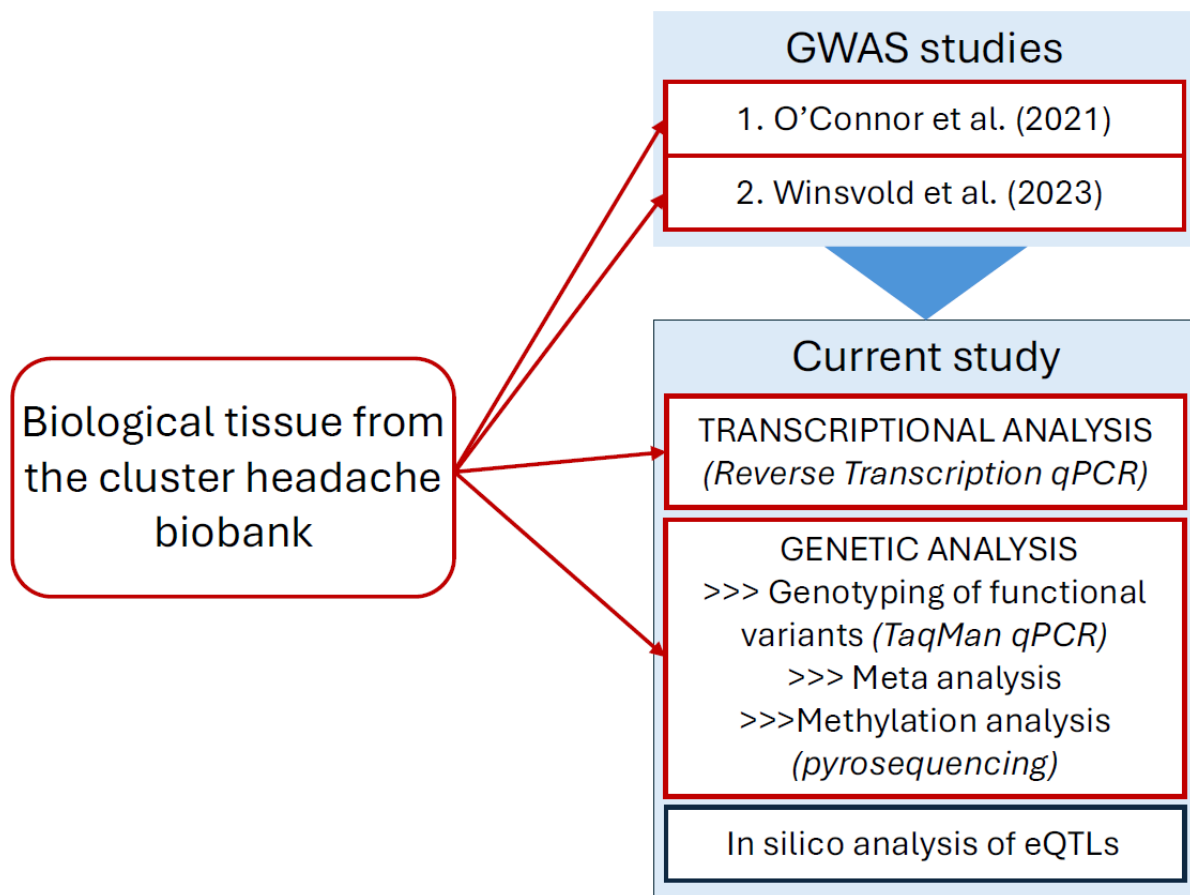
All three variants were in HWE: rs2230515 $P = 0.69$, rs2273621 $P = 0.6$ and rs2274224 $P < 1$. Logistic regression (additive model) with sex as covariate was utilised to analyse allele association. CH = cluster headache, OR = Odds ratio, CI = Confidence interval. Adj P = P-value adjusted for multiple testing using Bonferroni correction.

Supplementary Table 3: Allele frequencies of rs2273621, rs2274224 and rs2230515 in subgroups of the replication cohort

Gene	Variant	Allele	Allele frequency % (n)			
			CH all	CH confirmed	CH not confirmed	Controls
MERTK	rs2230515	G	67.79 (221)	69.31 (140)	65.32 (81)	62.87 (635)
		A	32.21 (105)	30.69 (62)	34.68 (43)	37.13 (375)
FHL5	rs2273621	A	59.26 (192)	58.41 (118)	60.66 (74)	66.36 (657)
		G	40.74 (132)	41.58 (84)	39.34 (48)	33.64 (333)
PLCE1	rs2274224	G	59.81 (195)	64.85 (131)	51.61 (64)	59.34 (591)
		C	40.18 (131)	35.15 (71)	48.39 (60)	40.66 (405)

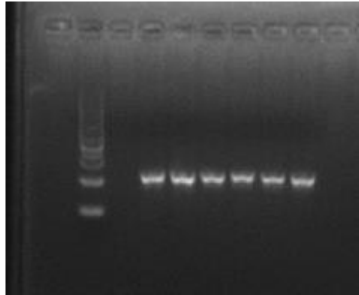
CH = cluster headache, confirmed = diagnosis confirmed by a neurologist, not confirmed = diagnosis validated by questionnaire data.

Supplementary Figure 1: Study design



Supplementary Figure 2: Methylation analysis by Pyrosequencing

A: PCR amplification

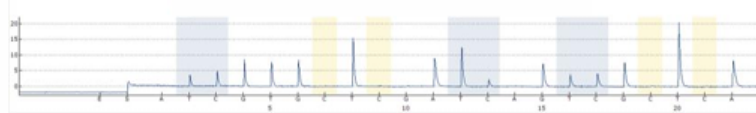


B: Pyro-methylation assay

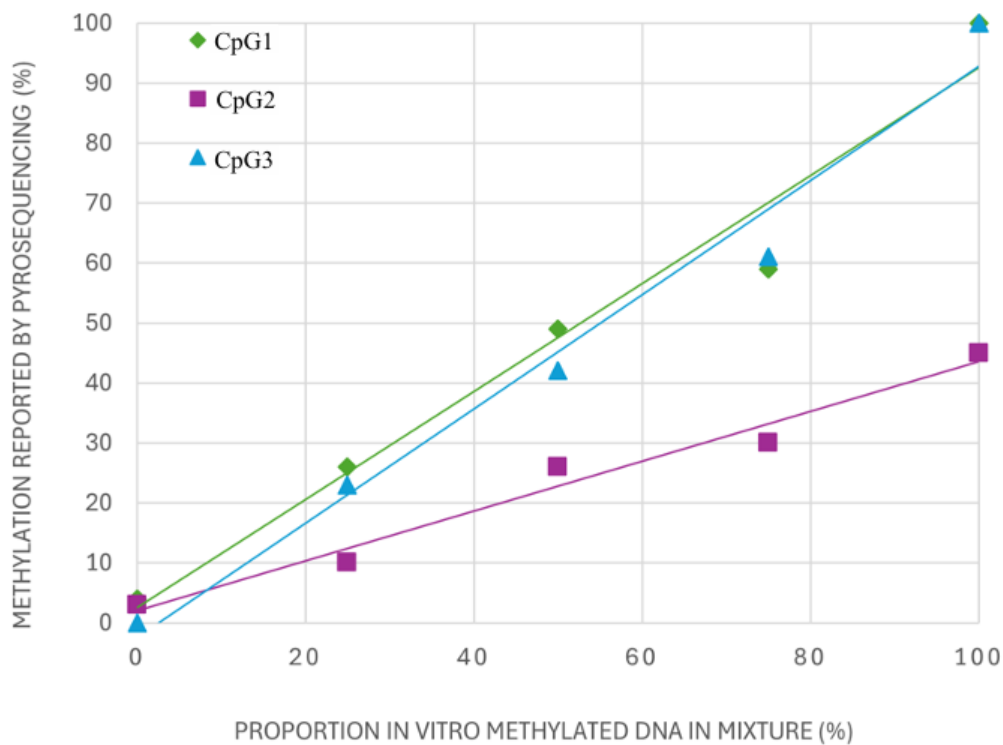
Histogram



PYROgram

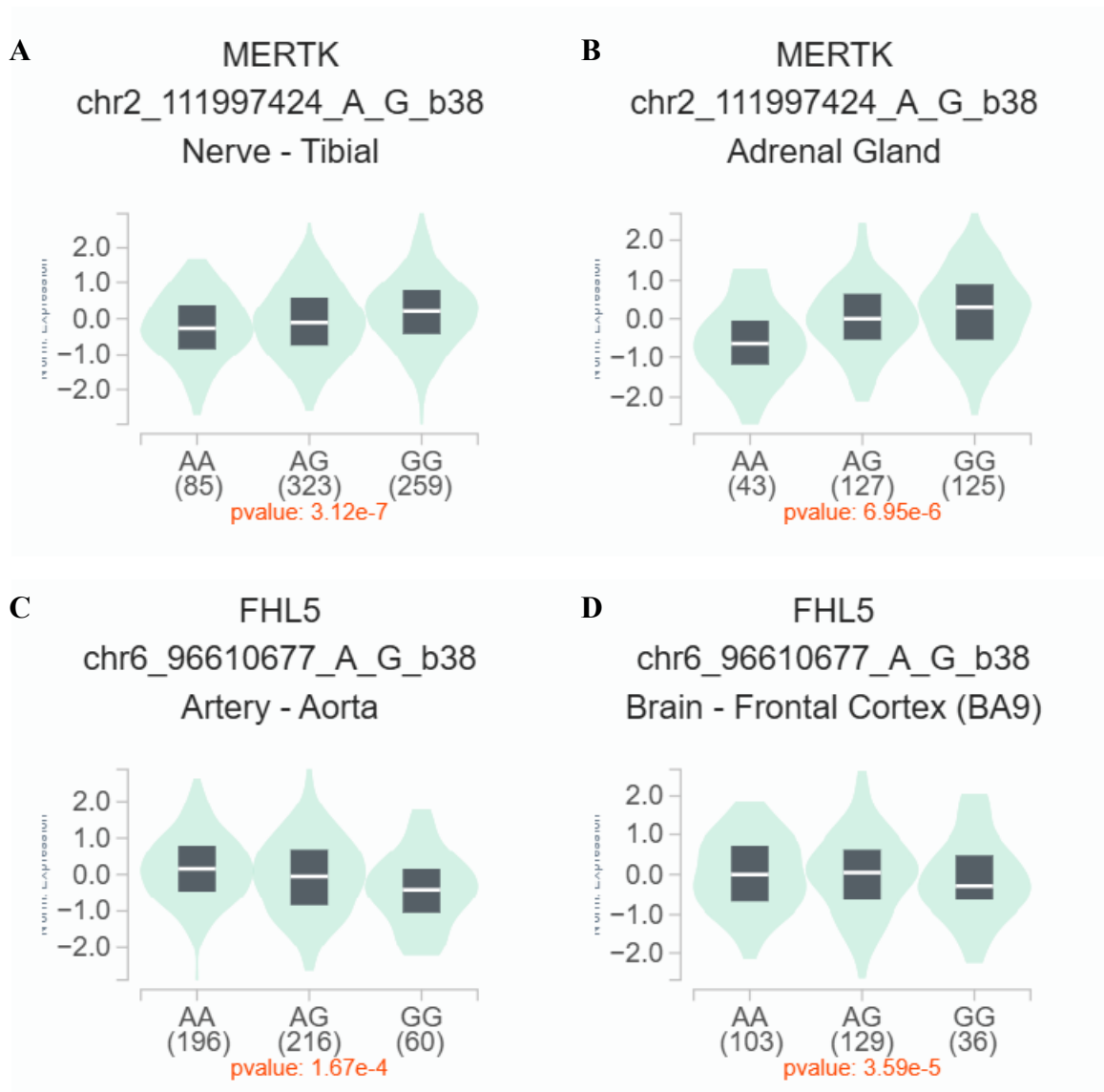


C: Methylation rate at each site



A: PCR product (219 bp) used to analyse methylation by Pyrosequencing. B: Methylation assay designed to capture the cg05979619 CpG site in the MERTK gene, position 112704577 on chromosome 2 (GRCh37 coordinates). The cg05979619 corresponds to Position 2 (CpG2) in the top panel (Histogram) and the bottom panel (example Pyrogram). C: Methylation assay test verifying a linear correlation for all three positions in test samples of methylated to unmethylated DNA at different ratios (0, 25, 50, 75 and 100 %).

Supplementary Figure 3: eQTL plots for rs2230515 and rs2273621



eQTL plots showing A-B: gene expression of MERTK (rs2230515) and C-D: FHL5 (rs2273621) showing that the risk allele in rs2230515 causes an increase of MERTK expression, while the risk allele of rs2273621 causes a decrease of FHL5 expression in different tissues. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS.¹ The data for this report was obtained from GTEx Analysis Release V10 (dbGaP Accession phs000424.v10.p2) on 06/04/2026.

1. Lonsdale J, Thomas J, Salvatore M, et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet.* 2013;45(6):580. doi:10.1038/NG.2653

Supplementary Figure 4: Correlation analysis of relative gene expression



Correlation analysis by pairwise comparisons between relative gene expression for each candidate gene in fibroblasts (A) and in blood (B), controls (C) in blue, cluster headache (CH) in pink. DUSP-10 = Dual specificity phosphatase 10, FTCDNL1 = Formiminotransferase cyclodeaminase N-terminal like, CAPN2 = Calpain-2 catalytic subunit, UFL1 = UFM1 specific ligase 1, WNT2 = Wingless-type MMTV integration site family, member 2, LRP1 = Low-density lipoprotein receptor-related protein 1.