**TITLE: Molecular genetic overlap between migraine and major depressive disorder**

**RUNNING TITLE: Genetic overlap between migraine and depression**

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**ABSTRACT**

Migraine and major depressive disorder (MDD) are common brain disorders that frequently co-occur. Despite epidemiological evidence that migraine and MDD share a genetic basis, their overlap at the molecular genetic level has not been thoroughly investigated. Using single nucleotide polymorphism (SNP) and gene-based analysis of genome-wide association study (GWAS) genotype data, we found significant genetic overlap across the two disorders. LD Score regression revealed a significant SNP-based heritability for both migraine (*h2* = 12%) and MDD (*h2* = 19%), and a significant cross-disorder genetic correlation (*rG* = 0.25; *P* = 0.04). Meta-analysis of results for 8,045,569 SNPs from a migraine GWAS (comprising 30,465 migraine cases and 143,147 control samples) and the top 10,000 SNPs from a MDD GWAS (comprising 75,607 MDD cases and 231,747 healthy controls), implicated three SNPs (rs146377178, rs672931, and rs11858956) with novel genome-wide significant association (*P*SNP ≤ 5 × 10‑8) to migraine and MDD. Moreover, gene-based association analyses revealed significant enrichment of genes nominally associated (*P*gene-based ≤ 0.05) with both migraine and MDD (*P*binomial-test = 0.001). Combining results across migraine and MDD, two genes, *ANKDD1B* and *KCNK5,* produced Fisher’s combined gene-based *P* values that surpassed the genome-wide significance threshold (*P*Fisher’s-combined ≤ 3.6 × 10‑6). Pathway analysis of genes with *P*Fisher’s-combined ≤ 1 × 10‑3 suggested several pathways, foremost neural-related pathways of signalling and ion channel regulation, to be involved in migraine and MDD etiology. In conclusion, our study provides strong molecular genetic support for shared genetically determined biological mechanisms underlying migraine and MDD.

**Keywords:**

migraine, depression, GWAS, meta-analysis, genetic correlation, shared genetics.

**INTRODUCTION**

Migraine and major depressive disorder (MDD) are among the most common neurological disorders, each affecting approximately 10–20% of the population with more females than males affected.[1-3](#_ENREF_1) Bidirectional comorbidity between migraine and MDD is widely recognised;[4-8](#_ENREF_4)it highly reduces the quality of life of patients with a huge impact on relatives and society.[9](#_ENREF_9) As epidemiological studies have revealed a moderate heritability for migraine and MDD with estimates ranging from 30–50%,[10](#_ENREF_10),[11](#_ENREF_11) shared genetic factors may underlie these disorders,[12-14](#_ENREF_12)although molecular evidence for such an association is lacking.

A number of genome-wide association studies (GWAS) have been conducted separately for migraine[15-21](#_ENREF_15) and MDD[22-33](#_ENREF_22). For both disorders an increasing number of loci have been identified. The largest, latest, migraine GWAS analysed 59,674 cases and 316,078 healthy controls and identified 38 genome-wide significant (*P*SNP ≤ 5 × 10‑8) loci containing 44 independent single nucleotide polymorphisms (SNPs) associated with migraine risk.[21](#_ENREF_21) While the largest, latest, MDD GWAS analysed a combined 130,620 self-reported and clinically evaluated lifetime major depression cases and 347,620 controls identified 15 genome-wide significant loci, containing 17 independent SNPs associated with MDD risk.[33](#_ENREF_33) Comparison of the genome-wide significant loci between the two disorders yielded no shared loci. It remains however of interest to investigate whether signals of other SNPs, below the threshold for genome-wide significance, reveal molecular genetic overlap between migraine and MDD.

Compared to epidemiological studies, analysing GWAS SNP data provides an opportunity to test for genetic overlap between migraine and MDD at the molecular genetic level and can yield genetic risk variants associated with *both* migraine and MDD. Also, extending the genetic overlap analysis from the SNP-level to the gene-level—given that genes are the predominant functional unit of the human genome and more closely related to biology than individual SNPs—can (i) provide novel evidence on the genetic association between migraine and MDD; (ii) give insight into shared biological pathways underlying the two disorders; and (iii) help identify target genes for drug development. Moreover, the identification of genetic overlap and specific genetic variants shared across disorders can be used to assess the validity of the clinical diagnosis and classification of patients.

Here we examined the genetic overlap across migraine and MDD by (i) evaluating SNP-based genetic overlap utilising LD (linkage disequilibrium) Score regression (LDSC) and SNP effect concordance analysis (SECA) using genome-wide summary statistics from the 2016 International Headache Genetics Consortium (IHGC) migraine and 2013 Psychiatric Genomics Consortium (PGC) MDD GWAS, and the ‘top’ 10,000 most significant SNP results from the 2016 23andMe MDD GWAS; (ii) identifying genetic risk variants associated with both migraine and MDD by meta-analysis of 2016 IHGC migraine and 2016 23andMe MDD GWAS results; (iii) evaluating gene-level genetic overlap across migraine and MDD to identify genes associated with migraine and MDD using gene-based association analysis of summary statistics from the 2016 IHGC migraine and 2013 PGC MDD GWAS; and (iv) exploring the biological pathways represented by the genes showing association to migraine and MDD.

**MATERIALS AND METHODS**

### Study samples

***2016 IHGC migraine GWAS***

The 2016 IHGC (<http://www.headachegenetics.org/>) migraine GWAS sample is comprised of 59,674 migraine cases and 316,078 healthy controls;[21](#_ENREF_21) all participants were of European ancestry. Migraine phenotypes were diagnosed by self-reported questionnaires or clinical interviews according to the International Classification of Headache Disorders (ICHD) criteria.[34](#_ENREF_34) Subjects in each individual GWAS had their specific standard genotyping platform and quality control criteria, which were summarised elsewhere;[21](#_ENREF_21) all subjects were imputed using the 1000 Genomes Project[35](#_ENREF_35) reference panel (Phase I, v3 release or later). Each individual GWAS also performed their association analysis independently, adjusted for sex and the top ten principal components to account for potential population stratification where required.[21](#_ENREF_21)A combined fixed-effect (FE) meta-analysis was then performed using the Genome-wide Association Meta-Analysis (GWAMA) program.[36](#_ENREF_36)After SNP filtering, the final 2016 IHGC migraine GWAS included association results for 8,045,569 SNPs. For more detailed descriptions of the migraine cohorts and statistical analyses, please refer to the original publication.[21](#_ENREF_21) Here, we utilised the GWAMA output after excluding results from the 23andMe GWAS sample (30,465 migraine cases and 143,147 controls), leaving a total migraine GWAS sample of 29,209 cases and 172,931 controls, to ensure there was no sample overlap between 2016 IHGC migraine GWAS and the 2016 23andMe MDD GWAS.

#### *2013 PGC MDD GWAS*

The 2013 PGC (<http://pgc.unc.edu>) MDD GWAS sample comprised 18,759 unrelated participants of European ancestry (9,240 MDD cases and 9,519 healthy controls) from nine MDD GWA case-control samples.[23](#_ENREF_23) All MDD cases were diagnosed by a structured clinical interview or clinical-based checklist according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria.[37](#_ENREF_37)The PGC performed a mega-analysis, which required centralising the genotype data from all GWA samples prior to performing consistent QC, imputation and association analysis. Individual genotypes were all imputed up to the CEU (Utah Residents with Northern and Western European Ancestry) and TSI (Toscani in Italy) HapMap3 reference panel. Association analysis was carried out using a logistic regression assuming an additive SNP effect (allelic association) model. The final 2013 PGC MDD GWAS comprised results for 1,232,794 SNPs.

***2016 23andMe MDD GWAS***

The 2016 23andMe MDD discovery GWAS sample[33](#_ENREF_33) recruited 307,354 subjects of European ancestry, including 75,607 self-reported MDD cases and 231,747 healthy controls. Subjects were systematically genotyped, QCd, and imputed using the 1000 Genomes Project[35](#_ENREF_35) Phase I reference panel. Under the assumption of additive allelic effects, GWA analysis was performed using logistic regression adjusted for age, sex, and the top five ancestry principal components. After removing SNPs with low quality imputation, 13,519,496 SNPs were included in the discovery GWAS; and only the top 10,000 most significant SNPs (<http://www.nature.com/ng/journal/v48/n9/full/ng.3623.html#supplementary-information>) with *P* ≤ 1 × 10-5 were available for download and used in our study.

In addition to the above details and original publications describing the GWAS summary statistics analysed in our study―including URLs from where the data can be obtained online―details and data from the analysed datasets are available from the GWAS Central database (<http://www.gwascentral.org/study/HGVST1855>).

**Genetic analyses**

***LD Score regression to evaluate genetic similarity***

LD Scores were calculated according to the European 1000 Genomes Project haplotype reference data (Phase I, v3). LD Score regression was performed using the LDSC software (<https://github.com/bulik/ldsc>).[38](#_ENREF_38)GWAS summary statistics from the 2016 IHGC migraine GWAS and the 2013 PGC MDD GWAS were utilised in this analysis. GWAS data were reformatted and harmonised utilising the “munge\_sumstats.py” script, based on the SNP list used in LD score calculation. As per the LDSC manual, SNPs were removed if they were not present in the relevant reference data, had a rare frequency (minor allele frequency [MAF] ≤ 0.01), were poorly imputed (INFO score ≤ 0.90) or strand-ambiguous. We first performed single-trait LD Score regression to evaluate the SNP-based liability heritability (*h*2SNP) for the 2016 IHGC migraine and 2013 PGC MDD GWAS, using their sample prevalence of 14.5% for migraine and 49.3% for MDD, and a population prevalence of 15% for both migraine and MDD; and then built a cross-trait LD Score regression to estimate the genetic correlation (*r*G) between migraine and MDD.

***SECA analysis to evaluate genetic overlap***

Whereas LD Score regression requires GWAS results for millions of SNPs spread evenly across the genome, SECA is able to assess genetic overlap for a subset of SNPs.[39](#_ENREF_39)Since only the top 10,000 most significant SNPs (*P* value ≤ 1 × 10-5) were available for the 2016 23andMe MDD GWAS, we utilised SECA to examine the genetic overlap between the 2016 IHGC migraine GWAS and 2016 23andMe MDD GWAS. SECA first aligned the SNP effects across the two GWA study summary results to the same effect allele, and then extracted a subset of independent SNPs via ‘*P* value informed’ linkage disequilibrium (LD) clumping. The approach iterated from the first to last SNP on each chromosome sorted from smallest to largest 2016 IHGC migraine GWAS *P* value that had not already been clumped (denoting this as the ‘index’ SNP) and formed clumps of all other SNPs that are within 1 Mb and in LD (*r*2 > 0.1, based on 1000G PhaseI v3 CEU genotype data) with the index SNP. A second round of LD clumping was performed to clump any of the round 1 index SNPs within 10 Mb of each other to account for long-range LD (*r*2 > 0.1). The approach identified the subset of independent (index) SNPs with the most significant association *P* values in the 2016 IHGC migraine GWAS. After subgrouping SNPs with *P* value thresholds *P* ≤ {0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0} in the 2016 IHGC migraine GWAS, two-sided exact binomial tests were performed to assess the concordance of SNP effect directions across the 2016 IHGC migraine GWAS and 2016 23andMe MDD GWAS results.

***Meta-analysis of migraine and MDD***

Meta-analysis of the 2016 IHGC migraine GWAS and 2016 23andMe MDD GWAS (for the top 10,000 SNPs) was performed using the METASOFT (URL: <http://genetics.cs.ucla.edu/meta/>) inverse variance‒weighted FE model,[40](#_ENREF_40)where the SNP effect size estimates (*β*) are weighted by their estimated standard errors (*se*) to calculate a meta-analysis *P* value. To allow for the presence of effect heterogeneity across studies, the METASOFT Han and Eskin’s random-effects (RE2) model[40](#_ENREF_40) was also utilised, as it is optimized to detect associations under heterogeneity. A total of 8,687 SNPs in both the 2016 IHGC migraine and 2016 23andMe MDD GWAS were included in the meta-analysis. Following the meta-analysis, we calculated the LD (*r2*) between the implicated SNPs (*P*SNP ≤ 5 × 10‑8) at each locus using PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>)[41](#_ENREF_41) and the European 1000 Genomes Project haplotype reference data (Phase I, v3).

***Gene-based analysis to evaluate genetic overlap***

#### A gene-based approach[42](#_ENREF_42) was also used to evaluate the genetic overlap across the 2016 IHGC migraine GWAS and 2013 PGC MDD GWAS. After downloading RefSeq gene information (hg19) from the UCSC genome browser (accessed 20 March 2014), overlapping isoforms of the same gene were combined to form a single full-length version of the gene, while isoforms that did not overlap were left as duplicates of that gene. This led to 23,438 unique genes. The common SNPs from the 2016 IHGC migraine and 2013 PGC MDD GWAS were then assigned to genes if they mapped to between 15 kb 5’ of the transcription start site (TSS) and 15 kb 3’ of the transcription end site (TES). This 15-kb gene boundary extension was chosen based on the observation that 90% of SNPs effecting expression quantitative trait loci (eQTLs) are within this proximity.[43](#_ENREF_43)Gene-based association tests were performed using the GATES test[44](#_ENREF_44) implemented in the Fast ASsociation Tests (FAST) package.[45](#_ENREF_45)GATES performs gene-based tests by adjusting the observed *P* value of the most significant SNP assigned to a gene by the total effective number of independent SNPs tested across the gene. GATES performs eigenvalue analysis of the *n*×*n* SNP correlation matrix (estimated from the 1000 Genomes Project [released on May 2012] CEU reference population) for each gene to estimate the effective number of independent SNPs. The original report introducing the GATES gene-based test used computer simulation to demonstrate that the test offers effective control of the type 1 error rate regardless of gene size and LD pattern among SNPs, and does not need permutation or simulation to validate significance.[44](#_ENREF_44)

#### Given that gene-based association results may be correlated across neighbouring genes due to LD between the most significant SNP assigned to each gene, we estimated the effective number of independent genes (i.e., number of independent gene-based tests) by examining the LD between the top (most) significant SNP assigned to each gene. This calculation was performed using the Genetic type I Error Calculator (GEC).[46](#_ENREF_46)The GEC approach first divides the input SNPs into LD blocks, and assumes LD blocks are independent by ensuring the SNPs between blocks are not in LD (*r*2 < 0.1). GEC subsequently performs eigenvalue analysis of the correlation matrix for each LD block to estimate the effective number of independent SNPs. In the original report introducing the GEC,[46](#_ENREF_46)computer simulation and permutation was used to demonstrate that using the GEC estimate of the effective number of independent SNPs in a Bonferroni procedure yields correct type I error rates and behaves similarly to the gold standard of permutation.

#### To test genetic overlap, we first generated gene sets for each disorder based on three levels of significance (i.e., gene-based *P* value ≤ 0.01, 0.05, or 0.1) to allow for differences in power across the different GWA studies, and then calculated the effective number of independent genes per disorder. Next, we set the 2016 IHGC migraine GWAS as the ‘discovery’ dataset and 2013 PGC MDD GWAS as the ‘target’ dataset to test for genetic overlap according to three *P* value significance levels (e.g., test whether the proportion of genes with a gene-based *P* value ≤ 0.05 for both migraine and MDD was more than expected by chance). The observed number of overlapping genes was defined as the effective number of genes with *P* values less than the threshold in both the discovery and target datasets. The observed proportion of overlapping genes was the observed effective number of overlapping genes divided by the effective number of genes with a *P* value less than the threshold in the discovery dataset. The expected proportion of overlapping genes was the effective number of genes with a *P* value less than the threshold in the target dataset divided by the total effective number of genes in the target dataset. The statistical significance of whether the number of overlapping genes was more than expected by chance was calculated using one-sided exact binomial tests. Moreover, to identify the individual genes associated across migraine and MDD, we combined gene-based evidence for association across the two disorders using the Fisher’s combined *P* value approach. This gene-based approach was recently utilised to show gene-based pleiotropy across migraine with aura and migraine without aura,[42](#_ENREF_42) as well as the five major disorders in the PGC: attention deficit hyperactivity disorder, autism spectrum disorder, bipolar disorder, MDD, and schizophrenia.[47](#_ENREF_47)

***Pathway analysis of overlapping genes***

To discover shared biological pathways underlying migraine and MDD, we performed a pathway analysis of the significant overlapping genes from the gene-based analysis using the g:GOSt tool of the g:Profiler web server (<http://biit.cs.ut.ee/gprofiler/>).[48](#_ENREF_48)The overlapping genes with Fisher’s combined *P* value lower than 1 × 10-3 were selected[49](#_ENREF_49) and evaluated using the g:Profiler web server. The g:GOSt tool can identify significantly enriched pathways through different functional databases including Gene Ontology (GO)[50](#_ENREF_50) (biological process, cellular component and molecular function), Kyoto Encyclopedia of Genes and Genomes (KEGG),[51](#_ENREF_51)and Reactome.[52](#_ENREF_52)Further advanced options are also available for term filtering, including the functional category size thresholds for limiting enrichment analyses and the significance threshold for multiple testing (e.g., Benjamini-Hochberg False Discovery Rate [FDR], and Bonferroni correction). For our analyses, no size boundaries were set for functional category and term intersection; Benjamini-Hochberg FDR was utilised for multiple testing correction; and other advanced options were kept as their default. Analyses were first run without including then run including electronic GO annotations. Lastly, given such pathway analyses may be biased in the presence of strong LD across neighbouring genes, we ensured the enriched pathways did not contain genes with top significant SNPs in LD (*r*2 > 0.1).

**RESULTS**

**LD Score-based genetic correlation between migraine and MDD**

As summarised in Table 1, using LD Score regression with no intercept constraining, we observed a significant SNP-based liability-scale heritability of 12% (95% CI: 9–15%) for 2016 IHGC migraine, and 19% (95% CI: 12–26%) for 2013 PGC MDD. For cross-trait analysis, a significant positive genetic correlation (*rG*) of 0.25 (95% CI: 0.01–0.48) was estimated between the 2016 IHGC migraine and 2013 PGC MDD GWAS.

**SECA-based genetic concordance between migraine and MDD**

SECA revealed a significant genetic concordance between genetic risk factors (SNP risk alleles) for 2016 IHGC migraine and 2016 23andMe MDD. For instance, the SNP effect concordance between migraine and MDD is considerable given that of the 358 independent SNPs with the smallest *P* values in the 2016 IHGC migraine GWAS, the risk increasing allele for MDD and migraine was the same for 202 (56.42%, two-sided binomial test *P* = 0.017). The SNP effect concordance was further enriched (by 31%) in the subset of independent SNPs with nominal MDD association (*P* ≤ 0.05), with 34 (73.91%) out of 46 independent SNPs having the same risk increasing allele for migraine and MDD (two-sided binomial test *P* = 0.0016). SNP effect concordance results for all 12 analysed *P* value thresholds are provided in Table 2.

**Genetic risk variants associated with both migraine and MDD**

A total of 683,106 participants were included in the meta-analysis of 2016 IHGC migraine and 2016 23andMe MDD GWAS. In total, 542 SNPs at 9 genomic loci produced evidence for genome-wide significant association (*P*SNP ≤ 5 × 10-8) based on the FE model (Supplementary Table 1 contains meta-analysis results for the 542 SNPs using both the FE and RE2 model). After examining LD between the most significant (‘top’), or ‘index’, risk SNPs, 9 independent SNPs were identified (Table 3).

Among these 9 independent SNPs, 5 of them (rs12127789 hg19.chr1:g.72740073G>T, rs2195636 hg19.chr3:g.158352440C>T, rs768705 hg19.chr5:g.87568710A>G, rs9536359 hg19.chr13:g.53691446C>T and rs5751069 hg19.chr22:g.41627775C>G) presented a significantly stronger association with MDD (either the SNP showed a genome-wide significant association or the SNP was in LD with a genome-wide significant SNP) compared to migraine, indicating that these SNPs are predominantly driven by the association signal in the 2016 23andMe MDD GWAS. However, one SNP (rs6476606 hg19.chr9:g.37005561A>G) showed near *suggestive* association (*P*SNP ≤ 1 × 10-5) with both MDD (*P*SNP = 1.5 × 10-5) and migraine (*P*SNP = 4 × 10-4), and was genome-wide significantly associated with MDD (*P*SNP = 1.2 × 10-8) in the published joint analysis of 23andMe discovery, PGC, and 23andMe replication MDD GWAS.[33](#_ENREF_33) Thus providing an ideal example where combining MDD GWAS and migraine GWAS results can improve power to identify risk loci for MDD. The remaining 3 SNPs (rs146377178 hg19.chr8:g.25386973C>T, rs672931 hg19.chr11:g.30920897T>C, and rs11858956 hg19.chr15:g.70261228T>C) showed *suggestive* association with MDD (and are not in LD [*r2* < 0.1] with genome-wide significant SNPs) also showed association with migraine (*P*SNP ≤ 0.005), and are novel genome-wide significant risk loci. SNP rs146377178 is located between *CDCA2* and *EBF2* on chromosome 8p21.2, rs672931 is located within *DCDC5* on 11p14.1, and rs11858956 is located between *RPLP1* and *TLE3* on 15q23.

**Gene-based genetic overlap between migraine and MDD**

As summarised in Table 4, similar to the SNP-based analysis, a significant gene-level genetic overlap between migraine and MDD was observed. For instance, a significant proportion of genes with gene-based *P* value ≤ 0.05 overlapped between migraine and MDD (*P*binomial-test = 0.001): the observed proportion (8.7%) of genes with a gene-based *P* value ≤ 0.05 in both migraine and MDD is significantly higher than the expected proportion (6.5%) of genes with *P* value ≤ 0.05 for MDD. Furthermore, the use of a less stringent gene-based *P* value threshold ≤ 0.1 produced more significant genetic overlap across two disorders (*P*binomial-test = 2.60 × 10-6), while the use of a more stringent gene-based *P* value threshold ≤ 0.01 produced fewer significant genetic overlap (*P*binomial-test = 0.045).

Combining gene-based evidence for association across the disorders (2016 IHGC migraine and 2013 PGC MDD) using Fisher’s combined test (Table 5), two genes were identified with combined *P* values that are below the genome-wide significance threshold adjusted for 13,524 independent gene-based tests (3.7 × 10-6 = 0.05/13,524), namely *ANKDD1B* on chromosome 5q13.3 and *KCNK5* on 6p21.1. Interestingly, the effect alleles of the top SNPs driving the *ANKDD1B* gene-based association (rs34358 hg19.chr5:g.74965122G>A for migraine and rs904743 hg19.chr5:g.74917862A>G for MDD) and the *KCNK5* association (rs9394578 hg19.chr6:g.39165859C>A for migraine and rs2815095 hg19.chr6:g.39156108T>C for MDD) had opposite effects on risk for migraine and MDD, and were in weak LD (*r2* = 0.24 and 0.39, respectively), providing evidence for allelic heterogeneity at these associated genes.

**Pathway analysis of the overlapping genes**

Pathway analysis was performed for a total of 86 overlapping genes with *P*Fisher’s-combined ≤ 1 × 10-3 (see Supplementary Table 2). After excluding pathways having common genes with top significant SNPs in LD *r2* > 0.1, 39 pathways with 21 genes were enriched (FDR ≤ 0.05) with at least one annotation of a human gene (summarised in Table 6), from which 10 pathways with 12 genes showed at least two human gene annotations.

Among these pathway terms, neural-related pathways were substantially over-represented, especially for pathways related to signalling (e.g., “Wnt signalling pathway”; KEGG:04310; *PLCB3*, *SERPINF1* and *DVL3*; FDR *P* value = 0.02) and the ion channel regulations (e.g., “potassium ion leak channel activity”; GO:0022841; *KCNK5* and *KCNK4*; FDR *P* value = 0.02), both of which were also strongly dominant in the pathways with at least two human gene annotations. Enzymatic activity-related pathways (e.g., “serine O-acyltransferase activity”; GO:0016412; *MBOAT4*; FDR *P* value = 0.05) and metabolic pathways (e.g., “arsonoacetate metabolic process”; GO:0018872; *AS3MT*; FDR *P* value = 0.05) were also observed to be enriched in migraine and/or MDD etiology. Notably, only one term “endocrine and other factor-regulated calcium reabsorption” (KEGG:04961; *PLCB3* and *AP2M1*; Bonferroni corrected *P* value = 0.05) remained after using more conservative Bonferroni correction for multiple testing, which is related to processes of intracellular signalling and neuronal excitability.[53](#_ENREF_53)

Extending pathway analyses to also include functional annotations of GO assigned by *in silico* curation methods (Inferred from Electronic Annotation [IEA]) provided further evidence for the molecular signalling-related pathways involved in migraine and/or MDD etiology, with 38 additional pathways (see Supplementary Table 3) represented by 11 genes (*ECM1,* *DLST*, *TMEM208*, *PLXNB1*, *RNF113B*, *FARP1*, *CLEC17A*, *GPR126*, *CENPH*, *GRK6*, and *TFB1M*). Importantly, this analysis highlighted seven pathways with at least two human gene annotations: “regulation of release of cytochrome c from mitochondria” (GO:0090199; *NOL3* and *BAD*; FDR *P* value = 0.03); “negative regulation of peptidase activity” (GO:0010466; *NOL3*, *ECM1*, *NGF* and *SERPINF1*; FDR *P* value = 0.03); “negative regulation of cytokine-mediated signaling pathway” (GO:0001960; *NOL3* and *ECM1*; FDR *P* value = 0.03); “Rac GTPase binding” (GO:0048365; *DVL3* and *FARP1*; FDR *P* value = 0.03); “cysteine-type endopeptidase regulator activity involved in apoptotic process” (GO:0043028; *NOL3* and *BAD*; FDR *P* value = 0.03); “extracellular matrix binding” (GO:0050840; *ECM1* and *GPR126*; FDR *P* value = 0.05); and “death receptor binding” (GO:0005123, *NOL3* and *NGF*; FDR *P* value = 0.05).

**DISCUSSION**

Here we performed a comprehensive analysis to assess the genetic overlap between migraine and MDD using three GWAS data sets, which is the first systematic study aimed at identifying shared genetic factors between migraine and MDD at the molecular genetic level. Several interesting findings are noteworthy.

Firstly, we estimated a significant SNP-based liability-scale heritability of 12% using the 2016 IHGC migraine GWAS data set and 19% using the 2013 PGC MDD GWAS data set. The SNP-based heritability estimates of migraine and MDD are lower than those estimated from twin and family studies.[10](#_ENREF_10),[11](#_ENREF_11)This so-called “missing heritability” is likely due to the combined effects of rare SNPs and SNPs with small effects that are difficult to capture using current GWAS sample sizes and analysis of common SNPs.[54](#_ENREF_54),[55](#_ENREF_55)

Our study reported a significant SNP-based *rG* of 0.25 between migraine (2016 IHGC) and MDD (2013 PGC), which is similar to estimates (*rG* = 0.30–0.36) from twin and family studies.[13](#_ENREF_13),[14](#_ENREF_14) Although the *rG* between 2016 IHGC migraine and 2016 23andMe MDD could not be assessed via LD Score regression (due to the restricted availability of genome-wide results for the 23andMe MDD GWAS), our SECA results provided strong evidence for a significant genetic overlap, indicated by the significant enrichment in concordant SNP effects across the 2016 IHGC migraine and 2016 23andMe MDD GWAS.

Given the strong evidence for shared genetic factors, we performed a meta-analysis of the 2016 IHGC migraine and 2016 23andMe MDD GWA studies. No substantial difference was observed when comparing results of the FE and the RE2 models (Supplementary Table 1), indicating negligible SNP effect heterogeneity across studies. The meta-analysis identified 3 novel (index) SNP loci near several genes: rs146377178 between *CDCA2* and *EBF2*, rs672931 within *DCDC5*, and rs11858956 between *RPLP1* and *TLE3*. Whereas little evidence exists that supports a biological role in migraine or MDD risk for *DCDC5* and *TLE3*, at least some evidence is reported in the literature for the other three genes. Specifically, *CDCA2*, which is related to cell division cycle, was previously observed to be involved in the overlapping pathways across migraine with aura and migraine without aura;[42](#_ENREF_42)*EBF2* is reported to play a role in regulating dopaminergic neurons in the midbrain periaqueductal grey matter, which is relevant to pain modulation,[56](#_ENREF_56)and therefore may contribute to both migraine and MDD risk; and *RPLP1* was revealed to be related to MDD in a mouse model, suggesting that the ribosome pathways of proteins synthesis/degradation were implicated in MDD etiology.[57](#_ENREF_57)

One SNP (rs6476606) showed association in both the 2016 IHGC migraine GWAS (*P*SNP = 0.0003) and 2016 23andMe MDD discovery GWAS (*P*SNP = 1.50 × 10-5), with genome-wide significant evidence for association in the FE meta-analysis (*P*SNP = 2.52 × 10-8), and genome-wide significant association with MDD in the joint analysis of 23andMe discovery, PGC, and 23andMe replication MDD GWAS (*P*SNP = 1.2 × 10-8).[33](#_ENREF_33) This indicates that combining migraine and MDD GWAS data has the potential to identify robust MDD risk loci. Interestingly, this finding is in line with previous results suggesting that in at least a subset of migraine patients with MDD, migraine may be a symptom or consequence of MDD.[58](#_ENREF_58)Further research will be required to determine whether combining migraine and MDD GWAS data can help to identify robust migraine risk loci (e.g., utilising genome-wide results from more powerful MDD GWAS).

Extending our analysis from SNP-level to gene-level revealed a significant genetic overlap across migraine and MDD, providing additional evidence for such overlap (‘pleiotropy’) between the disorders. Application of Fisher’s combined test identified two genes with genome-wide significant gene-based *P* values (*ANKDD1B* and *KCNK5*). Although minimal data exists for *ANKDD1B*, it may be relevant to migraine and MDD susceptibility due to its role in coding ankyrin-repeat proteins, which have been associated with a number of human disorders, and include the Notch protein (a key component of cell signalling pathways) in which mutations can cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)―for which the most common clinical manifestations are migraine headaches and transient ischemic attacks.[59](#_ENREF_59)In contrast, the two-pore forming potassium channel gene *KCNK5* is an attractive candidate for disorders of the central nervous system and other members of this protein family have already been linked to migraine or MDD susceptibility. For instance, although the genetic evidence has been debated,[60](#_ENREF_60),[61](#_ENREF_61)the TWIK-related spinal cord potassium channel (TRESK, encoded by *KCNK18*) has been associated with migraine susceptibility;[62](#_ENREF_62),[63](#_ENREF_63)and the inactivation of the TWIK-related potassium channel (TREK, encoded by *KCNK4*) produces a depression-resistant phenotype in a mouse model.[64](#_ENREF_64)

Based on the significant overlapping genes identified in gene-based association analyses, multiple pathways were observed, which were over-presented in neural-related pathways such as metal ion channel regulations, signalling pathways, and enzymatic activity. These results provide evidence for the importance of neurological mechanisms on triggering comorbid migraine and MDD, suggesting that comorbid migraine and MDD may be induced by their shared neurological symptoms.

Our study has limitations. Firstly, LD Score regression defaults with calculating heritability based on the observed scale. Although we converted the conditions onto the liability scale by setting a fixed population and sample prevalence of migraine and MDD, the estimates may still be underestimated due to the relatively high sample prevalence of PGC MDD (around 50%). In addition, we identified a very small genetic covariance intercept of 0.04, indicating a small sample overlap between 2016 IHGC migraine GWAS and 2013 PGC MDD GWAS that may have influenced our gene-based association analyses. However, we believe such effects will be negligible since the intercept is very close to zero. Moreover, the restricted availability of genome-wide GWAS summary statistics for the 2016 23andMe MDD GWAS limited our findings: (1) we could not estimate a genetic correlation between the 2016 IHGC migraine and 2016 23andMe MDD GWAS using LD Score regression; hence, we utilised SECA to test for their genetic overlap; (2) it is possible most of the genome-wide significant SNPs from meta-analysis of migraine and MDD showed a stronger signal for MDD compared to migraine because the 2016 23andMe MDD GWAS was limited to the top 10,000 SNPs (i.e., ‘weaker’ MDD SNP associations were not meta-analysed); and (3) we could not perform a complete genome-wide meta-analysis of the 2016 IHGS migraine and 2016 23andMe MDD GWAS; nor could we perform gene-based genetic overlap analysis across the 2016 IHGC migraine and 2016 23andMe MDD GWAS. Lastly, because our gene-based association tests assigned SNPs to genes based on close proximity (i.e., within 15 kb 5’ of the TSS site and 15 kb 3’ of the TES), more distant SNPs associated with gene-to-phenotype risk may influence the interpretation of our gene-based overlap analyses.

In conclusion, we have shown a significant genetic overlap across migraine and MDD at both the SNP- and gene-level. Importantly, we identified three novel independent genome-wide significant SNPs (rs146377178, rs672931, and rs11858956; located between *CDCA2* and *EBF2*, within *DCDC5*, and between *RPLP1* and *TLE3*, respectively), and two genome-wide significant genes (*ANKDD1B* and *KCNK5*). Multiple pathway terms, especially the neural-related pathways of signalling and metal ion channel regulation, were implicated. Overall, our study provides strong molecular genetic support for shared genetically controlled mechanisms underlying migraine and MDD risk, and provide impetus to perform further combined analyses of migraine and MDD GWAS data.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGMENTS**

Huiying Zhao was supported by a National Health and Medical Research Council (NHMRC) Early Career Fellowship (APP1091816). Dale R. Nyholt was supported by an NHMRC Research Fellowship (APP0613674). This work was supported by an NHMRC project grant (APP1075175), the European Union’s Seventh Framework Program (2007–2013) under grant agreement no. 602633 (EUROHEADPAIN) and the US National Institutes of Health (AA07535, AA011998, AA017688, AA10249, AA13320, AA13321, AA13326, AA14041, MH66206, DA12854, DA019951). We are grateful to Lisanne S. Vijfhuizen (Leiden University Medical Center) for assistance with the revision. We thank the PGC for making their 2013 MDD GWAS summary statistics publicly available.

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