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Blood gene expression studies in migraine: Potential and caveats

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Abstract

Background: Global gene expression analysis may be used to obtain insights into the functional processes underlying migraine. However, there is a shortage of high-quality post-mortem brain tissue samples for genetic analysis. One approach is to use a more accessible tissue as a surrogate, such as peripheral blood.

Purpose: Discuss the benefits and caveats of blood genomic profiling in migraine and its potential application in the development of biomarkers of migraine susceptibility and outcome. Demonstrate the utility of blood-based expression profiles in migraine by analysing pilot Illumina HT-12 expression data from 76 (38 case, 38 control) whole-blood samples.

Conclusion: Current evidence suggests peripheral blood is a biologically valid substrate for genetic studies of migraine, and may be used to identify biomarkers and therapeutic pathways. Pilot blood gene expression data confirm that expression profiles significantly differ between migraine case and non-migraine control individuals.

Keywords

Migraine, association, gene, mRNA, expression, blood, biomarkers, genomic profiling

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Introduction

Migraine is among the most pervasive medical conditions to afflict humankind, with a 1-year prevalence of 12% (1–4). It currently ranks as the most disabling neurological condition in terms of years lost to disability, and the seventh most disabling of all diseases worldwide (5). Current molecular evidence suggests migraine may be a disease of vascular origin, or one that involves vascular changes as a result of neuronal dysfunction (6,7). However, the exact causes are unknown, and there are no reliable pathological markers for clinical testing. Diagnosis is therefore based on the personal evaluation of headache symptoms, described by the diagnostic manual of the International Headache Society (8). Two migraine subtypes account for 90% of diagnoses, and include migraine without aura, characterised by moderate to severe headache and nausea accompanied in various combinations by vomiting and sensitivity to light and/or sound, and migraine with aura, which includes transient aura symptoms that manifest as visual, sensory or motor disturbances, followed by a headache phase similar to migraine without aura. The overlap of symptom profiles between these migraine subtypes, as well as other less prevalent forms of headache, means patients often satisfy multiple diagnoses. Both acute and prophylactic treatments are available to relieve

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migraine symptoms and reduce the severity and frequency of attacks, but are associated with a substantial amount of non-response, and their overuse can cause a rebound effect (9). Therefore, identifying new molecular targets could lead to improvements in the diagnosis of migraine and inform the development of more effective treatments.

Migraine is an inherited disorder with an estimated heritability in the order of 60% (10–14). Genome-wide association (GWA) studies have identified 13 common (minor allele frequency > 0.05) single-nucleotide polymorphism (SNP) loci associated with migraine (15–18), but their biological role in migraine susceptibility is currently unknown. As a complementary approach, the biological pathways underlying migraine may be identified using global gene expression analysis. Gene expression has an important role in the underlying susceptibility to complex disease and is influenced by genetic polymorphisms, as indicated by expression quantitative trait loci (eQTL). Epigenetic marks, including DNA methylation and post-translational histone modifications, also modify gene expression, in addition to a host of other factors, including age, sex, gonadal hormones, diet and medication use. Therefore, the gene expression patterns associated with migraine may reflect genetic and non-genetic effects and inform the development of biomarkers of migraine susceptibility and outcome.

Migraine is most likely caused by the dysfunction of multiple tissues, including blood vessels and the brain, so it is not obvious *a priori* where causal pathways manifest themselves. Thus, gene expression studies must carefully consider the choice of tissue for genetic dissection. This is particularly important for diseases with a neurological component due to the relative inaccessibility of brain tissue from living individuals and various technical biases associated with the use of post-mortem samples. Venepuncture is commonly used to monitor blood levels of medicinal agents and identify markers of disease for diagnostic purposes. Blood is a transcriptionally active and ubiquitous fluid composed of numerous cell types, and it is critical for the normal functioning of every tissue in the human body. Circulating blood leukocytes are equipped with receptors and pathways that respond to foreign substances and tissue damage, and can respond to inflammatory signals released from the brain following injury (19). Blood may therefore provide valuable surrogate information on the metabolic and disease pathways that are thought to occur in the cerebral vasculature and brain parenchyma of migraine sufferers.

A blood gene expression signature in migraine would greatly improve the clinical management of patients. In this article, we discuss the validity and potential of blood genomic profiling studies in migraine, including such profiling's utility as a substrate for the interpretation of DNA polymorphisms. We present pilot data to support these methods of investigation and suggest blood constitutes a highly accessible and promising source of genomic biomarkers for migraine.

Post-mortem gene expression of brain tissue

Genes are differentially expressed between tissues. It is therefore desirable to perform genetic tests of association in the tissue primarily affected by disease. It can be argued that brain tissue is most appropriate for expression studies of migraine, where progressive structural and functional changes associated with the disorder have been observed (20–22). However, the collection of brain biopsies from living and otherwise healthy individuals is not feasible. Researchers must instead use samples collected post-mortem. Genetic studies of post-mortem tissues have been used to identify the genes and pathways of many brain-related diseases, including Alzheimer's disease (23–25), Parkinson's disease (26), amyotrophic lateral sclerosis (27) and schizophrenia (28). These and other neurodegenerative diseases are well characterised, can be diagnosed using objective clinical tests and are generally associated with a reduced lifespan, so the collection of post-mortem tissues is possible. Migraine, on the other hand, is non-fatal and clinically heterogeneous, lacks objective clinical biomarkers and the frequency of attacks varies over time and often shows a marked decline in later life. These factors preclude the collection of sufficient numbers of high-quality, well-diagnosed post-mortem samples required for a controlled experiment.

The use of post-mortem brain tissues is also beset with analytical issues that may impact study results. First, pre-analytical factors such as the manner of death, length of post-mortem interval, method of tissue dissection and length of storage all impact RNA quality and may confound downstream analyses, resulting in spurious associations (29). Second, the brain is composed of heterogeneous cell types and different regions of the brain express unique transcripts (30). Thus, there is a level of cell type specificity within the brain itself that means an additional choice must be made in terms of the brain region for genetic dissection. While single-cell purification assays such as flow cytometry (31) and laser capture microdissection (32) are available in order to mitigate tissue heterogeneity, they introduce a level of technical variability that may diminish study power. Finally, matching appropriate control subjects and controlling for potential confounders is difficult using post-mortem samples because phenotypic data are often limited and rely on

retrospective data collection from patient medical records or hospital administrative databases.

Peripheral gene expression in migraine

Gene expression-based markers of disease, regardless of their tissue of origin, have inherent variation. Expression variation is especially pronounced in blood, where physiological factors, such as circulating hormones, circadian processes and cell type proportions, as well as age and gender, are known to influence the gene activity (33–36). Establishing the clinical utility of a biomarker often depends on the ability to control for these sources of variation and bias. This requires the evaluation of large numbers of tissue specimens in a controlled and prospective manner, including repeated measurements from the same individual over time. As noted above, post-mortem brain tissues cannot meet these criteria and can never be used in a controlled trial combining the use of genetic markers and various treatment interventions. Researchers must instead rely on a more accessible tissue, such as peripheral blood, and use carefully designed studies in order to limit unwanted variation in gene expression and extract biologically meaningful associations that can be generalised to the brain.

Peripheral blood is an attractive candidate for genomic studies of migraine due to the ease of acquisition and the ability to collect multiple samples with little discomfort to the patient. Current evidence suggests that the vast majority of genes expressed in brain tissue are also found in blood, with tissue-specific expression driven by a small number of genes (37). A study of matched whole blood and brain tissue in humans found that genes expressed in both tissues were significantly correlated and enriched for specialised brain processes, including axon guidance and neurogenesis (38). More recently, a direct comparison of peripheral blood monocytes and brain tissue reported a high correlation ($r = 0.98$) between genes with similar detection levels between the tissues (representing 37% of the transcript dataset), as well as the presence of many genes with putative roles in various neuropsychiatric disorders (39). These data suggest that blood possesses a subset of molecular pathways and gene networks involved in the pathophysiology of brain-related traits. Therefore, it is reasonable to suggest that blood gene expression may be used to complement other genetic/molecular data in order to uncover pathological processes in brain-related traits, as well as to mine for clinically useful biomarkers of disease processes.

Global gene expression studies of peripheral blood have a number of other advantages. Blood can be collected from a large and representative group of age and sex-matched cases and controls, each with detailed phenotypic and anthropomorphic data, as well as follow-up information on migraine frequency and characteristics. Follow-up information may then be used to select more homogenous patient subgroups for analysis, which will increase the power to detect genetic associations. Blood can also be collected at a time and in a manner that is similar for all participants, and samples can be enriched for a cell type of interest (e.g. neutrophils or monocytes), thereby reducing unwanted variation and improving gene expression resolution. However, the post-extraction methods required to enrich a cell type of interest exposes the sample to pre-analytical factors (e.g. blood collection systems, cell purification methods and temperature changes) that can alter RNA and produce artefactual changes in expression, and the cell type of interest has to be defined *a priori*.

An alternative method is to simply use expression profiles derived from whole blood, which captures RNA profiles of all blood cell types, including erythrocytes, granulocytes, lymphocytes, monocytes and platelets. Popular blood sampling methods for gene expression profiling include the PAXgene™ and TEMPUS™ tube systems, which contain proprietary compounds that lyse blood cells and immediately stabilise RNA. While several studies have reported non-trivial differences in gene expression profiles between whole-blood samples and blood cell type-specific RNA sources (40,41), no single blood RNA source has outperformed the others. Therefore, the choice of blood RNA source should be based upon the measurement criteria of practicality and reliability. Whole blood is practical because it does not require complex post-extraction methods and produces more stable expression profiles over repeated measurements within the same individual compared with cell type-specific methods (42). In addition, it is possible to impute cell type proportions in whole blood and to adjust for their impact on disease associations in downstream analyses (43). As such, whole blood is arguably the most appropriate for large-scale genomic epidemiological studies of complex traits such as migraine.

Blood genomic profiling of migraine is supported by parallel studies of rodents, in which surgically induced neurological injuries designed to mimic ischaemia, stroke and hypoxia produced overlapping gene expression changes in matched peripheral blood and brain samples (44–46). In humans, unique blood signatures have been reported for several neurological disorders, including stroke (47–49), multiple sclerosis (50) and Alzheimer's disease (51), as well as neuropsychiatric disease such as schizophrenia (52) and major depressive disorder (53). One large whole-blood expression study of major depression, which shares a higher than

expected comorbidity with migraine (54), reported many experiment-wide significant (False Discovery Rate (FDR) < 0.05) gene associations, as well as clear differences between the expression profiles of depressed and non-depressed individuals (55). Furthermore, a follow-up investigation of potential confounders found that depression status–gene expression associations were largely unrelated to medication use and cell type counts. These data provide further support for blood genomic studies of complex brain-related traits.

Distinct blood gene expression patterns have been measured between migraine and non-migraine subjects (56–58) (Table 1). For example, one small study reported global differences in expression between acute migraine and chronic migraine sufferers when compared with non-migraine controls, as well as differences between each migraine subtype (56). A group of platelet-related genes were over-expressed in both migraine subtypes, supporting a possible association between platelets, abnormal haemostasis and blood vessel dysfunction in migraine (59,60). However, this study failed to consider the impact of medication use on gene expression. This is particularly important for expression studies of chronic migraine, which is thought to develop from medication overuse (61). Thus, the authors could not convincingly establish whether platelet-related gene expression differences were due to a disease-related process or simply the result of differences in medication use between migraine cases and controls.

A follow-up study of gene expression in chronic migraine sufferers with and without medication overuse headache was able to characterise the impact of medication use on chronic migraine (57). To do this, gene expression from whole blood in chronic migraine sufferers with and without medication overuse headache was measured at baseline and at 30–60 days following a standard treatment regimen (Table 1). Subjects who responded to treatment had a unique blood genomic expression pattern compared to non-responders at baseline and follow-up, suggesting that it may be possible to develop a predictive biomarker in order to inform treatment options for medication overuse headache. In addition, migraine-gene expression associations showed clear enrichment of the genes expressed in neuronal and epithelial/smooth muscle cells, supporting a role for both tissues in migraine pathophysiology and further demonstrating the utility of whole blood in studying the molecular pathways underlying migraine.

A genomic profile of gene expression variation in blood

The observed difference in gene expression between individuals is not only the product of physiological and environmental variation, but also genetic regulatory variation. The impact of regulatory variation on gene expression can be determined through association testing in order to identify regions of the genome where there is a significant correlation between genetic polymorphism and gene expression, known as eQTL mapping (62,63). eQTL data may then be used to prioritise gene candidates at a migraine GWA study locus, and also to characterise the genomic profile of regulatory variation that impacts on the activity of the numerous genes underlying migraine susceptibility.

Many eQTL, particularly those associated with disease, are specific to a given tissue, and the fold change in expression and direction of effect of shared eQTL can differ across tissues (64,65). Therefore, it is not clear whether the eQTL found in one tissue will be replicated in others. This impedes the biological interpretation and extrapolation of eQTL associations when measured in a substitute tissue. Migraine pathogenesis is thought to involve processes in multiple tissues, so tissue-specific effects can be expected. Many eQTL associated with human traits have been identified in blood (66–68) and show a high level of sharing with many tissues (69). However, it is not possible to characterise all disease-relevant eQTL using blood alone. This caveat supports detailed eQTL studies of multiple tissues – including blood and brain – in order to dissect the complex regulatory systems that govern migraine susceptibility.

Several studies have examined eQTL specificity or sharing between the blood and brain using samples derived from different patients. Two recent meta-analyses compared gene expression and genotype data from post-mortem brain samples with catalogued eQTL from large surveys of peripheral blood. The first study reported that 24% of eQTL in peripheral blood overlapped with genes with local eQTL in the brain, which was significantly greater than expected by chance ($p = 9.4 \times 10^{-21}$), and increased to 71% in a subset analysis of genes expressed in both tissues (70). The second analysis reported a similar level of overlap between the tissues – ranging between 13% and 23% after adjustment for sample size – and found that genes with overlapping eQTL had similar functional roles (71). A third study associated the variants underlying specific traits from the National Human Genome Research Institute GWA study catalogue with gene expression in the blood and brain (72). Many eQTL were shared between the blood and brain; however, there were instances where the detection of eQTL was limited by the tissue studied. Taken together, these findings suggest that blood gene expression has the capacity to inform regulatory variation in brain tissue, but will not provide complete coverage of tissue-specific eQTL effects.

Table 1. Blood gene expression profiling studies in migraine.

		Sample Size											
		Cases			Controls								
Author	Diagnosis	M	F	T	M	F	T	Age	Ethnicity	Treatment/ therapy	Sample collection	Genes (*n*)	Gene ontology
Hershey et al. (2004)	M (IHS)	3	4	7	30	26	56	11–17	European	NSAID Triptans[a] Prev.[b]	Ictal phase	40	Platelet expression markers, *c-fos*, *jun-B*, *cox-2, MGSA*
Hershey et al. (2004)	CM (IHS)	7	8	15	30	26	56	10–16	European, African–American, racially mixed	Ibuprofen Triptans[a] Prev.[b]	Non-migraine state	353	Platelet expression markers, mitochondrial genes
Hershey et al. (2011)	CM, CM-MOH (ICHD-II)	–	–	19	–	–	14	5–18	European, African–American	Acute Preventive Medicine. Bio-behavioural	Prior treat. (D1) Follow-up (D2)	43 (D1) 206 (D2)	Cell signalling, apoptosis, leukocyte transendothelial migration, neurodegenerative disorders, Soluble NSF Attachment Protein Receptor (SNARF) interactions in vesicular transport, natural killer cell-mediated cytotoxicity
Hershey et al. (2012)	MRM(ICHD-II)	–	18	18	–	20	20	13–17	European, African–American, Racially mixed	Acute	Prior treat. state	77	Mitochondrial functioning, oxidative phosphorylation and metal ion binding
Hershey et al. (2012)	M (ICHD-II)	–	18	18	–	20	20	13–17	European, African–American, mixed	Acute	Prior treat. state	127	Cell signalling, glycophosphorylation, disulphide bonding, immune response, mitochondrial functioning
Gerring et al. (2016)	Identification (ID) migraine	15	15	30	23	23	46	42–78	European	Unknown	Retrospective	70	Not applicable

Table 1 presents, from left to right, the author; the International Headache Society (IHS) or International Classification of Headache Disorders (ICHD-II) diagnostic criteria used (M: migraine; CM: chronic migraine; MOH: medication overuse headache; MRM: menstrual-related migraine); sample sized utilised (M: males; F: females; T total); age (years) of the participants; ethnicity; treatment or therapy followed by the participants prior or during the time of blood collection (NSAID: nonsteroidal anti-inflammatory drugs; [a]zolmitriptan, sumatriptan, almotriptan; [b]amitriptyline, divalproex sodium, topiramate, propranolol); the blood sample collection (prior treat.: prior treatment; D1: blood obtained prior to treatment; D2: first follow-up after treatment); number of genes upregulated in cases; functional categories or names of the genes of upregulated transcripts.

Amalgamating eQTL data with other molecular information that is known to influence gene expression, such as epigenomic (e.g. DNA methylation) assays, and drawing on data from experimental genomic compendia, such as the Epigenomics Roadmap Project (73), will further characterise gene-regulatory variation between the blood and brain. In addition, tissue-specific effects of eQTL across the blood and brain may be assessed using data from the Genotype-Tissue Expression (GTEx) study (69), which contains genotype data from blood linked to genome-wide gene expression patterns across 54 distinct body tissues, including 13 brain regions. The use of diverse data types will aid with the characterisation of gene expression and causal variants in blood and improve the biological interpretation of the brain-related processes underlying migraine activity.

Blood gene profiles can separate migraine cases and non-migraine controls

We demonstrate the potential utility of blood genomic profiling in migraine by comparing blood gene expression profiles between migraine cases and non-migraine controls. Blood gene expression data were collated from the Brisbane Systems Genetics Study (BSGS), a family-based study designed to investigate the genetic factors affecting gene expression and the role of gene regulation in mediating phenotypes and complex disease (74). Informed written consent was obtained from each participant, and the study was approved by the Health Research Ethics Committee (HERC) of the QIMR Berghofer Medical Research Institute (Queensland Institute of Medical Research (QIMRB)). The baseline sample comprised 962 individuals from 314 families. Whole blood for gene expression profiling was collected directly into PAXgene^{TM} tubes (QIAGEN, Valencia, CA). Total RNA was extracted using QIAGEN whole-blood gene RNA purification kits. All gene expression profiles were generated on Illumina HumanHT-12 v3 or v4 bead arrays, and the data were normalised as described in detail elsewhere (75,76). After quality control, 17,994 probes representing 13,355 RefSeq genes were available for analysis. Gene expression data are available at the Gene Expression Omnibus under the accession code GSE53195.

Unrelated migraine cases were selected using the ID Migraine^{TM} screening tool, which has been shown to accurately identify 93% of migraine sufferers in a clinical setting (77). A total of 38 unrelated adult individuals (15 male, 23 female; aged 42–78 years) screened positive for migraine and were age and sex matched with unrelated individuals who screened negative for migraine. In order to assess the differential expression between the migraine cases and non-migraine control groups, we fitted generalised linear models for each of the 17,994 gene expression probes, adjusted for sex, age and age^2. A total of 54 genes were identified as differentially expressed at a liberal threshold (FDR < 0.1). A Q–Q plot showed clear elevation of small p-values ($p < 0.05$) for differential expression above the 95% confidence interval (grey shaded area) of the null identity line (shown in white) (Figure 1A), suggesting that many of the signals likely represent true associations. A histogram of p-values (Figure 1B) is also strongly inflated towards zero, suggesting the presence of many differentially expressed genes that were not

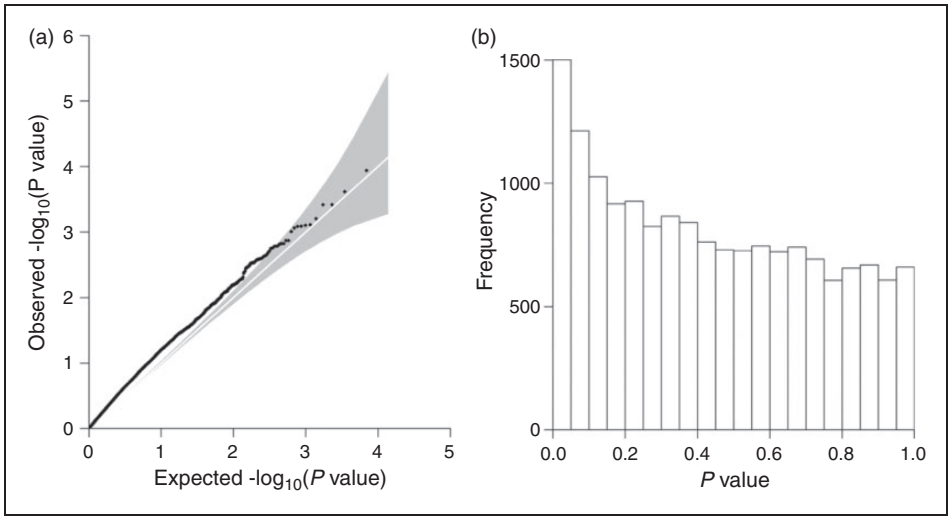

Figure 1. Q–Q plot of -$\log_{10}$ p-values (A) and histogram of p-values (B) from association tests for differential gene mRNA expression between migraine cases and non-migraine controls.

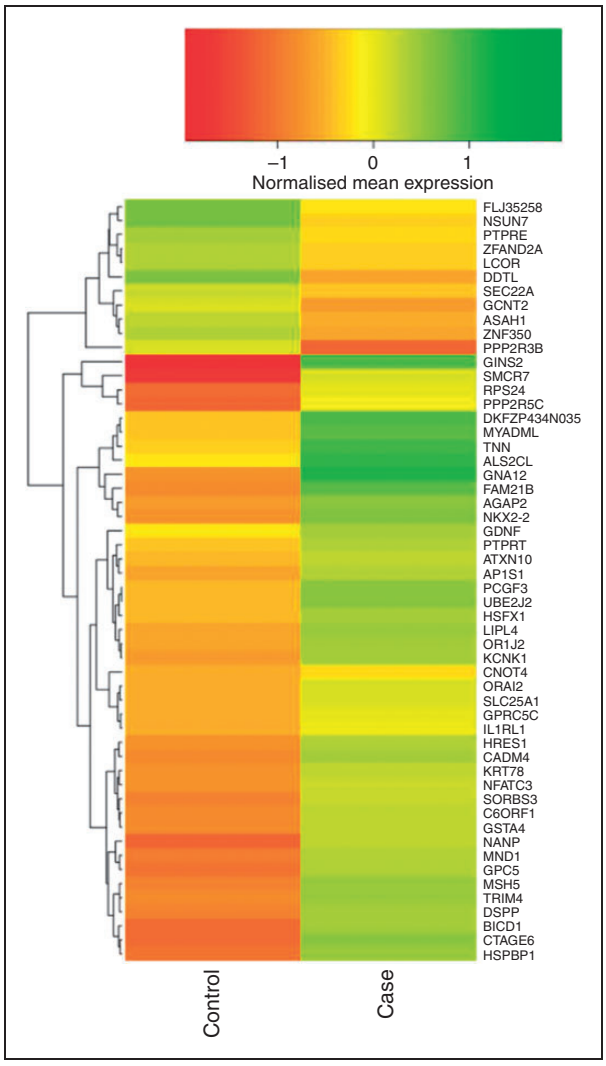

Figure 2. Heatmap of mean expression across migraine case and non-migraine control groups. For the 54 genes that were differentially expressed between cases and controls (False Discover Rate (FDR) < 0.1), mean expression was colour coded for the non-migraine control and migraine case groups. Genes were ordered according to the dendrogram of the hierarchical cluster analysis on the basis of Euclidean distance.

detectable at an experiment-wide significance threshold that was conservatively adjusted for 17,994 tests using the current sample size. A heatmap of mean gene expression values clearly differentiates between migraine cases and controls (Figure 2), and the observed clustering of case/control status is significant (Fisher's Exact test, $p = 2.11 \times 10^{-4}$).

Concluding remarks

In migraine, brain tissue that may hold the key to identifying causal genes and molecular pathways cannot be directly assessed from living patients in a clinical setting, and there is a lack of high-quality post-mortem samples for genetic dissection. Due to its accessibility, blood has been used as a substitute for brain tissue in order to gain insight into gene expression and gene regulation. There is debate as to whether peripheral blood provides biologically relevant information on pathological mechanisms within the brain (78). As we have discussed, current results suggest that many genes expressed in the brain are found in blood and may reflect brain-related disease processes. By analysing blood gene expression data from 38 migraine cases versus 38 non-migraine controls, we show that gene expression significantly differs between migraine and non-migraine individuals, adjusting for age and sex. These lines of evidence suggest that blood is a biologically valid substrate for the characterisation of the gene expression and genetic variation underlying migraine; however, confounding effects of blood cell counts and medication use cannot be excluded.

There are several important caveats in using blood as a surrogate for capturing gene expression and regulatory effects in brain tissue. First, genes are differentially expressed between tissues, so a surrogate may misrepresent both the expression direction and magnitude of effect in the tissue of interest. Moreover, genes expressed in one tissue may simply not be expressed in another, and therefore cannot contribute to a transcriptional profile of disease (37). Second, transcription in whole blood is dominated by a relatively small number of genes (37), which decreases the power to evaluate gene expression variation. This is especially problematic for complex diseases such as migraine, where small changes in the activity of many genes are thought to collectively drive disease susceptibility. Third, there are well-recognised differences in gene expression and regulatory effects between brain regions (79), limiting the ability to extrapolate findings from blood to the whole brain structure. Finally, more than any other tissue, blood gene expression is confounded by biological, clinical and technical variables, most of which are unobserved and introduce unwanted variation in gene expression. Taking these sources of variation into account, blood expression studies may require more samples in order to detect meaningful differences in gene expression compared to in the brain. For example, assuming a standard deviation of 0.7 for the gene intensity measurements on the $\log_2$ scale (realistic for genes expressed at moderate to high levels), for a two-sample two-sided t-test and a conservative (due to non-independence resulting from gene co-expression) Bonferroni-adjusted significance threshold of $p = 0.05/17{,}994 = 2.78 \times 10^{-6}$, analysis of 438 migraine case and 438 non-migraine control individuals is required in order to have 80% power to detect a 1.20-fold difference in expression level. Increased power may also

be provided by utilising single-cell assays in order to filter a cell type of interest, but prior knowledge is required in order to identify the cell type of interest, and important biological signals could be missed.

Notwithstanding these caveats, if a biomarker is to be used in a clinical setting to inform the diagnosis and treatment of migraine, it must be measureable in blood. The current evidence suggests that blood provides a window into the state of the brain in the presence of disease. While small sample sizes and the lack of matched brain tissue specimens has impeded the development of reliable blood biomarkers in migraine, studies have shown that blood gene expression differences exist between migraine cases and controls and can capture brain-related expression changes. Differential expression analyses using basic migraine information from the Brisbane Systems Genetics Study, representing one of the largest blood-based expression studies of migraine to date, corroborate these findings. The use of larger, more highly selected cohorts with detailed phenotype information will improve the power to detect the blood gene expression associated with migraine. Blood-based global gene expression levels also provide an ideal substrate for the integration of genomic factors that influence gene expression, such as DNA (SNP) polymorphism and epigenomic alterations. Linking these data with ongoing experimental genomic compendia will further prioritise blood gene expression signals and aid in the discovery of a genomic profile of migraine susceptibility and outcome.

Article highlights

- Whole blood is an accessible and reliable source of RNA and has been widely used in gene expression studies of neurological and neurodegenerative traits for disease profiling and biomarker identification.
- Global gene (RNA) expression data can be used in order to study the genetic and non-genetic effects underlying migraine susceptibility.
- Analysing pilot Illumina HT-12 expression data from 76 (38 case, 38 control) PAXgene^{TM} whole-blood samples, we found significant differences in gene expression between migraine cases and non-migraine controls.

Declaration of conflicting interests

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References

1. Rasmussen BK, Jensen R, Schroll M, et al. Epidemiology of headache in a general population – A prevalence study. *J Clin Epidemiol* 1991; 44(11): 1147–1157.
2. Henry P, Michel P, Brochet B, et al. A nationwide survey of migraine in France: Prevalence and clinical features in adults. *Cephalalgia* 1992; 12(4): 229–237.
3. Stewart WF, Lipton RB, Celentano DD, et al. Prevalence of migraine headache in the United States: Relation to age, income, race, and other sociodemographic factors. *J Am Med Assoc* 1992; 267(1): 64–69.
4. Diamond S, Bigal ME, Silberstein S, et al. Patterns of diagnosis and acute and preventive treatment for migraine in the United States: Results from the American migraine prevalence and prevention study. *Headache* 2007; 47(3): 355–363.
5. Murray CJL, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; 380(9859): 2197–2223.
6. Pietrobon D and Striessnig J. Neurobiology of migraine. *Nat Rev Neurosci* 2003; 4(5): 386–398.
7. Dodick DW. Examining the essence of migraine – Is it the blood vessel or the brain? A debate. *Headache* 2008; 48(4): 663–667.
8. Headache Classification Committee of the International Headache Society (HIS). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 2013; 33(9): 629–808.
9. Loder E. Triptan therapy in migraine. *N Engl J Med* 2010; 363(1): 63–70.
10. Gervil M, Ulrich V, Kaprio J, et al. The relative role of genetic and environmental factors in migraine without aura. *Neurology* 1999; 53(5): 995–999.
11. Russell MB and Olesen J. Increased familial risk and evidence of genetic factor in migraine. *Br Med J* 1995; 311(7004): 541–544.

12. Ulrich V, Gervil M, Kyvik K, et al. Evidence of a genetic factor in migraine with aura: A population-based Danish twin study. *Ann Neurol* 1999; 45: 242–246.
13. Ulrich V, Gervil M, Kyvik K, et al. The inheritance of migraine with aura estimated by means of structural equation modelling. *J Med Genet* 1999; 36: 225–227.
14. Mulder EJ, Van Baal C, Gaist D, et al. Genetic and environmental influences on migraine: A twin study across six countries. *Twin Res* 2003; 6(5): 422–431.
15. Anttila V, Stefansson H, Kallela M, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. *Nat Genet* 2010; 42(10): 869–873.
16. Chasman DI, Schurks M, Anttila V, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nat Genet* 2011; 43(7): 695–698.
17. Freilinger T, Anttila V, de Vries B, et al. Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nat Genet* 2012; 44(7): 777–782.
18. Anttila V, Winsvold BS, Gormley P, et al. Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nat Genet* 2013; 45(8): 912–917.
19. Sofroniew MV. Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci* 2015; 16(5): 249–263.
20. Kruit MC, Van Buchem MA, Hofman PAM, et al. Migraine as a risk factor for subclinical brain lesions. *J Am Med Assoc* 2004; 291(4): 427–434.
21. Palm-Meinders IH, Koppen H, Terwindt GM, et al. Structural brain changes in migraine. *J Am Med Assoc* 2012; 308(18): 1889–1897.
22. Amin FM, Asghar MS, Hougaard A, et al. Magnetic resonance angiography of intracranial and extracranial arteries in patients with spontaneous migraine without aura: A cross-sectional study. *Lancet Neurol* 2013; 12(5): 454–461.
23. Blalock EM, Geddes JW, Chen KC, et al. Incipient Alzheimer's disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A* 2004; 101(7): 2173–2178.
24. Colangelo V, Schurr J, Ball MJ, et al. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: Transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J Neurosci Res* 2002; 70(3): 462–473.
25. Dangond F, Hwang D, Camelo S, et al. Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter. *Physiol Genomics* 2004; 16: 229–239.
26. Mandel S, Grunblatt E, Riederer P, et al. Gene expression profiling of sporadic Parkinson's disease substantia nigra pars compacta reveals impairment of ubiquitin-proteasome subunits, SKP1A, aldehyde dehydrogenase, and chaperone HSC-70. *Ann N Y Acad Sci* 2005; 1053: 356–375.
27. Jiang Y-M, Yamamoto M, Kobayashi Y, et al. Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. *Ann Neurol* 2005; 57(2): 236–251.
28. Ray MT, Weickert CS and Webster MJ. Decreased BDNF and TrkB mRNA expression in multiple cortical areas of patients with schizophrenia and mood disorders. *Transl Psychiatry* 2014; 4: e389.
29. Atz M, Walsh D, Cartagena P, et al. Methodological considerations for gene expression profiling of human brain. *J Neurosci Methods* 2007; 163(2): 295–309.
30. Ramasamy A, Trabzuni D, Guelfi S, et al. Genetic variability in the regulation of gene expression in ten regions of the human brain. *Nat Neurosci* 2014; 17(10): 1418–1428.
31. Schwartz A and Fernández-Repollet E. Quantitative flow cytometry. *Clin Lab Med* 2001; 21(4): 743–761.
32. Emmert-Buck MR, Bonner RF, Smith PD, et al. Laser capture microdissection. *Science* 1996; 274(5289): 998–1001.
33. Whitney AR, Diehn M, Popper SJ, et al. Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci U S A* 2003; 100(4): 1896–1901.
34. Radich JP, Mao M, Stepaniants S, et al. Individual-specific variation of gene expression in peripheral blood leukocytes. *Genomics* 2004; 83(6): 980–988.
35. Eady JJ, Wortley GM, Wormstone YM, et al. Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. *Physiol Genomics* 2005; 22: 402–411.
36. Dumeaux V, Olsen KS, Nuel G, et al. Deciphering normal blood gene expression variation – The NOWAC postgenome study. *PLoS Genet* 2010; 6(3): e1000873.
37. Melé M, Ferreira PG, Reverter F, et al. The human transcriptome across tissues and individuals. *Science* 2015; 348(6235): 660–665.
38. Sullivan PF, Fan C and Perou CM. Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet* 2006; 141B(3): 261–268.
39. Rollins B, Martin MV, Morgan L, et al. Analysis of whole genome biomarker expression in blood and brain. *Am J Med Genet Part B Neuropsychiatr Genet* 2010; 153(4): 919–936.
40. Debey S, Schoenbeck U, Hellmich M, et al. Comparison of different isolation techniques prior gene expression profiling of blood derived cells: Impact on physiological responses, on overall expression and the role of different cell types. *Pharmacogenomics J* 2004; 4(3): 193–207.
41. Joehanes R, Johnson AD, Barb JJ, et al. Gene expression analysis of whole blood, peripheral blood mononuclear cells, and lymphoblastoid cell lines from the Framingham Heart Study. *Physiol Genomics* 2012; 44(1): 59–75.
42. Min JL, Barrett A, Watts T, et al. Variability of gene expression profiles in human blood and lymphoblastoid cell lines. *BMC Genomics* 2010; 11: 96.
43. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015; 12(5): 453–457.
44. Tang Y, Lu A, Aronow BJ, et al. Blood genomic responses differ after stroke, seizures, hypoglycemia,

and hypoxia: Blood genomic fingerprints of disease. *Ann Neurol* 2001; 50(6): 699–707.
45. Tang Y, Lu A, Aronow BJ, et al. Genomic responses of the brain to ischemic stroke, intracerebral haemorrhage, kainate seizures, hypoglycemia, and hypoxia. *Eur J Neurosci* 2002; 15(12): 1937–1952.
46. Tang Y, Nee AC, Lu A, et al. Blood genomic expression profile for neuronal injury. *J Cereb Blood Flow Metab* 2003; 23(3): 310–319.
47. Moore DF, Li H, Jeffries N, et al. Using peripheral blood mononuclear cells to determine a gene expression profile of acute ischemic stroke: A pilot investigation. *Circulation* 2005; 111(2): 212–221.
48. Tang Y, Xu H, Du X, et al. Gene expression in blood changes rapidly in neutrophils and monocytes after ischemic stroke in humans: A microarray study. *J Cereb Blood Flow Metab* 2006; 26(8): 1089–1102.
49. Sharma R, Macy S, Richardson K, et al. A blood-based biomarker panel to detect acute stroke. *J Stroke Cerebrovasc Dis* 2014; 23(5): 910–918.
50. Sharp FR, Xu H, Lit L, et al. The future of genomic profiling of neurological diseases using blood. *Arch Neurol* 2006; 63(11): 1529–1536.
51. Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med* 2007; 13(11): 1359–1362.
52. Glatt SJ, Everall IP, Kremen WS, et al. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. *Proc Natl Acad Sci U S A* 2005; 102(43): 15533–15538.
53. Glahn DC, Curran JE, Winkler AM, et al. High dimensional endophenotype ranking in the search for major depression risk genes. *Biol Psychiatry* 2012; 71(1): 6–14.
54. Frediani F and Villani V. Migraine and depression. *Neurol Sci* 2007; 28: S161–S165.
55. Jansen R, Penninx BWJH, Madar V, et al. Gene expression in major depressive disorder. *Mol Psychiatry*. Epub ahead of print 26 May 2015. DOI: 10.1038/mp.2015.57.
56. Hershey AD, Tang Y, Powers SW, et al. Genomic abnormalities in patients with migraine and chronic migraine: Preliminary blood gene expression suggests platelet abnormalities. *Headache* 2004; 44(10): 994–1004.
57. Hershey AD, Burdine D, Kabbouche MA, et al. Genomic expression patterns in medication overuse headaches. *Cephalalgia* 2011; 31(2): 161–171.
58. Hershey A, Horn P, Kabbouche M, et al. Genomic expression patterns in menstrual-related migraine in adolescents. *Headache* 2012; 52(1): 68–79.
59. Gretchen E and Tietjen MD. Migraine and ischaemic heart disease and stroke: Potential mechanisms and treatment implications. *Cephalalgia* 2007; 27(8): 981–987.
60. Dalkara T, Nozari A and Moskowitz MA. Migraine aura pathophysiology: The role of blood vessels and microembolisation. *Lancet Neurol* 2010; 9(3): 309–317.
61. Bigal ME, Serrano D, Buse D, et al. Acute migraine medications and evolution from episodic to chronic migraine: A longitudinal population-based study. *Headache* 2008; 48(8): 1157–1168.
62. Morley M, Molony CM, Weber TM, et al. Genetic analysis of genome-wide variation in human gene expression. *Nature* 2004; 430(7001): 743–747.
63. Westra H-J, Peters MJ, Esko T, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013; 45(10): 1238–1243.
64. Nicolae DL, Gamazon E, Zhang W, et al. Trait-associated SNPs are more likely to be eQTLs: Annotation to enhance discovery from GWAS. *PLoS Genet* 2010; 6(4): e1000888.
65. Nica AC, Parts L, Glass D, et al. The architecture of gene regulatory variation across multiple human tissues: The MuTHER study. *PLoS Genet* 2011; 7(2): e1002003.
66. Powell JE, Henders AK, McRae AF, et al. Genetic control of gene expression in whole blood and lymphoblastoid cell lines is largely independent. *Genome Res* 2012; 22(3): 456–466.
67. Mehta D, Heim K, Herder C, et al. Impact of common regulatory single-nucleotide variants on gene expression profiles in whole blood. *Eur J Hum Genet* 2013; 21(1): 48–54.
68. Raj T, Kuchroo M, Replogle JM, et al. Common risk alleles for inflammatory diseases are targets of recent positive selection. *Am J Hum Genet* 2013; 92(4): 517–529.
69. The GTEx Consortium. The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. *Science* 2015; 348(6235): 648–660.
70. Kim Y, Xia K, Tao R, et al. A meta-analysis of gene expression quantitative trait loci in brain. *Transl Psychiatry* 2014; 4: e459.
71. McKenzie M, Henders AK, Caracella A, et al. Overlap of expression quantitative trait loci (eQTL) in human brain and blood. *BMC Med Genomics* 2014; 7: 31.
72. Hernandez DG, Nalls MA, Moore M, et al. Integration of GWAS SNPs and tissue specific expression profiling reveal discrete eQTLs for human traits in blood and brain. *Neurobiol Dis* 2012; 47(1): 20–28.
73. Romanoski CE, Glass CK, Stunnenberg HG, et al. Epigenomics: Roadmap for regulation. *Nature* 2015; 518(7539): 314–316.
74. Powell JE, Henders AK, McRae AF, et al. Genetic control of gene expression in whole blood and lymphoblastoid cell lines is largely independent. *Genome Res* 2012; 22(3): 456–466.
75. Powell JE, Henders AK, McRae AF, et al. The Brisbane Systems Genetics Study: Genetical genomics meets complex trait genetics. *PLoS One* 2012; 7(4): e35430.
76. Powell JE, Henders AK, McRae AF, et al. Congruence of additive and non-additive effects on gene expression estimated from pedigree and SNP data. *PLoS Genet* 2013; 9(5): e1003502.
77. Lipton RB. A self-administered screener for migraine in primary care: The ID Migraine™ validation study. *Neurology* 2003; 61(3): 375–382.
78. Davies MN, Lawn S, Whatley S, et al. To what extent is blood a reasonable surrogate for brain in gene expression studies: Estimation from mouse hippocampus and spleen. *Front Neurosci* 2009; 3: 54.
79. Yeo G, Holste D, Kreiman G, et al. Variation in alternative splicing across human tissues. *Genome Biol* 2004; 5(10): R74.