



Eising, E., de Leeuw, C., Min, J. L., Anttila, V., Verheijen, M. H., Terwindt, G. M., Dichgans, M., Freilinger, T., Kubisch, C., Ferrari, M. D., Smit, A. B., de Vries, B., Palotie, A., van den Maagdenberg, A. M., Posthuma, D., & International Headache Genetics Consortium (2016). Involvement of astrocyte and oligodendrocyte gene sets in migraine. *Cephalalgia*, 36(7), 640-647.
<https://doi.org/10.1177/0333102415618614>

Peer reviewed version

Link to published version (if available):
[10.1177/0333102415618614](https://doi.org/10.1177/0333102415618614)

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Involvement of astrocyte and oligodendrocyte gene sets in migraine

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Running title: Glial gene sets enriched in migraine GWAS

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Abstract

Background

Migraine is a common episodic brain disorder characterized by recurrent attacks of severe unilateral headache and additional neurological symptoms. Two main migraine types can be distinguished based on the presence of aura symptoms that can accompany the headache: migraine with aura and migraine without aura. Multiple genetic and environmental factors confer disease susceptibility. Recent genome-wide association studies (GWAS) indicate that migraine susceptibility genes are involved in various pathways, including neurotransmission, which have already been implicated in genetic studies of monogenic familial hemiplegic migraine, a subtype of migraine with aura.

Methods

To further explore the genetic background of migraine, we performed a gene set analysis of migraine GWAS data of 4,954 clinic-based patients with migraine, as well as 13,390 controls. Curated sets of synaptic genes and sets of genes predominantly expressed in three glial cell types (astrocytes, microglia, and oligodendrocytes) were investigated.

Discussion

Our results show that gene sets containing astrocyte- and oligodendrocyte-related genes are associated with migraine; which is especially true for gene sets involved in protein modification and signal transduction. Observed differences between migraine with aura and migraine without aura, indicate that both migraine types, at least in part, seem to have a different genetic background.

Key words: Migraine, GWAS, pathway analysis, astrocytes, oligodendrocytes, synapse

Introduction

Migraine is a common brain disorder affecting 15% of the population and characterized by recurrent attacks of severe, often unilateral headache associated with additional neurological symptoms such as nausea, vomiting and an increased sensitivity to light and sound (1). The presence of an aura accompanying the headache phase, consisting of transient neurological symptoms that are usually visual in nature, distinguishes between migraine with aura and migraine without aura.

Genome-wide association studies (GWAS), which have become a widely used approach to study the genetic background of common disorders, already yielded thirteen genome-wide significant loci for migraine (2-5). Several of the migraine candidate genes (i.e. *MTDH*, *LRP1*, *PRDM16*, *MEF2D*, *ASTN2*, *PHACTR1*, *FHL5*, *MMP16*) that have been brought up by these GWAS loci can be linked to (glutamatergic) neurotransmission (2-5). This seems in line with previous genetic findings in familial hemiplegic migraine, a monogenic subtype of migraine with aura (1), that revealed that all three causal genes identified so far (*CACNA1A*, *ATP1A2* and *SCN1A*) play an important role in glutamatergic neurotransmission (6).

All currently known migraine GWAS loci combined explain only a minor fraction of the genetic heritability that has been estimated at 50% in twin studies (7), which is not different from GWAS findings in other common disorders (8). Part of the missing heritability may be explained by variants that do not reach the threshold for genome-wide significance, which is not unexpected given the small effect sizes that GWAS hits have and the large samples sizes that are needed to detect them. Such yet unidentified variants might be enriched in genes that play a role in disease-related pathways. Therefore, additional pathophysiological insights may be obtained by investigating GWAS data for specific disease-relevant gene sets. Here, we

used three expert-curated glial cell-related gene lists (i.e. astrocyte, oligodendrocyte and microglia cell type-related gene lists) (9), and a synaptic gene list (10) to study the role of genes involved in synaptic mechanisms and glial cells in common migraine. The four gene lists were subdivided into functional gene sets and investigated for association with migraine. This approach has been applied successfully in e.g. schizophrenia, thus identifying a role for synaptic genes (12) as well as specific astrocyte and oligodendrocyte functions (9). For the analysis, we used GWAS data of 13,588 control individuals and 5,037 migraine patients (2,733 patients with migraine with aura and 2,304 patients with migraine without aura) that were recruited via specialized headache clinics (2,5). We used clinic-based migraine GWAS cohorts, as phenotypic information is more complete and accurate than in population-based cohorts. This might reduce the clinical and genetic heterogeneity within the cohorts, thereby increasing the likelihood of identifying associations with migraine. Our gene set analyses results indicated a role for astrocyte- and oligodendrocyte-specific genes in migraine pathophysiology with a different contribution in both migraine types.

Material and Methods

Subjects

In this study we investigated genetic data of 5,037 migraine cases (2,733 patients with migraine with aura and 2,304 patients with migraine without aura) and 13,588 controls that were part of two previously published clinic-based GWA studies (2,5). Patients were collected via specialized headache centers within Europe (the Netherlands, Finland, and Germany). Migraine diagnoses were established using a combination of questionnaires and individual interviews, based on the ICHD-IIIbeta guidelines (1).

Genotyping and quality control

Patient and control samples were genotyped using Illumina arrays, except for the German control cohort that was used for the migraine without aura GWAS, which was genotyped partly using Illumina and partly using Affymetrix arrays (for details see Anttila *et al.* (2) and Freilinger *et al.* (5)). Sample and genotype quality controls were performed specifically for this study using the raw genotyping data of the discovery cohorts of Anttila *et al.* (2) and Freilinger *et al.* (5), using the protocol described in Anderson *et al.* (11). In short, samples were removed in case of non-European ancestry, as measured with principal components analysis with EIGENSTRAT, sex discrepancies between genotypic and phenotypic data, missingness (> 3% of SNPs), outlying heterozygosity rate (> 5SD) and relatedness (IBS > 0.125). Samples were also removed if no valid principal components for stratification correction could be computed. We retained 4,954 migraine cases and 13,390 controls after QC (Table 1). SNPs were removed from the analysis in case of: (i) a minor allele frequency < 1%; (ii) a deviation from Hardy-Weinberg equilibrium ($P < 10^{-5}$) in cases or controls; (iii) missing data for a SNP in > 3% of the individuals; (iv) a significantly different missing data rate between cases and controls ($P < 10^{-5}$); or (v) a non-unique genomic localization of SNPs.

Gene lists and gene sets

Four published, expert-curated gene lists, i.e. astrocytic, oligodendrocytic, microglia, and synaptic, were used for the analysis (9,10). In brief, the glial gene lists (astrocytic, oligodendrocytic, microglial) contain genes with enriched expression in at least one of these cell types based on: (i) microarray data of FACS-sorted oligodendrocytes, astrocytes and microglia from developing and adult mouse brain (12); (ii) genes that co-express with cell type markers in microarray data of human brain tissue (13,14); (iii) genes that are differentially expressed in microglia cell cultures after an inflammatory stimulus or in tissue of patients or animal models relevant to an inflammatory brain disorder (15-19); and (iv) a

list of oligodendrocyte transcription factors (20). The synaptic gene list consists of: (i) a manually curated list of genes of presynaptic proteins that with confirmation of proteomics data from isolated synapses (10,21-23), and (ii) a postsynaptic gene list based on proteomics data of synapses or the postsynaptic density (21-23). After combining the information from above mentioned resources into three lists with cell type-enriched genes, the lists were further curated by removing genes that were present in more than one of these gene lists, or that were reported to be highly expressed in neurons. The final lists consisted of 1,395 oligodendrocytic, 1,688 astrocytic, 239 microglial and 914 synaptic genes.

The genes from each of the glial gene lists (astrocytic, oligodendrocytic, microglial) were categorized into functional gene sets based on Gene Ontology (GO) biological process annotations (9). Due to the hierarchical structure of GO, gene sets can be related and can contain overlapping genes. Supplemental Table 1 shows the hierarchical structure of the functional gene sets. The synaptic gene list was categorized into functional gene sets based on ‘expert knowledge’ (10). This resulted in a total of 34 astrocytic, 29 oligodendrocytic, 19 microglial and 18 synaptic gene sets.

Statistical analysis

Statistical analyses were performed using the in-house developed software tool JAG (Joint Analysis of Genetic variants) (24) for: (i) the four gene lists, (ii) the 100 gene sets, and (iii) the 4,236 single genes in the four gene lists. JAG performs two types of statistical tests: the *self-contained test* and the *competitive test*. All P-values in this study were calculated using JAG. For each GWAS dataset (Table 1), SNPs were assigned to genes if located between the transcription start and stop site of the respective gene, based on NCBI human assembly build 37.3 and dbSNP release 135. Either 10 (for the Finnish and the German datasets) or 15

principal components (for the Dutch datasets) were used to correct for population stratification.

Self-contained P-values (P_{SC}) were calculated for the four gene lists, the 100 gene sets and for 4,236 single genes using at least 15,000 permutations of the case-control status (at $\alpha = 0.05$). The P_{SC} -values were calculated separately for each GWAS dataset, and then combined using Stouffer's Z-score method (25), weighted by the square root of the sample size. Bonferroni correction was used to account for multiple testing, correcting each gene set P_{SC} -value for the number of gene sets in each of the four gene lists. Hence the P_{SC} -values of the astrocytic, oligodendrocytic, microglial, and synaptic gene sets were corrected for 34, 29, 19 and 18 tests, respectively.

A *competitive test* was performed for each of the four gene lists and for each of the 100 gene sets that was significant (at $\alpha = 0.05$) on the self-contained test. The competitive test calculates P_{SC} -values for 150 random gene sets containing the same number of genes as the original gene list or gene set, and calculates the competitive P-value (P_{COMP}) as the proportion of random gene sets with a lower P_{SC} -value. For gene sets found to be significant in the competitive test (at $\alpha = 0.05$), the contribution of each gene was calculated as the drop in P_{SC} -value when all SNPs from that gene were removed from the analysis.

The self-contained test in JAG tests the null hypothesis that a set of genes is not associated with migraine, while the competitive test provides insight into whether a set of genes is more strongly associated to migraine than other comparably-sized sets of genes. For polygenic traits, self-contained tests are likely to show a significant P-value, simply due to the fact that a gene set contains many genes. Competitive tests control for the polygenic nature of a trait

by evaluating the association of a gene set relative to other gene sets. We thus only interpret the results of the competitive test.

Results

Association analysis of the four gene lists with migraine

We performed a gene set analysis using migraine GWAS data of 4,954 migraine patients (2,652 patients with migraine with aura and 2,302 patients with migraine without aura) and 13,390 controls to further explore the involvement of glial and synaptic genes in migraine pathophysiology. First, we conducted an overall analysis for the four gene lists, i.e. the three glial gene lists (astrocyte, oligodendrocyte and microglia) and the synaptic gene list (Table 2). A significant association (P_{SC} and $P_{COMP} < 0.05$) was found for oligodendrocyte-related genes with migraine, as well as with migraine with aura and migraine without aura, separately ($P_{COMP} = < 0.001, 0.040$ and 0.033 , respectively). No significant associations were found for the astrocytic, microglial and synaptic gene lists.

Association analysis of functional gene sets with migraine

Next, we studied specific functional gene sets that were defined within the four gene lists (Supplemental Table 1). Significant associations (P_{SC} and $P_{COMP} < 0.05$) with migraine were found for three astrocytic functional gene sets (“Protein transport”, “Cell proliferation” and “Miscellaneous”) and three oligodendrocyte functional gene sets (“Protein modification”, “Signal transduction” and “Miscellaneous”) (Table 3; Supplemental Table 1). Associations with functional gene sets were also calculated for migraine with and migraine without aura separately. Two functional gene sets showed a significant association with migraine with aura (the astrocytic gene set “Cell proliferation” and the oligodendrocytic gene set “Miscellaneous”) and two gene sets showed association with migraine without aura (the

astrocytic gene set “Protein transport” and the oligodendrocytic gene set “Signal transduction”) (Table 3). Interestingly, no overlap was found between functional gene sets that associated with migraine with aura and those that were associated with migraine without aura.

Gene-based analysis

To understand whether the identified association of the functional gene sets with migraine was due to single genes or the gene set as a whole, a gene-based analysis was carried out. The association signal of the astrocytic gene sets “Protein transport” and “Cell proliferation” was mainly explained by the effect of *GLI3* (Supplemental Table 2). The associations of the other gene sets were based on a joint effect of the genes in the set (Supplemental Table 2).

Discussion

We performed a gene set analysis using glial and synaptic gene sets (9,10) combined with data of the two clinic-based migraine GWA studies (2,5). The gene set analysis for the total oligodendrocyte gene list showed significant association with migraine. This suggests that in addition to neuronal dysfunction also oligodendrocytic dysfunction seem to play a possibly modulating role in migraine pathophysiology. A role for oligodendrocytes in migraine has not yet been proposed. Studies have shown that oligodendrocytes communicate actively with neurons, probably to regulate their energy supply - and thereby their activity - or to stimulate their myelination (26,27). By modulating neuronal excitatory signaling, oligodendrocytes may play a role in migraine pathophysiology. Of note, a recent study identified myelin abnormalities in trigeminal nerves from migraine patients (28). Sensitization of the trigeminal system and subsequent abnormal processing of pain signals is likely to play a causal role in generation of migraine headache (29).

Subsequently, we studied the 100 functional gene sets within the glial and synaptic gene lists, and found that several astrocytic and oligodendrocytic gene sets were associated with migraine. Astrocyte dysfunction has already been implicated in migraine. For instance, the ATP1A2 protein, which is expressed in astrocytes in adults, is mutated in familial hemiplegic migraine type 2 (30), various sporadic hemiplegic migraine patients (31), and in two families with clustering of common migraine (32). Moreover, astrocytes have been shown to play a role in cortical spreading depression, the neurophysiological correlate of the migraine aura (33). For some of the astrocyte gene sets, the associations were mainly driven by the gene *GLI3*, for which the predominant expression in astrocytes (9) was recently confirmed by Zhang *et al.* 2014 (34). *GLI3* is involved in brain development, specifically the proliferation and cell fate specification of neural progenitors (35-37), and astrocyte function (37). Several migraine GWAS top hits pinpointed genes (i.e. *MEF2D*, *ASTN2*, *PRDM16*, *PHACTR1* and *TGFBR2*) that are involved in brain development as well (38). Notably, *GLI3* was recently shown to be differentially expressed in the cortex of two familial hemiplegic migraine type 1 mouse models (39), further supporting a relation between this gene and migraine pathophysiology.

A combined effect of all genes was observed for several astrocytic and oligodendrocytic gene sets. The oligodendrocytic gene sets “Signal transduction” and “Protein modification” might relate to the active role that oligodendrocytes can play in the regulation of the energy supply and possibly the activity of neurons (26,27). Interestingly, the “Miscellaneous” gene sets contain a large percentage of membrane-associated and transmembrane genes. Furthermore, *PHACTR1*, one of the migraine GWAS top hits, contributes together with *PHACTR3* strongly to the association of the oligodendrocytic “Miscellaneous” gene set. Both genes regulate

protein phosphatase 1, a protein with a main role in synaptic plasticity (40). This indicates that the genes from the “Miscellaneous” gene sets may have related functions involved in migraine that are not identified yet when classifying genes into GO-based functional categories.

There are limitations of the study design used. Foremost, the validity of GO to classify glia cell and synaptic genes into gene sets based on their function is inherently limited to current, surely incomplete, knowledge about gene functions as well as the incomplete use of this knowledge within GO. As a consequence, for instance, the size of the “Miscellaneous” gene sets is quite large. By using well-defined, expert-curated gene sets (mostly based on experimental data), we tried to do our utmost to minimize this limitation. Expected improvements and expansions of GO and similar public databases will increase the size and specificity of functional gene sets for future studies. A second limitation of this study is that by limiting our analyses to glia cell and synaptic gene sets, we potentially missed associations with other cell types, such as those involved in the brain vasculature, that have been implicated in migraine (41). The reason for this omission is that we intended to increase the reliability of the results by using only well-defined, expert-curated gene sets, which are not available for other cell types.

Our separate analyses for migraine with and without aura showed different results. Of all functional gene sets that associated with migraine, none associated with both subtypes. This limited overlap in genetic risk for both migraine subtypes was previously observed in the migraine GWA studies. For example, the only identified migraine with aura GWAS locus that contains the *MTDH* gene (2) showed no signal of association in the migraine without aura GWAS (3,5). This suggest that despite the clinical overlap, different and additional

genes may play a role and may explain the aura phenomenon. Of note, the diagnosis of migraine with aura patients is based on ICHD-IIIbeta guidelines and includes patients with migraine with aura that either experience an aura in all of their attacks and patients that experience also attacks without an aura (1). Differences between migraine with aura and migraine without aura identified in the current study may even be more pronounced when including only migraine with aura patients that experience an aura before each attack. At the moment, we lack genotyping data of a sufficiently large number of patients that exclusively have migraine with aura attacks to perform such an analysis.

For a similar reason we did not perform our analyses separately for males and females. The low percentage of males in our cohorts (ranging from 13% in the German migraine without aura cohort and 20% in the Finnish migraine with aura cohort) (Table 1), the male subgroup would be underpowered for our gene set analyses. It is uncertain whether such a gender analysis would have given different insights as: (i) heterogeneity analyses reported in the migraine GWAS meta-analysis (3) showed very low heterogeneity between female and male subgroups, and (ii) a SNP effect concordance analysis of migraine GWAS data (42) showed very high SNP concordance of GWAS signal between male and female subgroups.

In conclusion, our results indicate that astrocyte- and oligodendrocyte-related gene sets may play a role in migraine susceptibility, especially those involved in protein modification and signal transduction. Furthermore, our results suggest that migraine with and migraine without aura may be conferred by – at least partly – different genetic factors.

Acknowledgements

This work was supported by grants from the Netherlands Organization for Scientific Research (NWO) VIDI (ZonMw) [grant number 91711319 to G.M.T.]; the EUROHEADPAIN project [grant number 602633 to M.D.F., A.M.J.M.v.d.M., & A.P.]; the Center for Medical Systems Biology (CMSB) established in the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research (NGI/NWO) [grant number 050-060-409 to A.M.J.M.v.d.M.]. The Wellcome Trust [grant number 098051 to A.P.]; the Academy of Finland [grant number 251704 to A.P., and 139795 to M.W.]; the Academy of Finland, Center of Excellence in Complex Disease Genetics [grant number 213506 and 129680 to A.P.]; ENGAGE Consortium [grant agreement HEALTH-F4-2007-201413]; EU/SYNSYS- Synaptic Systems [grant number 242167 to A.P.]; the Sigrid Juselius Foundation (to A.P.); the Folkhälsan Research Foundation (to M.W.); the Medicinska Understödsföreningen Liv & Hälsa (to M.W.); the Helsinki University Central Hospital (to M.K., V.A); and NWO Complexity project [grant number 645.000.003 to C.A.d.L]. Statistical analyses were carried out on the Genetic Cluster Computer (<http://www.geneticcluster.org>), which is financially supported by the Netherlands Scientific Organization (NWO) [grant number 480-05-003 to D.P].

Conflict of Interest Statement

The authors report no conflict of interest

Article highlights

- Gene-based analysis of GWAS data indicated that astrocytes and oligodendrocytes are genetically involved in migraine pathophysiology;

- The association of functional gene sets differed between migraine with aura and migraine without aura, indicating that both migraine types – at least in part – have a different genetic background.

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Supplemental Tables

Supplemental Table 1: Association of all functional gene sets with migraine, migraine with and migraine without aura.

Supplemental Table 2: Gene-based analysis showing the contribution to the total association signal with migraine for each gene in the gene set.

Supplemental Table 3: Gene-based analysis showing the contribution to the total association signal with migraine with aura for each gene in the gene set.

Supplemental Table 4: Gene-based analysis showing the contribution to the total association signal with migraine without aura for each gene in the gene set.