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Research Submission

Migraine Genetic Variants Influence Cerebral Blood Flow

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Objective.—To investigate the association of migraine genetic variants with cerebral blood flow (CBF).

Background.—Migraine is a common disorder with many genetic and non-genetic factors affecting its occurrence. The exact pathophysiological mechanisms underlying the disease remain unclear, but are known to involve hemodynamic and vascular disruptions. Recent genome-wide association studies have identified 44 genetic variants in 38 genetic loci that affect the risk of migraine, which provide the opportunity to further disentangle these mechanisms.

Methods.—We included 4665 participants of the population-based Rotterdam Study (mean age 65.0 ± 10.9 years, 55.6% women). Cross-sectional area (mm^2), flow velocity (mm/s), and blood flow (mL/min) were measured in both carotids and the basilar artery using 2-dimensional phase-contrast magnetic resonance imaging. We analyzed 43 previously identified migraine variants separately and calculated a genetic risk score (GRS). To assess the association with CBF, we used linear regression models adjusted for age, sex, and total brain volume. Hierarchical clustering was performed based on the associations with CBF measures and tissue enrichment.

Results.—The rs67338227 risk allele was associated with higher flow velocity and smaller cross-sectional area in the carotids ($P_{minimum} = 3.7 \times 10^{-8}$). Other variants were related to CBF with opposite directions of effect, but not significantly after multiple testing adjustments ($P < 1.4 \times 10^{-4}$). The migraine GRS was not associated with CBF after multiple testing corrections. Migraine risk variants were found to be enriched for flow in the basilar artery ($\lambda = 2.39$).

Conclusions.—These findings show that genetic migraine risk is complexly associated with alterations in cerebral hemodynamics.

Key words: genetics, migraine disorders, perfusion imaging, cerebrovascular circulation, regional blood flow, brain imaging

Abbreviations: CBF cerebral blood flow, CI confidence interval, GRS genetic risk score, GWAS genome-wide association study, MRI magnetic resonance imaging, OR odds ratio

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INTRODUCTION

Migraine is a severe recurrent headache disorder affecting more than 800 million people worldwide.[1] Many genetic and non-genetic factors are known to be related to the risk of migraine, making it a complex multifactorial disease. Non-genetic factors affecting migraine risk include age and gender, as well as hormonal changes, diet, and stress among others. Genetic factors underlying its occurrence include 44 genetic variants in 38 independent genetic loci, which have been identified in recent genome-wide association studies (GWAS).[2] However, the biological pathways these genetic factors are acting upon remain poorly understood.

Most etiologic research on migraine revolves around 2 leading hypotheses, ie, the neuronal and the vascular hypothesis.[3] Briefly, the neuronal hypothesis postulates that migraine is caused by neuronal events with changes in the cerebral blood flow (CBF) as a consequence, whereas the vascular hypothesis states that vascular and hemodynamic changes are the causal trigger of migraine with subsequent activation of perivascular nerves.[4] Hemodynamic changes in the acute phase of the disease include vasospasm and constriction, but in the interictal period between migraine attacks, the blood flow to the brain is increased in patients with migraine compared to healthy individuals.[5]

Recently, genetic loci underlying migraine have shown to be enriched for both vascular and neuronal pathways.[2] Now, more and more ground has been gained by the "neurovascular hypothesis," assuming an interplay of both vascular and neuronal factors to be involved in the development of migraine.[6,7] Yet, while many genetic loci have been replicated to associate with migraine, it remains unclear how these affect cerebral hemodynamics.

Therefore, in this study, we further investigated the relationship between genetic variation associated with migraine and cerebral vascular changes, which may underlie the pathophysiology of migraine.

*These authors contributed equally to this work.

Conflicts of Interest: None

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MATERIAL AND METHODS

Study Population.—This study was conducted within the Rotterdam Study, an ongoing population-based cohort study which aims to investigate the causes and determinants of diseases affecting elderly people.[8] This longitudinal cohort study was initiated in 1990 and extended in 2000 and 2006, with a total of 14,926 participants aged 45 years and over. At baseline, blood samples were drawn to perform genotyping (N = 11,496). In 2005, magnetic resonance imaging (MRI) was added to the study protocol, and all individuals were invited to undergo MRI scanning from that time onward. Since this scanning protocol was implemented 15 years after the initiation of the Rotterdam Study in 1990, brain imaging was only available in a subset of 4865 individuals with genotyping data. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and the Ministry of Health, Welfare and Sports of the Netherlands (Population Screening Act WBO, license number 1071272-159521-PG). All participants have given written informed consent.[8]

We included 4865 participants for whom both genotyping and phase-contrast brain MRI was performed between 2005 and 2015. Of those, participants with cortical brain infarcts (N = 200) were excluded from the analyses, since this can interfere with imaging processing which can consequently limit the reliability of MRI-derived metrics.

Genotyping.—At baseline, genotyping was performed in 11,496 individuals of the Rotterdam Study using Illumina 550K, 550K duo, or 610 quad arrays. The generation and management of genotype data for the Rotterdam Study were executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands. Samples with a call rate below 97.5%, as well as gender mismatches, excess autosomal heterozygosity, duplicates or family relations, ethnic outliers, variants with call rates lower than 95.0%, failing missingness test, Hardy-Weinberg equilibrium $P < 10^{-6}$, and allele frequencies below 1%, were removed. Genotypes were imputed using the MaCH/minimac software to the 1000 Genomes phase I version 3 reference panel.[9]

Genetic Risk Scores.—We calculated a weighted genetic risk score (GRS) using the summary statistic data of the most recent migraine GWAS meta-analysis.[2] We included all independent genome-wide significant autosomal variants (N = 43) and excluded rs12845494 located on chromosome X, since imputations of X chromosome variants were not available. For these genetic variants, we multiplied the reported effect estimate by the allele dosage for every participant. These weighted effects were then added up to create a GRS, which was subsequently standardized into a *Z*-score.

Measurement of CBF.—The brain MRI procedure has been described in more detail elsewhere.[10] In short, 2-dimensional phase-contrast imaging was performed on a dedicated 1.5 tesla MRI scanner. In the basilar artery and in the left and right carotid arteries, the vessel's cross-sectional area (mm^2) measured, as well as flow velocity (mm/s) and blood flow (mL/min), were measured.[11] These values were standardized to facilitate comparisons across individuals. Values more than 3.5 standard deviations from the mean were considered outliers and excluded from the analyses. Total CBF was calculated by adding up the blood flow estimates of the 3 arteries (mL/min). Total brain volume was computed as the sum of gray matter, white matter, and white matter lesion volumes, as obtained by the *k*-nearest neighbor classifier.[12]

Migraine Assessment.—Migraine assessment was done either by home or phone interview. We used a questionnaire based on the International Classification of Headache Disorders – 2nd edition (ICHD-II) migraine headache criteria,[13] slightly modified from the validated questionnaire of the Genetic Epidemiology of Migraine Study.[14] Migraine was defined as a lifetime occurrence of more than 5 headache attacks with a duration of 4-72 hours when untreated, accompanied with typical characteristics of migraine headaches and photophobia or nausea. Migraine was classified as the active migraine subtype if the last migraine attack occurred less than 1 year ago. The probable migraine subtype was defined as having 2 or more attacks and all other above-mentioned criteria, or persons with 5 attacks with a duration of 4-72 hours and all but one of the other criteria.

Data Analysis.—As a validation of the migraine GWAS results, we performed logistic regression analyses adjusted for age and sex to estimate the association of the migraine GRS with migraine, as well as migraine subtypes. Linear regression models were used to assess the association of the GRS and genetic variants separately with CBF parameters, adjusted for age, sex, and total brain volume. Since CBF measures within and between blood vessels in the brain can be correlated, we performed permutation testing (N = 10,000) to define the number of independent CBF measures. Hereto, we rerun the above-mentioned linear regression models for the different CBF parameters, and replaced the genetic variables with a random variable, which we repeated 10,000 times. Each time, the minimum *P* value of all regressions for the different outcomes was saved. These *P* values were sorted, and the *P* value threshold dividing the 5% lowest and the 95% highest *P* values was defined. Subsequently, we divided .05 by this threshold in order to calculate the number of independent tests. This showed that, out of the 10 measures for CBF (cross-sectional area, flow velocity and blood flow in 3 arteries, and total CBF), 8.2 were independent. This number was used to correct for multiple testing, resulting in a *P* value threshold of 6.1×10^{-3} (.05/8.2) for the GRS analyses. In the single-variant analyses, we additionally adjusted for the number of genetic variants tested, which resulted in a *P* value threshold of 1.4×10^{-4} (.05/[43 × 8.2]), because these 43 genetic variants were previously shown to be located in independent loci. Since individual genetic variants may be in the same pathway, and thus act in concordance with each other, we grouped the 43 genome-wide significant autosomal variants using hierarchical clustering. In a first approach, clusters were created based on their association with vessel area, velocity, and flow in the basilar and carotid arteries, in order to create groups of genetic variants with similar effects on CBF. In a second approach, we created groups by clustering the genetic variants according to similarities in their associated genes' *P* value for tissue enrichment in brain, vascular, and gastrointestinal tissues from the GTEx collection, as previously reported.[2] We created a tanglegram to visualize the overlap be-

tween the clusters created by the 2 different hierarchical clustering approaches, enabling us to explore whether genetic variants with similar tissue enrichments also had similar associations with CBF parameters. Additionally, we created GRS of the genetic variants in the different tissue clusters and studied the combined effect of the variants by relating these GRS to CBF measures. Still, effects on CBF within tissue clusters may be heterogeneous. Therefore, we also studied the enrichment of the genetic variants' associations for CBF in the different tissue clusters by comparing their genomic inflation factor λ for the different CBF measures, and calculated confidence intervals around the null, ie, $\lambda = 1$, using 10,000 permutations. R version 3.2.5 was used to create GRS, and to perform logistic and linear regression analyses, hierarchical clustering, and permutation testing. The R package "dendextend" was used to perform hierarchical clustering.

Table 1.—Descriptive Statistics

Variable	N = 4665
Age (years), mean ± SD	65.0 ± 10.9
Female, N (%)	2592 (55.6)
Migraine, N (%)	703 (16.7)
Migraine with aura	145 (4.0)
Active migraine	282 (7.4)
Probable migraine	177 (4.8)
Total CBF (mL/min), mean ± SD	521.6 ± 101.1
Basilar artery	
Area (mm^2), mean ± SD	28.7 ± 5.0
Flow (mL/min), mean ± SD	1.7 ± 0.6
Velocity (mm/s), mean ± SD	11.1 ± 4.4
Left carotid artery	
Area (mm^2), mean ± SD	35.4 ± 6.7
Flow (mL/min), mean ± SD	3.5 ± 0.9
Velocity (mm/s), mean ± SD	15.1 ± 4.6
Right carotid artery	
Area (mm^2), mean ± SD	36.3 ± 7.0
Flow (mL/min), mean ± SD	3.5 ± 0.9
Velocity (mm/s), mean ± SD	14.8 ± 4.5

N = number of participants; SD = standard deviation.

RESULTS

We included 4665 participants of the Rotterdam Study with an average age of 65.0 ± 10.9 years, of which 55.6% were women, and of which 16.7% were classified as having migraine. A complete overview of the descriptive statistics is presented in Table 1.

Validation GRS Migraine.—The GRS for migraine was associated with an increased risk of migraine (adjusted odds ratio [OR] 1.30, 95% confidence interval [CI] 1.19-1.41) and the different migraine subtypes (Fig. 1). Also, a quantile-quantile plot of the 43 migraine risk variants showed enrichment for migraine ($\lambda = 2.39$) (Fig. 2). Since there was a small overlap of individuals who were included in the

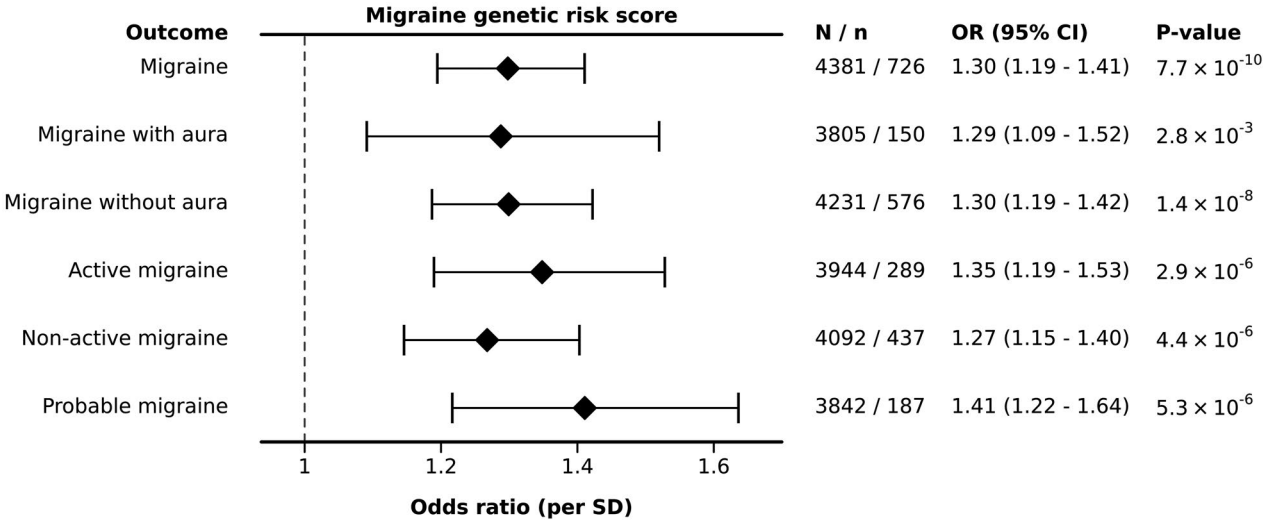

Fig. 1.—Associations between the migraine GRS and migraine diagnoses. Forest plot showing association results between the migraine GRS and different migraine subtypes. Confidence interval (CI); sample size (N); number of cases (n); odds ratio (OR); standard deviation (SD).

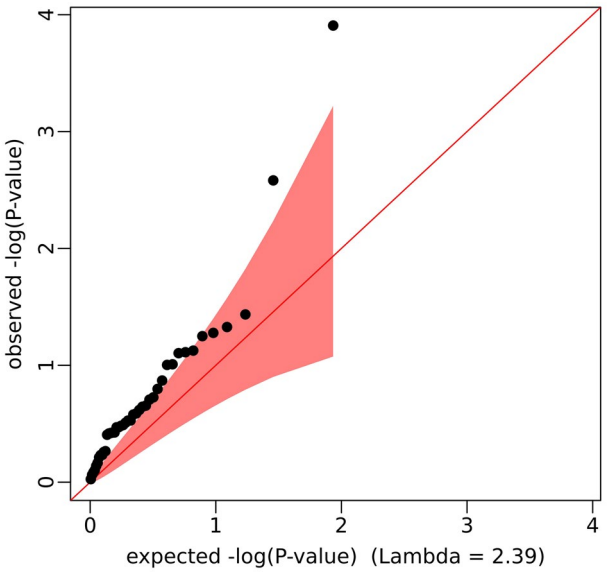

Fig. 2.—Quantile-quantile plot of the migraine genetic variants and prevalence of migraine. [Color figure can be viewed at wileyonlinelibrary.com]

original migraine GWAS (0.6% of the total GWAS sample), we also removed this subset to verify that there was no overfitting and found similar effect estimates (Supporting Table S1).

Migraine Genetic Variants and CBF.—The migraine GRS was associated with blood flow in the basilar artery ($\beta = 0.03$, $P = .040$), although this did not survive correction for multiple testing ($P < 6.1 \times 10^{-3}$) (Fig. 3). One genetic variant, rs67338227, was significantly related to smaller vessel area and higher velocity in both carotid arteries (minimal $P = 3.7 \times 10^{-8}$), but no relation was found with blood flow in any of the arteries. Interestingly, other genetic variants showed associations with CBF measures in the opposite direction of effect compared to rs67338227 (minimal $P = 2.6 \times 10^{-4}$) (Supporting Table S2). These associations survived adjustments for the number of independent outcomes under investigation, but were not significant after adjusting for both the number of independent outcomes and the number of genetic variants tested ($P < 1.4 \times 10^{-4}$).

Hierarchical Cluster Analysis.—Because the genetic variants' risk alleles showed heterogeneous associations with CBF, we hierarchically clustered the variants according to their estimated effect on measures of CBF (Supporting Fig. S1 and Supporting Table S3). Two out of the 4 created clusters mainly included variants associated with CBF; 1 cluster mainly contained genetic

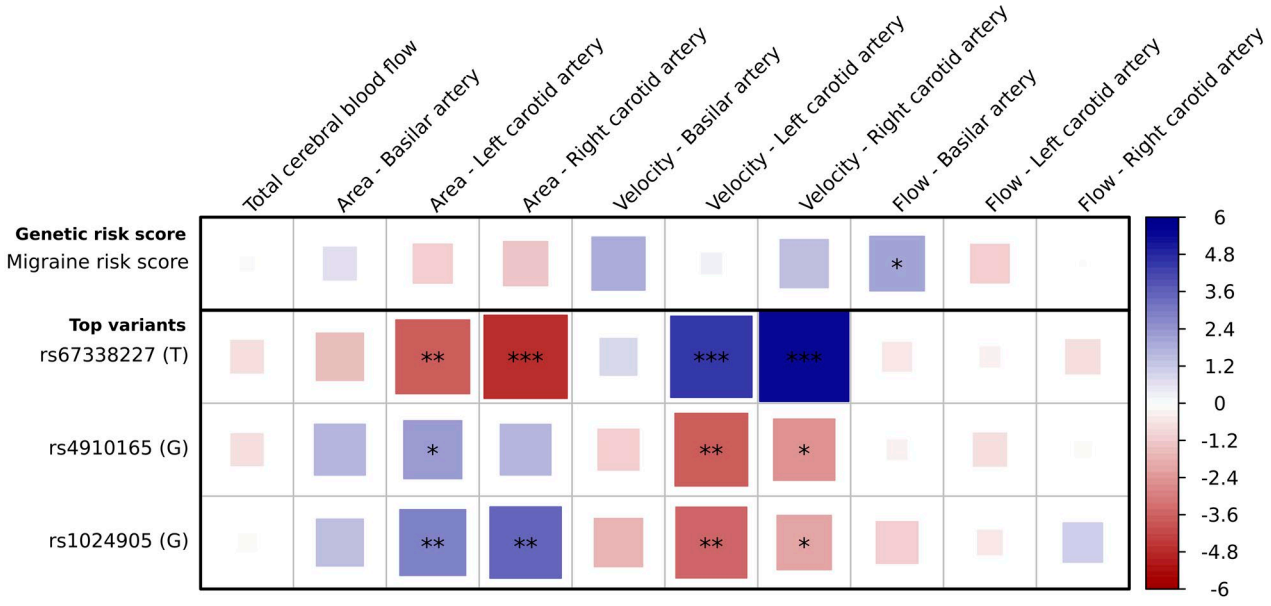

Fig. 3.—Associations between genetic risk variants for migraine and CBF. Plot showing association results between the migraine GRS and the 3 top genetic variants. Colors and sizes of the blocks correspond to *t* values, with blue and red indicating positive and negative associations, respectively. Larger blocks indicate stronger associations, and significance levels are noted by asterisks: * for *P* value <.05, ** for *P* value <.05/8.2 = 6.1×10^{-3}, and * for *P* value <.05/(43 × 8.2) = 1.4×10^{-4}. [Color figure can be viewed at wileyonlinelibrary.com]**

variants linked to larger vessel areas and lower velocities, whereas the other cluster primarily contained variants related to smaller vessel areas and higher velocities. We additionally clustered the genetic variants according to the tissue enrichment in brain, vascular, and gastrointestinal tissues of their associated genes, which was available for 39 genetic variants (Supporting Table S4 and Fig. 4). Four clusters were created, containing genetic variants linked to genes with enrichment in the following tissues: enrichment in all 3 tissues (brain, vascular, and gastrointestinal; N = 9); enrichment in brain tissue (N = 5); enrichment in vascular tissue

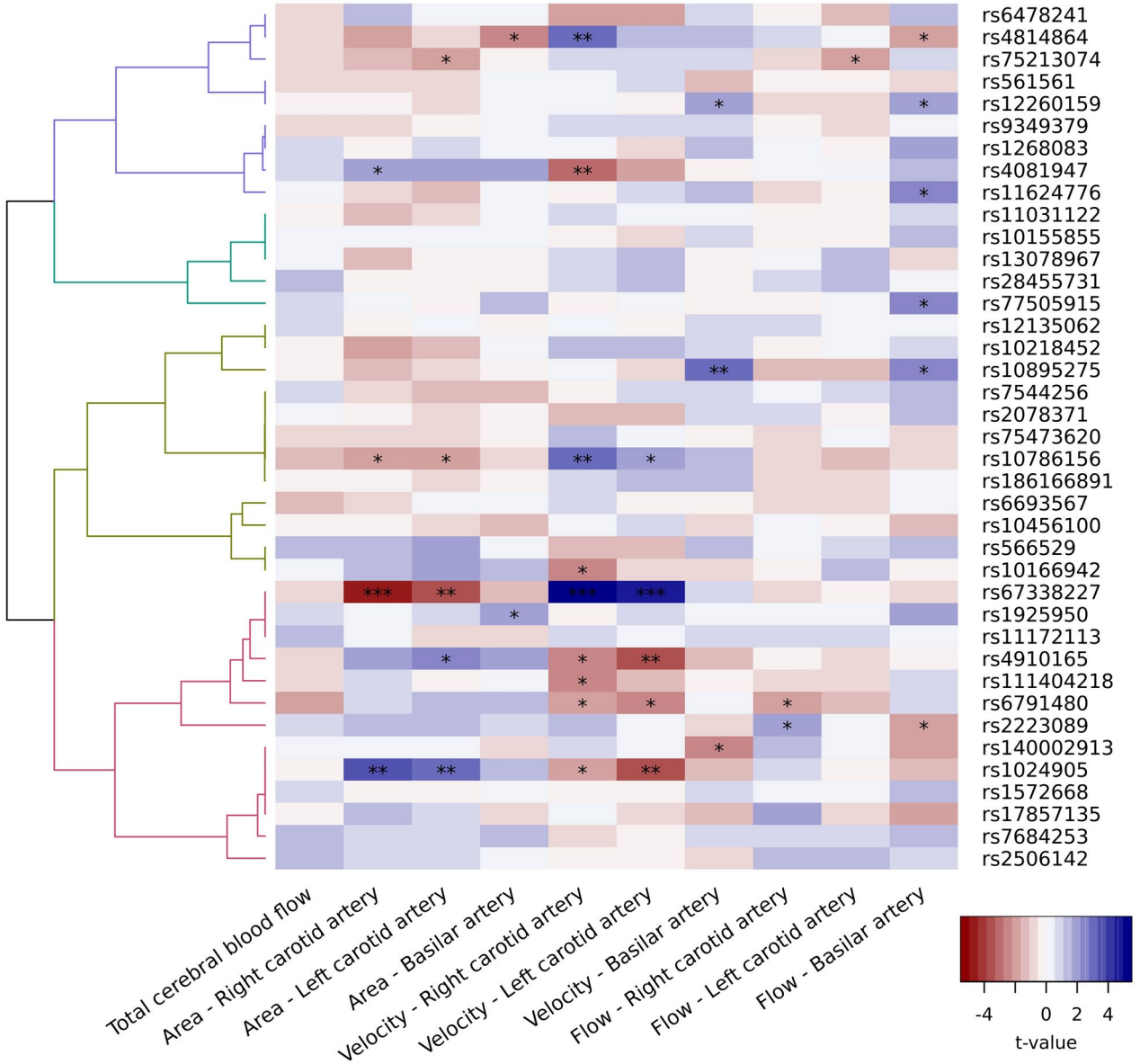

Fig. 4.—Associations between genetic risk variants for migraine and CBF, hierarchically clustered based on the enrichment of their associated genes in brain, vascular, and gastrointestinal tissues. Plot showing association results between 39 autosomal migraine genetic risk variants and measures of CBF, including a dendrogram based on the tissue enrichment in brain, vascular, and gastrointestinal tissues of their associated genes. The genetic variants in each cluster are linked to genes that are differentially enriched in tissues: vascular, brain, and gastrointestinal tissues (purple branches); brain tissue (green branches); vascular and gastrointestinal tissues (yellow branches); vascular tissue (pink branches). Colors and sizes of the blocks correspond to *t* values, with blue and red indicating positive and negative associations, respectively. Significance levels are noted by asterisks: * for *P* value <.05, ** for *P* value <.05/8.2 = 6.1 × 10^{-3}, and * for *P* value <.05/(43 × 8.2) = 1.4 × 10^{-4}. [Color figure can be viewed at wileyonlinelibrary.com]**

(N = 13); enrichment in vascular and gastrointestinal tissues (N = 12). As shown in Supporting Table S5 and Supporting Fig. S2, the grouping based on CBF and tissue enrichment clustering were not strongly correlated. However, the cluster with enrichment in vascular tissue only (N = 13) included all 3 top genetic variants presented in Figure 3. GRS combining the associations of the genetic variants in these clusters did not relate to CBF measures significantly (Fig. 5). Nonetheless, we did observe enrichment of the migraine risk variants for flow in the basilar artery ($\lambda = 2.39$) (Fig. 6, Supporting Table S6 and Supporting Fig. S3), which

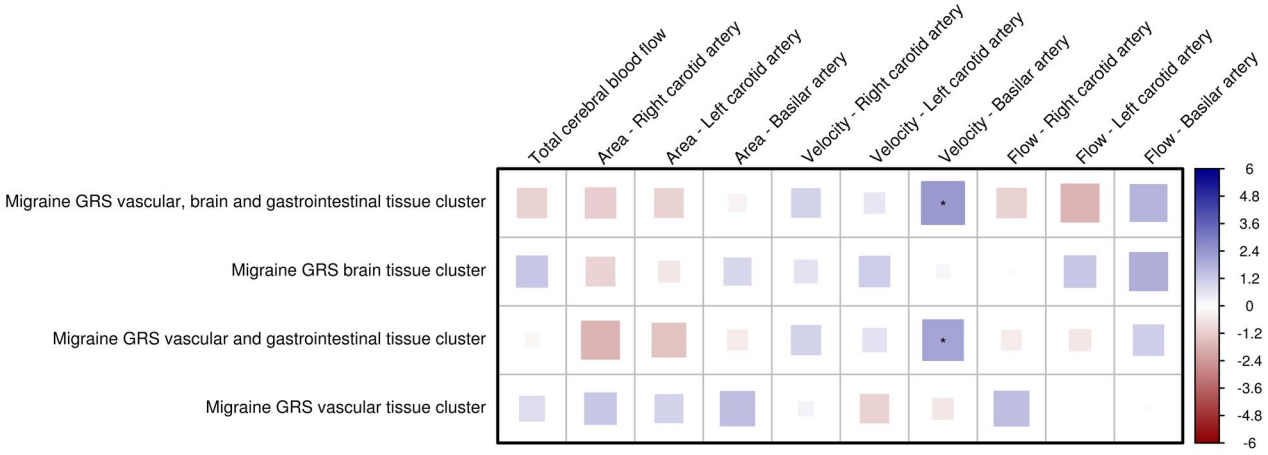

Fig. 5.—Associations between 4 migraine GRS, containing genetic variants based on tissue enrichment patterns, and CBF. Plot showing association results between migraine GRS and measures of CBF. Clusters of 39 genome-wide significant genetic variants for migraine were created based on the tissue enrichment in brain, vascular, and gastrointestinal tissues of their associated genes, as presented in Figure 4. Weighted GRS of the genetic variants in each cluster were calculated to estimate the combined effect of the variants in each cluster. Colors and sizes of the blocks correspond to *t* values, with blue and red indicating positive and negative associations, respectively. Larger blocks indicate stronger associations, and significance levels are noted by asterisks: * for *P* value <.05, ** for *P* value <.05/8.2 = 6.1 × 10^{-3}. Genetic risk score (GRS). [Color figure can be viewed at wileyonlinelibrary.com]

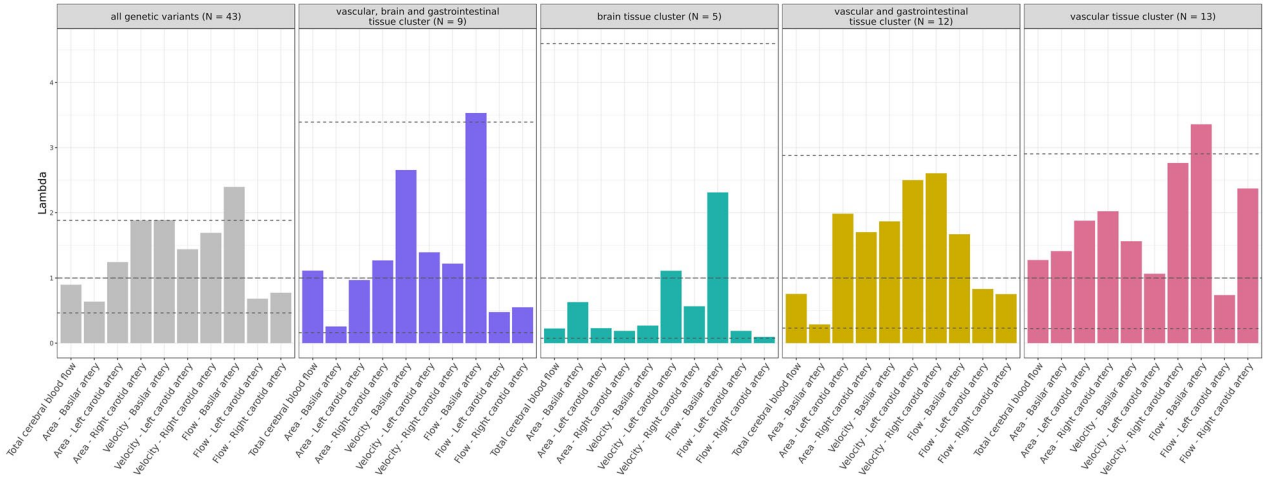

Fig. 6.—Enrichment of the migraine genetic variants for measures of CBF, overall and stratified by tissue enrichment cluster. Plot showing enrichment results of migraine genetic risk variants for measures of CBF. The overall λ for all 43 genetic variants is represented, as well as for clusters of variants linked to genes that are differentially enriched in tissues: vascular, brain, and gastrointestinal tissues (cluster 1, purple bars, N = 9); brain tissue (cluster 2, green bars, N = 5); vascular and gastrointestinal tissues (cluster 3, yellow bars, N = 12); vascular tissue (cluster 4, red bars, N = 13), as shown in Figure 4. Confidence intervals around the null, ie, $\lambda = 1$ (long-dashed gray lines), are shown in gray dashed lines. [Color figure can be viewed at wileyonlinelibrary.com]

was equal to the enrichment observed for migraine. The enrichment was even stronger in the first cluster, containing variants nearby genes expressed in brain, gastrointestinal, and vascular tissues (λ = 3.53), and in the fourth cluster, containing variants nearby genes expressed in vascular tissue (λ = 3.36).

DISCUSSION

In this study, we found that the known migraine variant rs67338227 was significantly associated with CBF measures after adjustments for multiple testing ($P < 1.4 \times 10^{-4}$). Other genetic variants identified for migraine showed associations in opposite directions of effect although they did not survive multiple testing correction. Significant enrichment was observed for the migraine variants' associations with flow in the basilar artery.

We found that genetic variants associated with migraine also showed associations with CBF. This finding builds upon a previously performed gene enrichment analysis, which showed enrichment of migraine variants for genes expressed in vascular and smooth muscle tissue.[2] Also, migraine has been shown to genetically overlap with other vascular phenotypes, such as coronary artery disease and ischemic stroke.[15,16] Moreover, in a GWAS for CBF performed in the Rotterdam Study, the 2 genome-wide significant variants were located in loci that had earlier been associated with migraine,[17] which already suggested a genetic overlap between migraine and CBF. This study thus adds to the evidence supportive of structural hemodynamic changes involved in migraine.

Interestingly, significant associations were observed between individual migraine variants and CBF, but not for the migraine GRS including all autosomal genome-wide significant variants for migraine. Usually, a GRS is considered a measure of the combined effect of multiple genetic variants. An underlying assumption is thus that the genetic variants' magnitude and direction of effect for the association with the outcome of interest is similar or equal to the effect estimate used to create the GRS. In our study, the individual variants indeed showed associations in the same direction in relation to migraine, but varying effect estimates in opposite directions were observed when the variants were related to measures of CBF. This characterizes the complex relation between migraine and CBF, and is in line with previous studies that showed both hypo- and hyperperfusion in migraine patients.[18,19] The varying associations with CBF might be attributed to a distorted autoregulation in people with migraine, resulting in higher fluctuations in CBF, as previous studies already suggested.[20,21] More research is needed to further explore possible pathways through which the identified migraine variants may affect CBF. A formal mediation analysis could be performed in future research to examine whether the migraine genetic variants are affecting disease risk through CBF alterations. Moreover, genetic variants that did not reach genome-wide significance for migraine could be taken into account, since this would increase the explained variance for migraine, and may therefore give a more complete picture of their association with CBF as well. Although clusters of genetic variants linked to genes with enrichment in vascular tissues contained the genetic variants with the strongest CBF associations, the combined effect estimates of the different clusters on CBF remained marginal. Therefore, alternative methods to separate clusters of genetic variants based on their pathophysiology could be considered, which in turn may help elucidating the different biological pathways leading to migraine. Furthermore, lead genetic variants identified in a GWAS are not necessarily the causal variant associated with disease. Hence, studies investigating which variants or genes are most likely causal would be valuable to gain more insight into the role of cerebral hemodynamics in migraine pathophysiology.

Findings of this study suggest that total CBF may not be a comprehensive measure when studying vascular changes involved in migraine. Although many nominal significant associations were shown between migraine risk variants and CBF measures, no nominal significant associations were found for total CBF. We showed that migraine variants associated with the vessel's area and its blood velocity with opposite directions of effects, resulting in a null effect on its blood flow since flow is a cross product of area and velocity. This observation is in line with findings of the CBF GWAS, in which one of the hits (rs2971609) was associated with vessel area and flow velocity, but had a null effect on the vessels'

flow rate.[17] Additionally, the migraine variants' associations were often not similar across blood vessels, consistent with previous studies that showed location-specific or asymmetric changes in CBF measures.[22-24] Consequently, blood flow might not seem to be affected when not all CBF measures are examined together, whereas there are in fact hemodynamic changes present in the brain.

Pathways associated with migraine include both neuronal and vascular components,[25] suggesting a shared vascular and neuronal component underlying the development of migraine. In our study, the genetic variant rs67338227, located in the 4 and a half LIM domains 5 and UFM1-specific ligase (*FHL5*/*UFL1*) locus, showed a significant association with measures of CBF. *UFL1* produces a protein located in the endoplasmic reticulum membrane, and is involved in hematopoietic stem cell function and hematopoiesis in mice,[26,27] as well as signaling pathways including NF-κB signaling and protein ufmylation.[28] *FHL5* produces a protein that interacts with the transcription factor cAMP-responsive element modulator.[29] Although this gene has mainly been described in the context of spermatid differentiation into mature spermatozoa, it is also highly expressed in vascular tissue.[30] Altogether, *FHL5*/*UFL1* is a plausible locus involved in the link between migraine and CBF as it is involved in both neuronal and vascular pathways. Functional studies are needed to further look into mechanisms through which these genes could affect both migraine and CBF, and whether intervening in these pathways could influence migraine symptoms or occurrence.

Strengths of this study are the large sample size and the population-based setting, which allowed us to include a broad spectrum of people with and without migraine, ie, also including those suffering from migraine not diagnosed by a medical specialist. There are also limitations we need to take into consideration. First, we only assessed CBF during the interictal period of participants with migraine, which did not allow us to look at the effect of the genetic variants on CBF during a migraine attack. Second, we only measured CBF in 3 large brain vessels, whereas vascular changes in smaller brain vessels may be more critical in pathophysiological mechanisms underlying migraine. Yet, this has most likely led to an underestimation of the effect estimates found. Finally, a part of the study population (N = 2392) was included in the discovery GWAS for migraine. However, this only accounts for 0.6% of the total study population in the meta-analysis,[2] and in a sensitivity analysis excluding overlapping participants, we did not observe substantial differences of the associations.

In conclusion, in this study, we found enrichment of migraine genetic variants for measures of CBF. These findings are supportive of an involvement of structural cerebral hemodynamic changes in migraine pathophysiology. Future studies are needed to further elucidate pathways through which CBF is influenced by migraine variants.

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