



Association between *PNPLA3* rs738409 G variant and MRI cerebrovascular disease biomarkers

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ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) has been associated with greater cerebral white matter hyperintensity (WMH) volume and microbleeds. The adiponutrin (*PNPLA3*) rs738409 G variant, a robust NAFLD susceptibility variant, has been variably associated with carotid atherosclerosis. We hypothesized that this variant is associated with WMH volume, microbleeds, covert brain infarction (CBI), and small perivascular spaces.

Methods: We performed a cross-sectional analysis of the Northern Manhattan Study-MRI Substudy. The associations between the rs738409 G variant allele and outcomes were assessed using linear regression for WMH volume, logistic regression for microbleeds and CBI, and Poisson regression for small perivascular spaces. Models were adjusted for age, sex, principal components, diabetes, and body mass index.

Results: We included 1063 Northern Manhattan Study participants who had brain MRI and genotype data available (mean age 70 ± 9 years, 61% women). The G allele frequency was 24%. The prevalence of any microbleeds and CBI were 8% and 18%, respectively. The median WMH volume and small perivascular space count score were 7.7 mL and 6, respectively. GG homozygosity, but not heterozygosity, was associated with WMH volume ($\beta = 0.27$; 95% CI, 0.03, 0.51) compared to non-carriers. Having at least one G allele was associated with the presence of microbleeds (Odds ratio, 1.78; 95% CI, 1.02, 3.12); the association was attenuated in other models. No associations were observed for CBI and small perivascular spaces.

Conclusion: The *PNPLA3* rs738409 G allele was associated with greater WMH volume, and inconsistent associations with microbleeds were seen.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) affects approximately 30% of Americans [1]. Some studies have reported an independent association between NAFLD and incident cardiovascular disease and mortality [2,3]. While a clear association between NAFLD and clinical stroke has not emerged [4,5], NAFLD has been associated with atrial fibrillation [6] and subclinical vascular disease including coronary artery disease [7–9], and carotid artery plaque and intimal media thickness [10]. Additionally, NAFLD, particularly when accompanied by liver fibrosis, has been associated with cerebral white matter

hyperintensities and microbleeds [11–13].

NAFLD is multifactorial. In addition to its association with obesity and diabetes, strong genetic risk factors have been identified [14,15]. The best-established NAFLD susceptibility variant is a single nucleotide polymorphism in the adiponutrin gene (*PNPLA3* [patatin-like phospholipase 3]; rs738409[C > G]) [14]. This variant is thought to impair lipid processing and mobilization, thereby resulting in hepatic lipid retention and lower circulating lipid levels, particularly low density lipoprotein (LDL) [16,17]. However, this variant is also associated with the development of nonalcoholic steatohepatitis [18] – an inflammatory, fibrotic form of NAFLD – which is known to be a strong

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determinant of cardiovascular mortality risk among individuals with NAFLD [2,19]. The impact of these seemingly opposing mechanisms on cerebrovascular disease are unclear.

Data regarding the association between the *PNPLA3* variant and cerebrovascular disease are limited and conflicting [20–22]. One study of Italian individuals reported an association with carotid intimal media thickness among younger individuals [22]. We found no studies that have investigated associations with brain magnetic resonance imaging (MRI) biomarkers of cerebrovascular disease, which include white matter hyperintensities (WMH), cerebral microbleeds, covert brain infarction (CBI), and small dilated perivascular spaces. In a cross-sectional analysis of the Northern Manhattan Study, we tested the hypothesis that *PNPLA3* rs738409 variant is associated with these brain MRI cerebrovascular disease biomarkers.

2. Materials and methods

2.1. Study population

We performed a cross-sectional analysis using data from the Northern Manhattan Study (NOMAS) – MRI Substudy. NOMAS is an ongoing, population-based cohort study of incident stroke in a racially and ethnically diverse population [23]. The NOMAS cohort was recruited from 1993 to 2001 by random digit dialing; community members were eligible for participation if they were stroke-free, age 40 or older, and resided in a Northern Manhattan home with a telephone. Participants underwent baseline interview, examination, and phlebotomy. They were followed annually by telephone for change in vital status, incident stroke, and other illnesses. The Institutional Review Boards at Columbia University Medical Center and the University of Miami approved the study. All participants provided written informed consent to participate.

This analysis was performed on the NOMAS-MRI Substudy sample, which was comprised of NOMAS participants and household members recruited to undergo brain MRI. In total, 1290 stroke-free participants age 50 or older and without contraindication to MRI were recruited for the MRI sub-study between 2003 and 2008; 1091 individuals were recruited from the original NOMAS prospective cohort, and 199 were their unrelated household members. Participants underwent history, examination, and phlebotomy at the time of MRI. We included all participants who underwent brain MRI and had available genotype data for *PNPLA3*. The sample size was fixed by the dataset; however, post hoc power calculations were performed to estimate necessary sample sizes for crude comparisons: using SAS Proc Power, we calculated that, for an alpha level of 0.05 and in order to achieve a power of 0.80, 782 total subjects would be needed to detect a partial correlation between *PNPLA* allele copy number and continuous outcomes of 0.1. For binary outcomes with reference proportion of 0.20, for an alpha level of 0.05, and in order to achieve a power of 0.80, 535 subjects would be needed per dichotomized genotype group to detect an odds ratio of 1.5.

2.2. Measurements

The genotyping procedures for NOMAS have been previously described [24]. In brief, stored frozen DNA samples were obtained from whole blood extraction and processed at the University of Miami according to Affymetrix procedures (Affymetrix Genome-Wide Human SNP Array 6.0) at the Genotyping Core of the John P. Hussman Institute for Human Genomics at the University of Miami. Genotype calling was performed using Affymetrix Power Tools v.1.15.0. *PNPLA3* allele frequencies were imputed with high fidelity.

We tabulated age, sex, race-ethnicity, vascular risk factors, and use of anti-thrombotic drugs and statin medications at the time of MRI. Race-ethnicity was self-reported, and principal component analysis was used to account for population-specific variation in allele frequencies [25]. Specifically, the principal components that were significantly

associated with *PNPLA3* allele frequencies ($P < .05$) were included in models as covariates to account for population substructure. Body mass index, hypertension, diabetes, and hypercholesterolemia were determined using self-reported diagnoses, medication use, examination, blood pressure measurement, and laboratory testing [26]. To determine whether lipid fractions differed with respect to the number of *PNPLA3* variant alleles in our sample, lipid fractions were also tabulated.

Participants underwent 1.5-Tesla brain MRI (Philips Medical Systems, Best, The Netherlands). The MRI processing protocol for NOMAS has been published [27–30]. Four MRI biomarkers of cerebrovascular disease were measured: WMH volume, cerebral microbleeds, CBI, and small dilated perivascular spaces. Microbleeds, CBI, and small perivascular spaces were adjudicated by vascular neurologists; WMH volume measurement was automated. WMH volume, CBI, and small perivascular spaces were adjudicated for all MRI scans, and microbleeds were adjudicated for 769 participants with available MRI gradient echo sequence data [31]. WMH volume was calculated by a semi-automated method with a QUANTUM 6.2 package on an Ultra 5 workstation (Sun Microsystems, Santa Clara, California). Cerebral microbleeds were adjudicated using the Brain Observer MicroBleed Scale applied to T2* gradient echo sequence images acquired in the coronal plan, as previously described [31]. In this analysis, microbleeds were categorized as present or absent. CBI was defined by the presence of a fluid-attenuated inversion recovery sequence lesion of at least 3 mm in size that was distinct from any adjacent vascular structures. As previously reported, there was excellent interrater agreement [30]. CBI was treated as a binary variable. Last, small dilated perivascular spaces (otherwise referred to as small perivascular spaces) were adjudicated using a previously published method [29]. Small perivascular spaces – parenchymal voids ≤ 3 mm in diameter on T1 images – were counted for a total score of 0 to 26 based on counts in 13 separate anatomic areas. The interrater agreement was excellent [29].

2.3. Statistical methods

Baseline characteristics were compared using standard descriptive statistics; categorical variables were compared with the chi-squared test, means were compared with Tukey's method, and medians were compared using the Kruskal-Wallis test. Multivariable regression models were created for each outcome of interest to calculate β parameters (and odds ratios for logistic models) and their 95% confidence intervals (CI). Linear regression was used for WMH volume, logistic regression for microbleeds and CBI, and Poisson regression for small perivascular space score. Models were incrementally adjusted: 1) unadjusted; 2) adjusted for age, sex, and principal components; and 3) additionally adjusted for body mass index and diabetes. Body mass index and diabetes were included as possible confounders in regression models given the interrelatedness of NAFLD and these conditions. Lipid levels were conceptualized as mediators given the known effects of this *PNPLA3* variant on lipid levels [17] and were therefore not included as possible confounders. WMH volume was natural log-transformed and adjusted to account for total intracranial volume in all models. Using these models, two genetic inheritance models were evaluated. First, in an additive model, variant homozygous (GG) individuals and heterozygous (GC) individuals were separately compared to non-carriers (CC). Second, in a dominant model, individuals with at least one variant allele (GG or CG) were compared to non-carriers (CC). These comparisons were specified based on the pattern of limited prior observations of associations between *PNPLA3* and premature coronary artery disease in the additive model and coronary artery calcification in the dominant model [32].

Two post-hoc models were separately created: 1) models were additionally adjusted for low density lipoprotein (LDL) levels; 2) models were adjusted for race/ethnicity in addition to principal components.

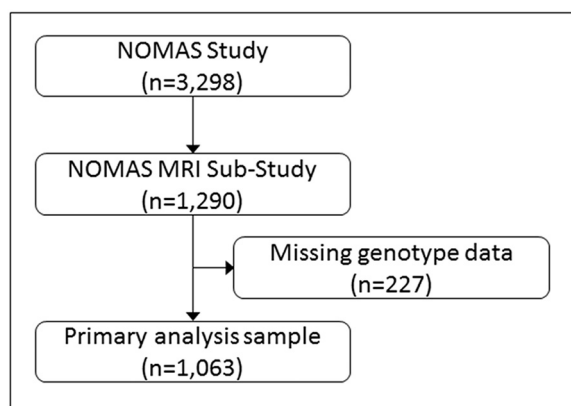


Fig. 1. Study population flow diagram.

The MRI Sub-Study cohort was assembled from the participants of the original Northern Manhattan Study cohort and their household members. Abbreviations: NOMAS, Northern Manhattan Study.

3. Results

From the 1290 participants in the NOMAS MRI-Substudy, we included 1063 participants with available genotype data (mean age 70 ± 9 years, 61% women) (Fig. 1).

There were no significant differences between those with and without missing data except that Hispanics were less likely to have missing genotype and microbleed data (Supplemental Tables 1 and 2).

There were 67 (6%) participants with two variant *PNPLA3* G alleles, and 381 participants (36%) who were heterozygous; the G allele frequency was 24%. Hispanics were more likely to have G variant alleles, and there were no other significant differences between genotype groups (Table 1). Lipid fraction levels did not significantly differ between genotype groups.

The median WMH volume was 7.7 mL (interquartile range [IQR], 2.3–8.5 mL). Having two variant G alleles was associated with greater WMH volume when compared to non-carriers after adjusting for age, sex, principal components, and total intracranial volume ($\beta = 0.26$; 95% CI, 0.02, 0.50) and after additionally adjusting for diabetes and body mass index ($\beta = 0.27$; 95% CI, 0.03, 0.51) (Table 2). This corresponds with a 31% greater WMH volume in GG individuals as compared to non-carriers. In post hoc models including hypertension, the association was similar ($\beta = 0.24$; 95% CI, 0.01, 0.47). This effect was not observed for heterozygous individuals or in the dominant inheritance models in which the presence of at least one G allele was compared to non-carriers.

Cerebral microbleeds were identified in 62 participants (8%; 95% CI, 6, 10%). Having two variant alleles, compared to non-carriers, was not significantly associated with cerebral microbleeds (odds ratio, 2.42; 95% CI, 0.87, 6.71) in adjusted models (Table 2). A similar direction and smaller magnitude of effect was observed for heterozygous participants, compared to non-carriers (odds ratio, 1.70; 95% CI, 0.95, 3.04). Last, in the dominant inheritance model, having at least 1 variant allele was significantly associated with cerebral microbleeds (odds ratio, 1.78; 95% CI, 1.02, 3.12) in adjusted models.

CBI was present in 187 participants (18%; 95% CI, 15, 20%). Having two variant alleles, compared to non-carriers, was not significantly associated with CBI (odds ratio, 1.59; 95% CI, 0.80, 3.18). There was no association in the dominant inheritance model, either, in which those with at least one G allele were compared to non-carriers (odds ratio, 0.93; 95% CI, 0.66, 1.32) (Table 2). The median small perivascular space count score was 6 (IQR, 3–8). No associations were observed for small perivascular spaces in any of the inheritance models (Table 2).

We evaluated two post-hoc models (Supplemental Table 3). After

Table 1

Characteristics of Participants, Stratified by *PNPLA3* rs738409 G variant allele number.

Characteristic ^a	0 (N = 615)	1 (N = 381)	2 (N = 67)	P [†]
Age, mean (SD), years	70.7 (9.0)	70.2 (8.6)	69.1 (8.0)	0.27
Female	373 (61)	231 (61)	41 (61)	0.996
Race [‡]				
Non-Hispanic White	89 (14)	55 (14)	4 (6)	0.15
Hispanic	389 (63)	293 (77)	58 (87)	< 0.01
Non-Hispanic Black	137 (22)	33 (9)	5 (8)	< 0.01
Hypertension [§]	486 (79)	300 (78)	53 (79)	0.99
Diabetes [§]	158 (26)	101 (27)	19 (28)	0.88
Congestive heart failure [§]	13 (2)	14 (4)	2 (3)	0.34
Atrial fibrillation [§]	27 (4)	16 (4)	0 (0)	0.22
Coronary artery disease [§]	143 (23)	98 (26)	21 (31)	0.29
Current smoking	76 (12)	42 (11)	7 (10)	0.77
Body mass index (kilograms/m ²)	28.5 (5.1)	28.6 (4.9)	28.2 (5.3)	0.85
Systolic blood pressure (mmHg)	136 (17)	137 (18)	136 (18)	0.61
Total cholesterol (mg/dL)	194 (39)	194 (41)	186 (41)	0.26
LDL (mg/dL)	116 (34)	115 (38)	114 (35)	0.90
HDL (mg/dL)	54 (17)	53 (17)	48 (13)	0.02
Triglycerides (mg/dL)	125 (73)	133 (88)	124 (53)	0.29
Anti-platelet medication	237 (39)	173 (45)	28 (42)	0.10
Statin therapy	147 (24)	108 (28)	14 (21)	0.20

Abbreviations: SD, standard deviation; LDL, low density lipoprotein; HDL, high density lipoprotein; mmHg, millimeters mercury; mg/dL milligrams per deciliter.

^a Data are reported as number (%) except as otherwise specified.

[†] Chi-squared test for categorical variables and ANOVA test for continuous variables.

[‡] Self-reported.

[§] Based on self-reported diagnoses, examination, medications, electrocardiographic, and laboratory data.

adjusting for LDL levels, results were similar except that, in the dominant inheritance model, having at least 1 variant allele was no longer associated with cerebral microbleeds by standard convention (odds ratio, 1.75; 95% CI, 0.99–3.10; $P = .06$). Results were similar to the primary analysis after additionally adjusting for race/ethnicity apart from principal components.

4. Discussion

In this cross-sectional analysis of the NOMAS-MRI Substudy, we investigated the relationship between the NAFLD-susceptibility *PNPLA3* rs738409 G variant allele and MRI biomarkers of cerebrovascular disease. This variant was associated with WMH volume and cerebral microbleeds, except after adjusting for LDL levels in the case of microbleeds. There was no association with CBI nor small perivascular spaces. Associations appeared after adjusting for demographics and principal components, suggesting that these factors biased crude results to the null.

We found no prior studies of *PNPLA3* variants and WMH volume and cerebral microbleeds. However, our findings are consistent with observations regarding clinical NAFLD. In observational studies, NAFLD, particularly with advanced liver fibrosis, was associated with WMH burden in two studies [11,13]. In our sample, the presence of two variant alleles was associated with WMH volume, but this association was not seen when heterozygotes were included. Individuals homozygous for the *PNPLA3* variant allele are at greater risk for inflammatory liver disease and cirrhosis [18]. Similarly, having at least one variant allele was associated with microbleeds in our sample, and associations were similar but not statistically significant in other inheritance models or when additionally adjusting for LDL levels. Clinical NAFLD, primarily when accompanied by liver fibrosis, has also variably been associated with more microbleeds [12,13]. While this *PNPLA3* variant is associated with lower LDL levels [17], the relationship

Table 2Associations* between *PNPLA3* rs738409 G variant and brain magnetic resonance imaging cerebrovascular disease biomarkers.

Outcome	Model 1	Model 2	Model 3
White matter hyperintensity volume [†]		<i>β</i> (95% confidence interval)	
GG vs CC	−0.01 (−0.31, 0.29)	0.26 (0.02, 0.50) <i>P</i> = .03	0.27 (0.03, 0.51) <i>P</i> = .02
GC vs CC	−0.18 (−0.35, −0.01)	−0.07 (−0.20, 0.07) <i>P</i> = .33	−0.06 (−0.20, 0.07) <i>P</i> = .35
GG or GC vs CC	−0.15 (−0.31, 0.004)	−0.03 (−0.16, 0.10) <i>P</i> = .66	−0.03 (−0.15, 0.10) <i>P</i> = .69
Cerebral microbleeds [‡]		Odds ratio (95% confidence interval)	
GG vs CC	1.80 (0.71, 4.56)	2.35 (0.86, 6.40) <i>P</i> = .10	2.42 (0.87, 6.71) <i>P</i> = .09
GC vs CC	1.46 (0.85, 2.53)	1.68 (0.94, 2.98) <i>P</i> = .08	1.70 (0.95, 3.04) <i>P</i> = .08
GG or GC vs CC	1.52 (0.90, 2.55)	1.76 (1.01, 3.06) <i>P</i> = .05	1.78 (1.02, 3.12) <i>P</i> = .04
Covert brain infarction [‡]		Odds ratio (95% confidence interval)	
GG vs CC	1.00 (0.53, 1.90)	1.60 (0.80, 3.19) <i>P</i> = .18	1.59 (0.80, 3.18) <i>P</i> = .19
GC vs CC	0.70 (0.50, 1.00)	0.86 (0.59, 1.24) <i>P</i> = .42	0.86 (0.59, 1.24) <i>P</i> = .41
GG or GC vs CC	0.75 (0.54, 1.03)	0.94 (0.66, 1.33) <i>P</i> = .71	0.93 (0.66, 1.32) <i>P</i> = .70
Small perivascular spaces [‡]		<i>β</i> (95% confidence interval)	
GG vs CC	−0.07 (−0.18, 0.04)	0.06 (−0.06, 0.18) <i>P</i> = .31	0.06 (−0.06, 0.17) <i>P</i> = .33
GC vs CC	−0.06 (−0.12, −0.01)	−0.01 (−0.07, 0.04) <i>P</i> = .62	−0.01 (−0.07, 0.04) <i>P</i> = .62
GG or GC vs CC	−0.06 (−0.12, −0.01)	−0.01 (−0.06, 0.05) <i>P</i> = .86	−0.01 (−0.06, 0.05) <i>P</i> = .85

* Model 1 is unadjusted. Model 2 is adjusted for age, sex, principal components. Model 3 is also adjusted for body mass index and diabetes. White matter hyperintensity volume was log-transformed for linear regression, and models were adjusted for total intracranial volume. Logistic regression was used for models of microbleeds and covert brain infarction. Poisson regression was used for small perivascular space score.

[†] Associations reported as *β* (95% confidence interval).

[‡] Associations reported as odds ratio (95% confidence interval).

between LDL levels and cerebrovascular small vessel disease biomarkers is complex. LDL levels were not associated with WMH volume or microbleeds in NOMAS [31,33], and there is also evidence of a negative association between hyperlipidemia and these biomarkers from other cohorts [34]. Clinically, low LDL levels have been associated with an increased risk of intracerebral hemorrhage in some but not all studies [35,36]. Taken together, given the known effects of the *PNPLA3* variant on lipid levels [17], these data suggest that the variant may be associated with WMH volume and microbleeds in part through effects on lipid levels. However, the addition of LDL to dominant inheritance models resulted in loss of statistical significance, which may be suggestive of mediation; larger mediation analyses are needed to explore mechanisms. Alternatively, if LDL is conceptualized as a confounder, then the loss of statistical significance would suggest that this variant allele is not associated with microbleeds. For WMH volume, increased systemic inflammation related to liver inflammation may also be implicated [37,38]. However, the neutral association between the *PNPLA3* variant and CBI and small perivascular spaces in our sample demonstrates that this variant may not contribute to small vessel disease uniformly. Alternatively, the heterogeneity of the pathophysiology [39,40] underlying these MRI biomarkers may explain discrepant findings among the imaging biomarkers.

The *PNPLA3* variant allele was not associated with CBI. Elevated LDL is an important risk factor for ischemic stroke [35], in contrast to its relationship with WMH and microbleeds. This variant's role in ischemic heart disease may help contextualize our findings. Specifically, a recent Mendelian Randomization study found no association between the *PNPLA3* rs738409 variant and ischemic heart disease [41]. An additional genome-wide association study also found no association, and perhaps an inverse association, with coronary artery disease [42]. However, a recent meta-analysis [43] of genome-wide association studies classified NAFLD susceptibility variants into two categories: variants such as *PNPLA3* rs738409 G that are associated with reduced lipid secretion from the liver, and variants that are not. Variants without impact on lipid levels were associated with increased coronary artery disease, whereas this was not the case for variants implicated in plasma lipid reduction [43]. This suggests that NAFLD increases coronary artery disease risk, perhaps through inflammation [37,38], but that this risk is mitigated by the presence of *PNPLA3* and other similar LDL-lowering variants that may reduce the propensity for atherosclerosis [44]. This may explain the neutral association between the *PNPLA3* variant and CBI in our sample, although the etiologies of CBI are

heterogeneous and include small vessel disease [39]. Prior studies that identified an association of *PNPLA3* variants with carotid and coronary atherosclerosis did so only in particular subgroups, and the validity of these findings may have been limited by their single-center or case-control designs [22,32]. Overall, our findings suggest that there may be a heterogeneous effect of the *PNPLA3* variant for MRI cerebrovascular disease biomarkers. A positive association may be limited to predominantly non-atherosclerotic processes such as WMH and microbleeds, for which inflammation may be more causative than LDL levels [45–48]. By extension, the neutral association between the *PNPLA3* variant and CBI may belie heterogeneity by CBI etiologic subtype, which should be explored in future studies.

Our study has several strengths related primarily to the well-characterized nature of the NOMAS cohort, including of exposures and MRI outcome measures. Limitations are several. First, this study was cross-sectional, so impact on progression of cerebrovascular disease biomarkers cannot be assessed. Second, while the NOMAS-MRI Substudy collected genomic and brain imaging data, it did not collect detailed liver imaging data. Our results speak to the association between the *PNPLA3* rs738409 variant and brain MRI cerebrovascular disease biomarkers; future studies will ideally include both genomic data and liver imaging data. Third, the most anterior/posterior sections were not examined for microbleeds, leading to underestimation of prevalence. Additionally, when using microbleed adjudications from different raters in post hoc analysis, our results were not significant; our findings regarding microbleeds in particular require replication and validation. Fourth, validated methods were used to discern small perivascular spaces, but the radiographic similarity to small lacunar CBI lesions raises the possibility of misclassification as the cause of neutral findings. Fifth, the small sample size precludes subgroup analyses and tests for interaction by possible effect modifiers such as diabetes. The small sample size also precluded demonstration of known effects of the variant allele on lipid levels. Last, the possibility of chance findings is not excluded; replication of our findings is necessary.

5. Conclusions

The NAFLD susceptibility *PNPLA3* rs738409 [C > G] variant allele was associated with WMH volume in the NOMAS cohort of clinically stroke-free individuals. An association with microbleeds was no longer significant after adjusting for LDL levels; the relevance of this variant allele for microbleeds is unclear. Confirmation of our findings and

further investigation may yield insights into the pathophysiology of cerebral small vessel disease.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2020.116981>.

References

- [1] M.H. Le, P. Devaki, N.B. Ha, D.W. Jun, H.S. Te, R.C. Cheung, et al., Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States, *PLoS One* 12 (2017) e0173499.
- [2] M. Ekstedt, H. Hagström, P. Nasr, M. Fredrikson, P. Stål, S. Kechagias, et al., Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up, *Hepatology* 61 (2015) 1547–1554.
- [3] G. Targher, C.D. Byrne, A. Lonardo, G. Zoppini, C. Barbui, Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis, *J. Hepatol.* 65 (2016) 589–600.
- [4] K.S. Alexander, N.A. Zakai, S.D. Lidofsky, P.W. Callas, S.E. Judd, R.P. Tracy, et al., Non-alcoholic fatty liver disease, liver biomarkers and stroke risk: the reasons for geographic and racial differences in stroke cohort, *PLoS One* 13 (2018) e0194153.
- [5] I. Ying, G. Saposnik, M.J. Vermeulen, A. Leung, J.G. Ray, Nonalcoholic fatty liver disease and acute ischemic stroke, *Epidemiology* 22 (2011) 129–130.
- [6] M.T. Long, X. Yin, M.G. Larson, P.T. Ellinor, S.A. Lubitz, D.D. McManus, et al., Relations of liver fat with prevalent and incident atrial fibrillation in the Framingham heart study, *J. Am. Heart Assoc.* 6 (2017).
- [7] J.L. Mellinger, K.M. Pencina, J.M. Massaro, U. Hoffmann, S. Seshadri, C.S. Fox, et al., Hepatic steatosis and cardiovascular disease outcomes: an analysis of the Framingham heart study, *J. Hepatol.* 63 (2015) 470–476.
- [8] L.B. VanWagner, H. Ning, C.E. Lewis, C.M. Shay, J. Wilkins, J.J. Carr, et al., Associations between nonalcoholic fatty liver disease and subclinical atherosclerosis in middle-aged adults: the coronary artery risk development in young adults study, *Atherosclerosis* 235 (2014) 599–605.
- [9] D.H. Sinn, D. Kang, Y. Chang, S. Ryu, S. Gu, H. Kim, et al., Non-alcoholic fatty liver disease and progression of coronary artery calcium score: a retrospective cohort study, *Gut* 66 (2017) 323–329.
- [10] S.A. Madan, F. John, N. Pyrsopoulos, C.S. Pitchumoni, Nonalcoholic fatty liver disease and carotid artery atherosclerosis in children and adults: a meta-analysis, *Eur. J. Gastroenterol. Hepatol.* 27 (2015) 1237–1248.
- [11] S. Petta, A. Tuttolomondo, C. Gagliardo, R. Zafonte, G. Brancatelli, D. Cabibi, et al., The presence of white matter lesions is associated with the fibrosis severity of nonalcoholic fatty liver disease, *Medicine (Baltimore)* 95 (2016) e3446.
- [12] Y.D. Kim, D. Song, J.H. Heo, S.U. Kim, B.K. Kim, J.Y. Park, et al., Relationship between cerebral microbleeds and liver stiffness determined by transient elastography, *PLoS One* 10 (2015) e0139227.
- [13] H. Jang, D. Kang, Y. Chang, Y. Kim, J.S. Lee, K.W. Kim, et al., Non-alcoholic fatty liver disease and cerebral small vessel disease in Korean cognitively normal individuals, *Sci. Rep.* 9 (2019) 1814.
- [14] S. Romeo, J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L.A. Pennacchio, et al., Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease, *Nat. Genet.* 40 (2008) 1461–1465.
- [15] N. Stefan, H.U. Häring, K. Cusi, Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies, *Lancet Diabetes Endocrinol.* 7 (2019) 313–324.
- [16] P. Dongiovanni, L. Valenti, Genetics of nonalcoholic fatty liver disease, *Metabolism* 65 (2016) 1026–1037.
- [17] D.J. Liu, G.M. Peloso, H. Yu, A.S. Butterworth, X. Wang, A. Mahajan, et al., Exome-wide association study of plasma lipids in > 300,000 individuals, *Nat. Genet.* 49 (2017) 1758–1766.
- [18] S. Stender, J. Kozlitina, B.G. Nordestgaard, A. Tybjaerg-Hansen, H.H. Hobbs, J.C. Cohen, Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci, *Nat. Genet.* 49 (2017) 842–847.
- [19] D. Kim, W.R. Kim, H.J. Kim, T.M. Therneau, Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States, *Hepatology* 57 (2013) 1357–1365.
- [20] A. Di Costanzo, L. D'Erasmo, L. Polimeni, F. Baratta, P. Coletta, M. Di Martino, et al., Non-alcoholic fatty liver disease and subclinical atherosclerosis: a comparison of metabolically- versus genetically-driven excess fat hepatic storage, *Atherosclerosis* 257 (2017) 232–239.
- [21] R. Zampino, A. Florio, N. Coppola, G. Cirillo, M. Macera, A. Marrone, et al., PNPLA3 I148M variant as a risk factor for carotid atherosclerosis in chronic hepatitis C, *Int. J. Cardiol.* 172 (2014) 291–292.
- [22] S. Petta, L. Valenti, G. Marchesini, V. Di Marco, A. Licata, C. Cammà, et al., PNPLA3 GG genotype and carotid atherosclerosis in patients with non-alcoholic fatty liver disease, *PLoS One* 8 (2013) e74089.
- [23] M.S. Elkind, R.R. Sciacca, B. Boden-Albala, T. Rundek, M.C. Paik, R.L. Sacco, Relative elevation in baseline leukocyte count predicts first cerebral infarction, *Neurology* 64 (2005) 2121–2125.
- [24] L. Wang, M.R. Di Tullio, A. Beecham, S. Slifer, T. Rundek, S. Homma, et al., A comprehensive genetic study on left atrium size in Caribbean Hispanics identifies potential candidate genes in 17p10, *Circ. Cardiovasc. Genet.* 3 (2010) 386–392.
- [25] J. Novembre, M. Stephens, Interpreting principal component analyses of spatial population genetic variation, *Nat. Genet.* 40 (2008) 646–649.
- [26] J. Gutierrez, M.S. Elkind, K. Cheung, T. Rundek, R.L. Sacco, C.B. Wright, Pulsatile and steady components of blood pressure and subclinical cerebrovascular disease: the Northern Manhattan Study, *J. Hypertens.* 33 (2015) 2115–2122.
- [27] C.B. Wright, M.C. Paik, T.R. Brown, S.P. Stabler, R.H. Allen, R.L. Sacco, et al., Total homocysteine is associated with white matter hyperintensity volume: the Northern Manhattan Study, *Stroke* 36 (2005) 1207–1211.
- [28] S. Prabhakaran, C.B. Wright, M. Yoshita, R. Delapaz, T. Brown, C. DeCarli, et al., Prevalence and determinants of subclinical brain infarction: the Northern Manhattan Study, *Neurology* 70 (2008) 425–430.
- [29] J. Gutierrez, M.S.V. Elkind, C. Dong, M. Di Tullio, T. Rundek, R.L. Sacco, et al., Brain perivascular spaces as biomarkers of vascular risk: results from the Northern Manhattan Study, *AJNR Am. J. Neuroradiol.* 38 (2017) 862–867.
- [30] J.Z. Willey, Y.P. Moon, M.C. Paik, M. Yoshita, C. DeCarli, R.L. Sacco, et al., Lower prevalence of silent brain infarcts in the physically active: the Northern Manhattan Study, *Neurology* 76 (2011) 2112–2118.
- [31] M.R. Caunca, V. Del Brutto, H. Gardener, N. Shah, N. Dequatre-Ponchelle, Y.K. Cheung, et al., Cerebral microbleeds, vascular risk factors, and magnetic resonance imaging markers: the Northern Manhattan Study, *J. Am. Heart Assoc.* 5 (2016).
- [32] R. Posadas-Sánchez, Á. López-Urbe, C. Posadas-Romero, N. Pérez-Hernández, J.M. Rodríguez-Pérez, W.A. Ocampo-Arcos, et al., Association of the I148M/PNPLA3 (rs738409) polymorphism with premature coronary artery disease, fatty liver, and insulin resistance in type 2 diabetic patients and healthy controls. The GEA study, *Immunobiology* 222 (2017) 960–966.
- [33] J.Z. Willey, H. Gardener, Y.P. Moon, M. Yoshita, C. DeCarli, Y.K. Cheung, et al., Lipid profile components and subclinical cerebrovascular disease in the northern Manhattan study, *Cerebrovasc. Dis.* 37 (2014) 423–430.
- [34] J. Jimenez-Conde, A. Biffi, R. Rahman, A. Kanakis, C. Butler, S. Sonni, et al., Hyperlipidemia and reduced white matter hyperintensity volume in patients with ischemic stroke, *Stroke* 41 (2010) 437–442.
- [35] L. Sun, R. Clarke, D. Bennett, Y. Guo, R.G. Walters, M. Hill, et al., Causal associations of blood lipids with risk of ischemic stroke and intracerebral hemorrhage in Chinese adults, *Nat. Med.* 25 (2019) 569–574.
- [36] C. Judge, S. Rutledge, M. Costello, R. Murphy, E. Loughlin, A. Alvarez-Iglesias, et al., Lipid lowering therapy, low-density lipoprotein level and risk of Intracerebral Hemorrhage - a meta-analysis, *J. Stroke Cerebrovasc. Dis.* 28 (2019) 1703–1709.
- [37] M. Al Rifai, M.G. Silverman, K. Nasir, M.J. Budoff, R. Blankstein, M. Szklo, et al., The association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic inflammation and subclinical atherosclerosis: the multi-ethnic study of atherosclerosis (MESA), *Atherosclerosis* 239 (2015) 629–633.
- [38] H.J. Lee, C.H. Lee, S. Kim, S.Y. Hwang, H.C. Hong, H.Y. Choi, et al., Association between vascular inflammation and non-alcoholic fatty liver disease: analysis by 18F-fluorodeoxyglucose positron emission tomography, *Metabolism* 67 (2017) 72–79.
- [39] J.P. Fanning, A.J. Wesley, A.A. Wong, J.F. Fraser, Emerging spectra of silent brain infarction, *Stroke* 45 (2014) 3461–3471.
- [40] J.M. Wardlaw, M.C. Valdés Hernández, S. Muñoz-Maniega, What are white matter hyperintensities made of? Relevance to vascular cognitive impairment, *J. Am. Heart Assoc.* 4 (2015) 001140.
- [41] B.K. Lauridsen, S. Stender, T.S. Kristensen, K.F. Kofoed, L. Køber, B.G. Nordestgaard, et al., Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279013 individuals, *Eur. Heart J.* 39 (2018) 385–393.
- [42] N. Simons, A. Isaacs, G.H. Koek, S. Kuč, N.C. Schaper, M.C.G.J. Brouwers, PNPLA3, TM6SF2, and MBOAT7 genotypes and coronary artery disease, *Gastroenterology* 152 (2017) 912–913.
- [43] M.C.G.J. Brouwers, N. Simons, C.D.A. Stehouwer, G.H. Koek, N.C. Schaper, A. Isaacs, Relationship between nonalcoholic fatty liver disease susceptibility genes and coronary artery disease, *Hepatol. Commun.* 3 (2019) 587–596.
- [44] S. Rüschbaum, K. Schwarzkopf, M. Friedrich-Rust, F. Seeger, F. Schöelzel, Y. Martinez, et al., Patatin-like phospholipase domain containing 3 variants differentially impact metabolic traits in individuals at high risk for cardiovascular events, *Hepatol. Commun.* 2 (2018) 798–806.
- [45] Y. Gu, J. Gutierrez, I.B. Meier, V.A. Guzman, J.J. Manly, N. Schupf, et al., Circulating inflammatory biomarkers are related to cerebrovascular disease in older adults, *Neurol. Neuroimmunol. Neuroinflamm.* 6 (2019) e521.

- [46] A. Shoamanesh, S.R. Preis, A.S. Beiser, R.S. Vasan, E.J. Benjamin, C.S. Kase, et al., Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham heart study, *Neurology*. 84 (2015) 825–832.
- [47] K.A. Walker, M.C. Power, R.C. Hoogeveen, A.R. Folsom, C.M. Ballantyne, D.S. Knopman, et al., Midlife systemic inflammation, late-life white matter integrity, and cerebral small vessel disease: the atherosclerosis risk in communities study, *Stroke*. 48 (2017) 3196–3202.
- [48] L. Lampe, R. Zhang, F. Beyer, S. Huhn, S. Kharabian Masouleh, S. Preusser, et al., Visceral obesity relates to deep white matter hyperintensities via inflammation, *Ann. Neurol*. 85 (2019) 194–203.