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The Role of the Gene–Gene and Gene–Environment Interactions of Polymorphic Loci of Matrix Metalloproteinases in Forming the Risk of Ischemic Stroke on the Background of Arterial Hypertension in Men

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Objectives. To analyze the role of genetic polymorphisms of matrix metalloproteinase (*MMP*) genes and their gene–gene and gene–environment interactions in the formation of ischemic stroke (IS) in men with arterial hypertension (AH). **Materials and methods.** The study included 523 men with hypertension: 201 with IS and 322 without stroke. The relationship between *MMP* loci and the formation of stroke in the presence of hypertension was determined by logistic regression analysis in dominant, recessive, and additive genetic models using PLINK v.2.050 software. Haplotype analysis was performed for five single nucleotide polymorphisms (SNP) co-located on chromosome 11 (314.3 kb), and associations of haplotypes with the development of stroke were identified using the EM algorithm. Gene–gene and gene–environment interactions between *MMP* and smoking and alcohol consumption in the development of stroke were assessed by the generalized multifactor dimensionality reduction (GMDR) method using GMDR v.0.9 software. **Results.** The rs3025058 polymorphic locus was found to be associated with IS in men in the dominant and additive genetic models (OR = 0.63–0.74, $p_{perm} = 0.03$). Four *MMP* haplotypes had protective effects in relation to the development of stroke in the presence of hypertension (OR = 0.48–0.50, $p_{perm} = 0.02$–0.03). Four models of gene–gene interactions of *MMP* polymorphic loci (OR = 2.19–2.55, $p_{perm} < 0.001$) and three four-way models of gene–environment interactions of *MMP* with alcohol abuse (OR = 2.82–3.11, $p_{perm} < 0.001$) were associated with high risks of developing IS in men with hypertension. rs3025058, rs1320632, rs11225395, and rs1799750 demonstrated the largest contributions to gene–gene and gene–environment interactions on formation of IS. **Conclusions.** The results obtained here indicate that the interactions of *MMP* genes with each other and with modifiable environmental factors play a significant role in the development of stroke on the background of AH in men.

Keywords: ischemic stroke, matrix metalloproteinases, gene–environment interactions.

In the Russian Federation, stroke accounts for over 20% of total mortality [1]. In the age group ≥65 years, 46% of patients have cognitive deficit and 26% are in constant need of outside help six months after stroke [2]. Adjusted for age, the incidence of ischemic stroke (IS) in men is 30% higher than in women [3, 4].

The main risk factor for stroke is arterial hypertension (AH). Arterial pressure (BP) has a directly proportional, differentiated, prognostically, and etiologically significant relationship with the development of stroke [5, 6]. Genetic risk factors for the development of acute cerebrovascular accident (aCVA) are also now under active investigation, including the roles of genetic polymorphisms of matrix metalloproteinases (MMP) [7, 8]. MMP are responsible for the breakdown of extracellular matrix components and are involved in all the reactions of the neuroinflammatory cascade which accompany cerebral infarction [9]. A number of studies have found that the extent of damage in stroke correlates with the level of *MMP* gene expression [10, 11]. However, the contribution

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TABLE 1. Clinical Characteristics of IS Patients and Men in the Control Group

Parameter	Patients with IS (n = 201)	Patients without IS (n = 322)	p
Age, years	58.11 ± 7.09	57.18 ± 7.62	0.42
BMI, kg/m^2	30.29 ± 5.27	31.29 ± 3.95	0.17
TC, mM	5.55 [5.00–6.60]	5.33 [4.80–6.20]	0.04*
HDL-C, mM	1.17 [0.99–1.60]	1.30 [1.06–1.55]	0.04*
LDL-C, mM	3.85 [3.10–4.80]	3.60 [2.93–4.20]	0.002*
TG, mM	2.01 [1.45–2.87]	1.62 [1.21–2.22]	0.001*
Smoking, n (%)	124 (62.00)	182 (56.70)	0.27
Alcohol abuse, n (%)	30 (15.23)	12 (3.92)	0.001*

Data are presented as absolute numbers of patients (n) and %, mean ± standard deviation ($M \pm SD$), and median and interquartile range (Me [Q1–Q3]); *statistically significant between-group differences.

of the interaction of genetic polymorphisms with each other and with environmental factors in the development of cerebrovascular pathology remains poorly understood.

The aim of the present work was to study the role of *MMP* gene polymorphisms and their gene–gene and gene–environment interactions in the formation of the risk of developing IS in men with AH.

Materials and Methods. The study cohort was formed at St. Joasaph Belgorod Regional Clinical Hospital (departments of neurology and cardiology) in 2013–2016. Study results from 523 men were analyzed: 201 with IS on the background of AH and 322 with AH without a history of acute cerebrovascular accident (aCVA) (control group).

Inclusion criteria: Russian nationality, natives of the Central Chernozem region, not related to each other, systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mmHg.

Exclusion criteria: symptomatic and secondary hypertension, liver and kidney failure, refusal to participate in the study. We have previously described the inclusion and exclusion criteria in more detail [8].

IS was diagnosed by specialists from the department of neurology, St. Joasaph Clinical Hospital by neurological examination, patients' complaints, and brain CT or MRI scans. The atherothrombotic subtype was recorded in 81 (40.29%) of the men, the cardioembolic subtype in 46 (22.89%), the hemodynamic subtype in 50 (24.87%), and the lacunar subtype in 24 (11.95%). There were no patients with arterio-arterial embolism or the hemorheological microocclusion type of stroke among the patients included in the study.

Diagnoses of AH were established in compliance with the recommendations of the All-Russian Scientific Society of Cardiology for the Diagnosis and Treatment of AH [12]. Body mass index (BMI, kg/m^2), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), triglycerides (TG), smoking status, and alcohol abuse were recorded for all respondents. Blood for biochemical analysis was taken after fasting for 8 h and analysis was carried out in the St. Joasaph Belgorod Regional Clinical Hospital. Smoking was defined as smoking one or more cigarettes daily during the past year. Alcohol abuse was defined as drinking 50 g of alcohol per day for 1 year or more [13].

The study was carried out in compliance with Good Clinical Practice and the principles of the Helsinki Declaration. The study protocol was reviewed and approved by the Ethics Committee of the Medical Institute of Belgorod State National Research University (protocol No. 8 of April 10, 2013). Informed consent for the study was obtained from all patients. The clinical characteristics of the men included in the study are presented in Table. 1. Patients with stroke and patients with AH without IS were comparable in terms of age, BMI, and smoking (p>0.05), but differed significantly in terms of lipid profiles and alcohol abuse ($p < 0.05$).

It should be noted that most of the men studied had severe (39.7%) or moderate (26.9%) hypertension, though only 34.9% of respondents were taking antihypertensive drugs on a constant basis. The most common complications of hypertension were cardiac (54.7%): 23.8% of men with hypertension had been diagnosed with coronary heart disease.

Genetic analysis. Patients included in the study underwent genotyping at seven polymorphic loci (rs1799750, rs243865, rs3025058, rs11568818, rs1320632, rs11225395, rs652438) selected on the basis of their regulatory potential and influences on gene expression [14, 15]. We have previously described the methods used for genomic DNA isolation and genotyping [8].

Statistical analysis. Correspondence of genotype frequencies to the Hardy–Weinberg equilibrium was assessed using the χ^2 test. The relationship between polymorphic *MMP* loci and the risk of stroke in the presence of hypertension in men was determined by logistic regression analysis (dominant, recessive, and additive genetic models) using PLINK v.2.050 software (http://zzz.bwh.harvard.edu/plink/plink2.shtml). Haplotype analysis was performed for SNPs rs11568818, rs1320632, rs11225395, rs1799750,

TABLE 2. Associations of Genotypes of Polymorphic Loci of *MMP* Genes with the Risk of Developing IS on the Background of AH in Men

Polymorphism	Model	OR (95% CI)	p_{perm}
rs11568818 *MMP7*	Dominant (AG/GG vs AA)	0.76 (0.51–2.14)	0.18
	Recessive (GG vs AG/AA)	0.71 (0.42–1.19)	0.19
	Additive (AG vs GG vs AA)	0.79 (0.60–1.05)	0.11
rs1320632 *MMP8*	Dominant (AG/GG vs AA)	1.46 (0.87–2.45)	0.15
	Recessive (GG vs AG/AA)	1.42 (0.27–2.58)	0.68
	Additive (AG vs GG vs AA)	1.39 (0.87–2.20)	0.17
rs11225395 *MMP8*	Dominant (CT/TT vs CC)	0.90 (0.59–1.36)	0.61
	Recessive (TT vs CT/CC)	1.01 (0.79–2.17)	0.33
	Additive (CT vs TT vs CC)	1.03 (0.79–1.35)	0.82
rs1799750 *MMP1*	Dominant (1G2G/2G2G vs 1G1G)	1.31 (0.86–2.01)	0.21
	Recessive (2G2G vs 1G2G/1G1G)	1.22 (0.77–1.94)	0.40
	Additive (1G2G vs 2G2G vs 1G1G)	1.20 (0.91–1.57)	0.19
rs3025058 *MMP3*	Dominant (5A6A/5A5A vs 6A6A)	0.63 (0.42–0.95)	0.03*
	Recessive (5A5A vs 5A6A/6A6A)	0.72 (0.44–1.18)	0.20
	Additive (5A6A vs 6A6A vs 5A5A)	0.74 (0.56–0.97)	0.03*
rs652438 *MMP12*	Dominant (GA/GG vs AA)	1.14 (0.65–2.01)	0.64
	Recessive (GG vs GA/AA)	2.10 (0.18–4.36)	0.55
	Additive (GA vs GG vs AA)	1.17 (0.69–1.96)	0.57
rs243865 *MMP2*	Dominant (TC/TT vs CC)	1.29 (0.88–1.90)	0.19
	Recessive (TT vs TC/CC)	1.63 (0.79–3.39)	0.19
	Additive (TC vs TT vs CC)	1.28 (0.94–1.73)	0.12

OR is odds ratio, 95% CI is the 95% confidence interval, *statistically significant differences.

and rs3025058, which are co-located on chromosome 11 (314.3 kb). The frequencies of combinations and their association with the development of stroke were evaluated using the EM algorithm; haplotypes with frequencies of <5% were excluded from the analysis. Results were corrected using an adaptive permutation test, where $p_{perm} < 0.05$ was regarded a statistically significant level. Gene–gene and gene–environment interactions of *MMP* with alcohol consumption in the development of stroke in men were assessed by the generalized multifactor dimensionality reduction (GMDR) method using GMDR software (v0.9, https://www.uab.edu/hcgs/bioinformatics). Three- and four-factor interaction models were selected with reproducibility (CVC) ≥9/10 and prediction accuracy (TBA) of >55%, at a significance level of $p \leq 0.0107$. The study results were validated using a permutation test, in which 1000 permutations were performed with 10 cross-validations, ensuring $p_{perm} < 0.001$. The nature and strength of the interactions of *MMP* genes with each other and with environmental factors during the formation of the risk of stroke were assessed in terms of percentage entropy by the MDR method and interactions were visualized as plots (MDR program, vers. 3.0.2) (http://sourceforge.net/projects /mdr).

Results. Allele and genotype frequencies for all single nucleotide polymorphisms (SNP) complied with Hardy–Weinberg equilibrium ($p > 0.05$) in both groups and were comparable with the frequencies of these loci in European populations. Logistic regression analysis results are presented in Table 2. The polymorphic locus rs3025058 was associated with IS in men in the dominant and additive models ($p_{perm} = 0.03$) and had a protective effect during the development of the disease (OR = 0.63–0.74).

Four *MMP* haplotypes were identified whose frequencies differed significantly in the groups of men with stroke on the background of hypertension and those without stroke ($p_{perm} = 0.02–0.03$) (Table 3). All the combinations identified had protective effects on the development of stroke in their carriers (OR = 0.48–0.50).

Application of the GMDR method identified the four most significant models of *MMP* gene–gene interactions

TABLE 3. *MMP* Haplotypes Associated with the Development of IS on the Background of AH in Men

No.	Polymorphic loci					Haplotype frequency		OR	p_{perm}
	rs1320632 *MMP8*	rs11225395 *MMP8*	rs1799750 *MMP1*	rs3025058 *MMP3*	rs652438 *MMP12*	Patients with IS (n = 203), %	Patients without IS (n = 322), %		
H1		C	1G	5A		12.09	18.13	0.50	0.03
H2		C	1G	5A	A	11.20	17.16	0.49	0.02
H3	A	C	1G	5A		12.31	19.08	0.50	0.03
H4	A	C	1G	5A	A	11.15	18.14	0.48	0.03

Obtained by logistic regression adjusted for BMI, total cholesterol, HDL-C, LDL-C, TG, and alcohol abuse.

TABLE 4. Models of *MMP* Gene–Gene Interactions Associated with the Development of IS in Men (n = 523)

Models of gene–gene interactions	OR (95% CI)	Test Bal. acc. (%)	p
rs1799750 *MMP1*×rs3025058 *MMP3*× rs11225395 *MMP8*	2.55 (1.49–4.37)	57.00	0.01
rs3025058 *MMP3*×rs1320632 *MMP8*× rs11225395 *MMP8*	2.45 (1.44–4.18)	58.70	0.01
rs1799750 *MMP1*×rs1320632 *MMP8*× rs11225395 *MMP8*	2.19 (1.28–3.74)	56.93	0.01
rs1799750 *MMP1*×rs11568818 *MMP7*× rs11225395 *MMP8*	2.40 (1.40–4.11)	56.60	0.01

Obtained by GMDR method taking account of covariates; Test Bal. acc. – model prediction accuracy (%), permutation tests involved performance of 1000 permutations with 10 cross-validations, ensuring $p_{perm} < 0.001$.

TABLE 5. Models of Gene–Environment Interactions of *MMP* Associated with Formation of the Risk of Developing IS in Men (n = 523)

Models of gene–environment interactions	OR (95% CI)	Test Bal. acc. (%)	p
Alcohol abuse×rs1799750 *MMP1*×rs3025058 *MMP3*×rs11225395 *MMP8*	3.11 (1.83–5.29)	56.71	0.01
Alcohol abuse×rs3025058 *MMP3*×rs1320632 *MMP8*×rs11225395 *MMP8*	3.02 (1.78–5.13)	57.99	0.01
Alcohol abuse×rs1799750 *MMP1*×rs1320632 *MMP8*×rs11225395 *MMP8*	2.82 (1.65–4.84)	59.76	0.001

Obtained by GMDR method taking account of covariates; alcohol abuse – alcohol abuse; permutation tests involved performance of 1000 permutations with 10 cross-validations, ensuring $p_{perm} < 0.001$.

associated with the development of stroke in men ($p_{perm} <$ < 0.001) (Table 4). Polymorphism rs11225395 was present in all the models identified, while the combination of rs1320632 and rs11225395 was included in two of the four models.

Analysis of gene–environment interactions of *MMP* polymorphic loci with alcohol abuse revealed three four-factor models associated with a high risk of stroke in men (OR = = 2.82–3.11, $p_{perm} < 0.001$) (Table 5). It should be noted that the combinations identified included rs3025058, rs1320632, rs11225395, and rs1799750, for all of which intergenic interactions in the formation of the risk of stroke in the presence of hypertension have previously been demonstrated.

The strength and direction of interactions between polymorphic *MMP* genes and alcohol abuse in the development of stroke are presented as plots (Fig. 1). Marked antagonistic-type interactions were found between alcohol consumption and rs11225395, rs3025058, rs11568818, and rs243865 (from –1.70% to –2.24% of entropy). It is of note that the combination of rs3025058 and rs11225395 occurred in two of the three models identified here, which confirms the contribution of these SNPs and their interactions with alcohol consumption to forming the predisposition to stroke in men.

Thus, six of the seven SNP studied here were associated with the development of IS in men, with the rs3025058, rs11225395, and rs1799750 loci making the largest contributions. We did not find any significant associations between the development of stroke in men and rs652438.

Discussion. MMP are among the main modulators of the extracellular matrix, providing proteolysis of its components in normal and pathological processes, and also cleaving cell adhesion molecules, neurotrophins, and cytokines [16, 17]. Early activation of MMP in the acute phase of IS mediates neuron death and disruption of the blood–brain barrier (BBB), leading to hemorrhagic injury and infiltration of inflammatory cells into the infarcted area [18]. Previous studies have demonstrated associations between *MMP* genes and the development of cardiovascular and cerebrovascular diseases [19–25]. The present study found that rs3025058, rs11568818, rs1320632, rs11225395, rs652438, and rs1799750 are involved in forming the risk of developing IS on the background of hyperten-

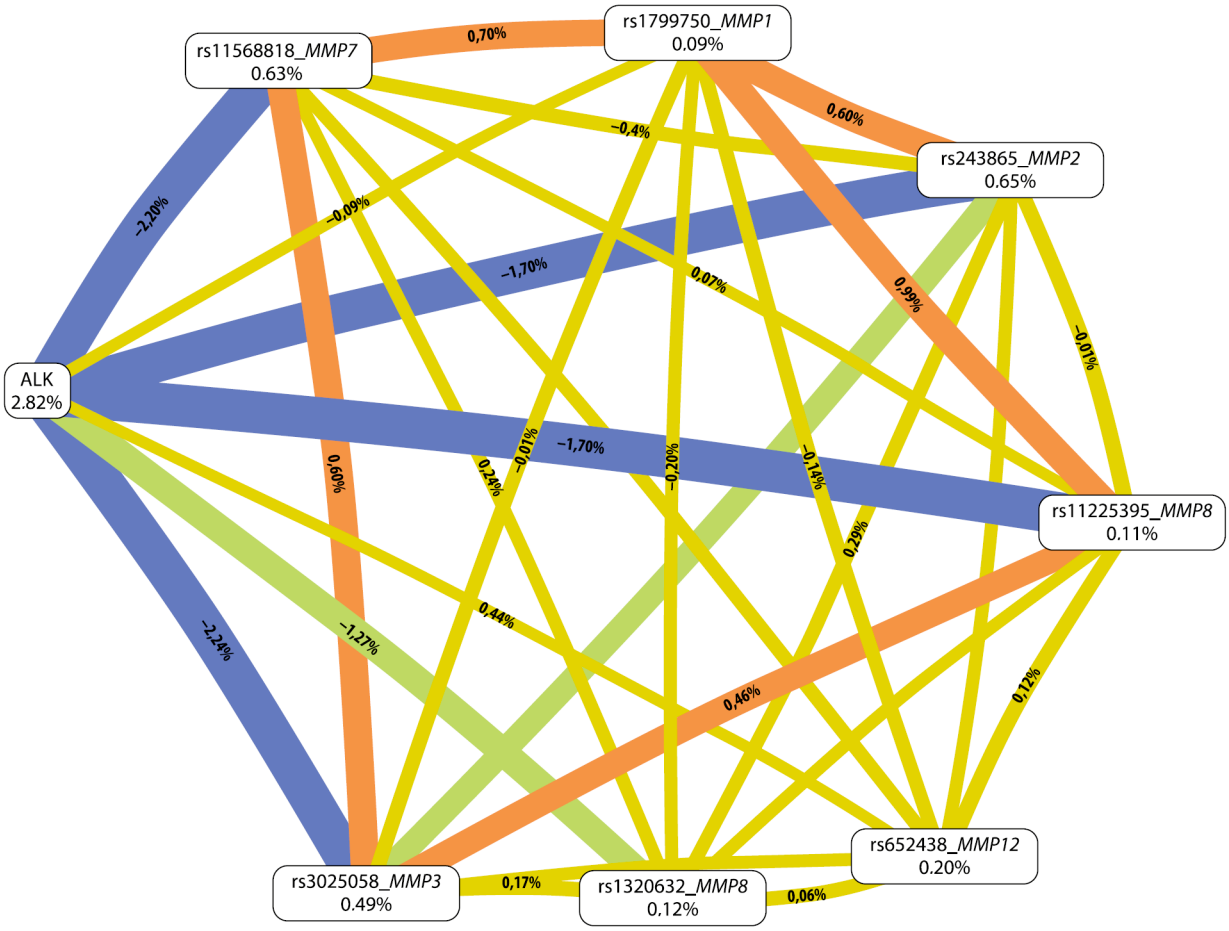

Fig. 1. Plot of gene–environment interactions of *MMP* with alcohol (ALK) on development of IS in men with AH. Line color reflects the nature of the interaction during phenotype formation: blue shows marked antagonism, brown shows additive interaction; orange shows moderate synergism, green shows moderate antagonism. Interaction strength and direction are given as percentage entropy.

sion in men, while SNP of the *MMP3*, *MMP8*, and *MMP1* genes demonstrated the greatest contributions.

The rs3025058 *MMP3* polymorphic locus was associated with the development of IS both independently and as part of haplotypes and models of gene–gene and gene–environment interactions (p = 0.01–0.03). This genetic variant is caused by insertion (6A) or deletion (5A) of a nucleotide at position –1612 of the promoter of the *MMP3* gene, which encodes MMP3 protein. MMP3 is a key member of the MMP family and is capable of activating other proteolytic enzymes, including MMP1, MMP8, MMP9, and MMP13 [26]. Recent data suggest that overexpression of MMP3 in atherosclerotic plaques leads to increases in their vulnerability and rupture, which is the main cause of IS [27]. Our data are consistent with studies in other populations. Thus, the frequency of allele 6A of rs3025058 in the Italian population was significantly higher in a group of patients with stroke than in the control group (OR = 1.58, $p < 0.02$) [21], while Sherva et al. [22] found a protective effect of the 5A/5A rs3025058 genotype in the development of IS in the American population (OR = 0.51, $p = 0.017$). At the same time, a study reported by Zhang [23], conversely, did not find a relationship between rs3025058 and the development of IS, which may be due to differences in the study designs.

The genetic polymorphisms rs11225395 and rs1799750 are elements of most haplotypes and models of gene–gene and gene–environment interactions associated with the development of stroke. The rs11225395 polymorphic variant is due to the substitution of C for T at position –799 of the promoter of the *MMP8* gene, while rs1799750 is due to the insertion of an additional guanine at position –1607 of the *MMP1* promoter region. Study data showed that in the Taiwanese population, the rs11225395 polymorphism of *MMP8* is associated with the development of IS in a recessive genetic model (OR = 1.24, $p < 0.01$), and also in combination with alcohol abuse (OR = 1.40, $p < 0.05$) [28]. Studies of the Italian population reported by Ghilardi et al. [21] showed a synergistic effect of the 2G rs1799750 polymorphism of *MMP1* and the 6A rs3025058 polymorphism of *MMP3* in the development of cerebrovascular pathology (OR = 2.66, p = 0.016), which is also consistent with our results. At

the same time, the association of the rs1799750 polymorphic locus of *MMP1* with the incidence of IS did not reach a statistically significant level in the Tunisian population ($p = 0.074$) [29]. Only a few reports in the available literature address the role of interactions of *MMP* genes with each other and with environmental factors in the formation of the predisposition to IS, which dictates the need for further research in this area. Thus, Hsieh et al. [28] found that the combined effects of the polymorphic loci of *MMP7*, *MMP8*, and *MMP26* and modifiable risk factors, including smoking and alcohol consumption, significantly increase the risk of developing IS (OR = 5.75, $p < 0.05$).

The present study established the existence of gene–environment interactions between *MMP* and alcohol abuse in the development of stroke. Alcohol is known to be an independent risk factor for the development of cerebrovascular diseases. Thus, Reynolds et al. [30] found an association between patients drinking more than 60 g of alcohol per day and the relative risk of II (OR = 1.69, 95% CI 1.34–2.15, $p < 0.01$). The concentrations of MMP1, MMP2, MMP3, and MMP7 have also been found to increase significantly in response to ethanol [31]. Ethanol-induced oxidative stress in brain tissues triggers molecular cascades mediating activation of MMP, leading to disruption of the BBB and increases in the susceptibility of cells to ischemic damage [32, 33].

Conclusions. Thus, the results obtained here indicate that the interactions of *MMP* genes with each other and with alcohol abuse play a significant role in forming the risk of stroke in men with hypertension. Four haplotypes were found ($p_{perm} = 0.02–0.03$), along with four models of gene–gene and three models of gene–environment interactions of *MMP* with alcohol ($p_{perm} < 0.001$), associated with the development of IS. The direction and strength of interactions between *MMP* genes and alcohol on formation of IS have been established. The present study has some limitations. Thus, only men were included in the study cohort; the analysis was limited to seven polymorphic loci of six *MMP* genes; analysis of gene–environment interactions did not include other potentially significant environmental risk factors for IS.

The authors declare no conflict of interest.

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