

NINO KRA VEISHVILI ^{1,3}, EKA KVARATSHELIA ^{1,2}, SANDRO SURMAVA ¹, MAIA GAGUA ²,
EKA MAISURADZE ², ELENE ABZIANIDZE ¹

ONE-CARBON METABOLISM GENE POLYMORPHISM CORRELATE WITH LEVELS OF DNA METHYLTRANSFERASES IN PATIENTS WITH MIGRAINE

¹Tbilisi State Medical University, Department of Molecular and Medical Genetics, Tbilisi, Georgia;

²Tbilisi State Medical University, V. Bakhutashvili Institute of Medical Biotechnology, Tbilisi, Georgia;

³Tbilisi Institute of Medicine, Tbilisi, Georgia

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ნინო კრავეიშვილი ³, ეკა კვარაცხელია ^{1,2}, სანდრო სურმავა ¹, მაია გაგუა ²,
ეკა მაისურაძე ², ელენე აბზიანიძე ¹

ერთი ნახშირბადის მეტაბოლიზმში მონაწილე გენის პოლიმორფიზმის კორელაცია დნმ-მეთილტრანსფერაზების დონეებთან შაკიკის მქონე პაციენტებში

¹თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, მოლეკულური და სამედიცინო გენეტიკის დეპარტამენტი, თბილისი, საქართველო; ²თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ვ.ბახუტაშვილის სახელობის სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი, საქართველო; ³თბილისის მედიცინის ინსტიტუტი, თბილისი, საქართველო

რეზიუმე

შაკიკი გავრცელებული დარღვევაა, რომელსაც ახასიათებს ხანგრძლივი (4-დან 72 სთ-მდე), შეტევითი ხასიათის თავის ტკივილი და თანმდევნი ნევროლოგიური სიმპტომები. წინამდებარე კვლევაში ჩვენ გავანალიზეთ დნმ-მეთილტრანსფერაზების (DNMT), DNMT1, DNMT3a და DNMT3b-ის, დონეები შაკიკით დაავადებულ პაციენტებში ჯანმრთელ კონტროლთან შედარებით. ასევე, შევისწავლეთ DNMT-ების დონეებსა და LINE-1 მეთილირების სტატუსს შორის კავშირი შაკიკით დაავადებულ პაციენტებში MTHFR C677T პოლიმორფიზმით.

მასალები და მეთოდები: კვლევაში მონაწილეობდა 48 პირი (24 პაციენტი შაკიკით და 24 ჯანმრთელი კონტროლი). კვლევის გეგმა დაამტკიცა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის ეთიკის კომიტეტმა.

შედეგები: ჩვენს მიერ ნანახი იქნა, რომ LINE-1-ის მეთილირება დადებითად კორელირებდა DNMT3a-თან. გარდა ამისა, DNMT1, DNMT3a და DNMT3b-ების დონეები MTHFR C677T პოლიმორფიზმის TT გენოტიპების მქონე პაციენტებში დაქვეითებული იყო CC გენოტიპების მქონე პაციენტებთან შედარებით.

დასკვნები: DNMT-ების დონეების ცვლილებები შესაძლებელია მნიშვნელოვან როლს თამაშობდეს შაკიკის პათოგენეზში. დნმ-ის მეთილირებაში მონაწილე ფერმენტების შემდგომმა გამოკვლევამ შესაძლოა მნიშვნელოვანი წვლილი შეიტანოს ეპიზოდური და ქრონიკული შაკიკის მკურნალობის ახალი მეთოდების შემუშავებაში.

Introduction: Migraine is one of the most common neurological diseases which affects 11% of the adult population worldwide. Clinical manifestation of the disease is classified into two main subtypes: migraine with aura (MA), the classic form, and migraine without aura (MO) [1]. The exact pathophysiology of migraine remains unclear, but it is believed that activation of the trigeminovascular system (TGVS) and cortical spreading depression (CSD) play an important role in these pathophysiological conditions [2].

Studies has shown that migraine has a three-time higher rate in women (15-18%) than in men (6-8%). The course of the disease may differ depending on gender, and it is related with ovarian hormones [3,4]. Moreover, women experience more frequent, longer and stronger headaches and their headaches are more susceptible to develop into a chronic form [5]. Women are also more prone to photo- and phonophobia and nausea as compared to men. According to population-based studies, genetic and environmental factors have almost equal input in the development of migraine [5,6].

Homocysteine-related dysfunction of the vascular endothelium may potentially influence susceptibility to migraine [7,8]. Hyperhomocysteinaemia-related endothelial injury may activate trigeminal fibers, leading to an inflammatory reaction occurring in the meninges, along with dilation of

the large cerebral vessels. It is this reaction that is believed to cause the characteristic headache common in patients with migraine with aura (MA) [9]. Various factors determine the levels of circulating plasma homocysteine, in particular, dietary deficiencies in the cofactors such as folic acid, and vitamin B12 and B6 essential for metabolizing homocysteine. Increasing evidence suggests an association between circulating homocysteine levels and common polymorphisms of genes involved in one-carbon metabolism, including *MTHFR* 677C>T, 1298A>C, *MTR* 2756A>G and methionine synthase reductase (*MTRR*) 66A>G [10,11,12].

DNA methylation that occurs at cytosine-phosphate-guanine (CpG) dinucleotide sites is the most common epigenetic modification event in the genome [13]. The DNA methylation process involves placing a methyl group onto the 5-position of cytosines situated in CpG dinucleotides and turning the cytosine into 5-methylcytosine (5mC), which is catalyzed by members of the DNA methyltransferase (DNMT) family [14]. DNMT1 is the primary enzyme responsible for copying methylation patterns after DNA replication because it localizes to replication foci and interacts with the proliferation cell nuclear antigen; DNMT3a and DNMT3b are responsible for de novo methylation. The overexpression of DNMT1, DNMT3a, and DNMT3b has been reported in various malignancies, including gastric, urothelial, and lung cancers, and may be related to tumorigenesis, tumor progression, and poor survival [15].

In this study, we examined the expression of DNMT1, DNMT3a, and DNMT3b in patients with migraine. The association between the levels of DNMTs and the LINE-1 methylation status was also investigated in migraine patients with C677T (rs1801133) polymorphism of one-carbon metabolism related gene *MTHFR*.

Material and methods. A total 48 individuals were enrolled in this study (24 patients with migraine and 24 age-matched healthy controls). Patients with migraine were recruited from the Tbilisi Institute of Medicine (Tbilisi, Georgia) from 2018 to 2021. The study protocol was approved by the Ethics Committee of Tbilisi State Medical University (N5-2017/65; Dec 13, 2017). Written informed consent was obtained from all patients and controls. Detailed information on medical history from all study subjects were recorded including demographic characteristic, headache features (pain duration, frequency and accompanied symptoms during attacks). Patient inclusion criteria were as follows: Adults>20, male and female, migraine without aura was diagnosed with neurological examination and based on international criteria of headache [ICHD-III] determined by HIS, not using pain management medicine at least 7 days prior to sample collection. Controls (n=24), which showed no evidence of headache disorder were recruited.

Sample collection and DNA preparation. Blood samples (5ml) were collected into EDTA-Vacationer tubes. PBMCs were isolated using Ficoll-Paque (Sigma-Aldrich, USA) gradient centrifugation. DNA from PBMCs was obtained by using QIAamp DNA mini kit (QIAGEN, Hilden, Germany).

MTHFR genotyping. For SNP genotyping of *MTHFR* rs1801133 variant TaqMan Assay (Thermo Fisher, USA) was performed. Each TaqMan SNP Genotyping Assay contained sequence specific forward and reverse primers to amplify the polymorphic sequence of interest and Two TaqMan minor groove binder (MGB) probes with nonfluorescent quenchers (NFQ): One VIC-labeled probe to detect Allele 1 sequence and one FAM-labeled probe to detect Allele 2 sequence. The allelic discrimination (AD) plot represents each sample well as an individual point on the plot. A typical AD plot shows Homozygote clusters, a Heterozygote cluster, and the no- template controls. The points in each cluster are grouped closely together and each cluster is located well away from the other clusters.

Measurement of DNMTs. Levels of DNMT1, DNMT3A and DNMT3B were measured in nuclear extracts of PBMC using DNMTs assay kits (Abcam, MA, USA) according to the manufacturer instruction.

Statistical analysis. Data were analyzed using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test was performed to evaluate the significance of any differences between the migraine and control groups. Spearman's rank correlation was used to examine the correlation between two continuous variables. All statistical analyses were two-sided and a *p*-value ≤ 0.05 was considered to indicate a statistically significant difference.

Results. Demographic and clinical characteristics of patients and control individuals provided in a Table 1.

Table 1. Demographic and clinical parameters of the study population

Characteristics	Migraine group (n=24)	Healthy group (n=24)
Age (years), mean (\pm SD)	38.9 \pm 5.7	41.2 \pm 7.6
Gender:		
Male	2	4
Female	22	20
Disease duration (years) mean (\pm SD)	11.6 \pm 7.5	-
Attack frequency/per month mean (\pm SD)	5 \pm 5.8	-
Attack duration (h)	34 \pm 16	-
Migraine without aura (MO) (n)	24	-

Data presented in \pm SD

The levels of DNMT1, DNMT3A, DNMT3B in patients with migraine and control samples. First, we examined the expression levels of DNMT1, DNMT3A and DNMT3B in 24 patients and compared with 24 control samples. Patients and controls showed similar levels of DNMTs (DNMT1, DNMT3A and DNMT3B) (Table 1). In addition, DNMT3B was expressed at lower levels than the other 2 DNMTs in both control and study groups.

Table 2. The levels of DNMT1, DNMT3A, DNMT3B in cases and the controls

DNA Methyltransferases	Case n=24	Controls n=24	<i>p</i>
DNMT1	0.72 \pm 0.15	0.58 \pm 0.21	0.56
DNMT3A	0.48 \pm 0.04	0.55 \pm 0.12	0.87
DNMT3B	0.22 \pm 0.13	0.14 \pm 0.09	0.07

Data presented in \pm SD

When considering the clinical parameter of the study population, such as attack frequency, we found no significant difference between DNMT1, DNMT3A, DNMT3B levels ($P > 0.05$) (Table 3).

Table 3. DNMT1, DNMT3A, DNMT3B and attack frequency in migraine patients

DNA Methyltransferases	Patients with 1-5 attack per month (n=14)	Patients with 6-10 attack per month (n=10)	<i>p</i>
DNMT1	0.53 \pm 0.13	0.68 \pm 0.12	0.62
DNMT3A	0.39 \pm 0.24	0.49 \pm 0.08	0.25
DNMT3B	0.25 \pm 0.09	0.15 \pm 0.10	0.15

Data presented in \pm SD

Correlation between the levels of DNMT1, DNMT3A, DNMT3B and methylation status of LINE-1 Interspersed Repetitive Element. In the previous study we observed high levels of unmethylated cytosines (uC) in the LINE-1 interspersed repetitive element (IRE) in the migraine group compared with the control subjects [16]. In the present study we analyzed correlations between DNMTs and methylation levels of LINE-1 and we found that DNA methylation levels of LINE-1 were correlated with levels of DNMT3a in migraine group and not with DNMT1 and DNMT3b. Interestingly, LINE-1 levels were slightly negatively correlated with DNMT3b ($r = -0.299$), however this was not statistically significant (Fig.1).

Correlation between the levels of DNMTs and rs1801133 polymorphism of MTHFR gene. Next, we examined the relationship between C677T (rs1801133) polymorphism of *MTHFR* gene and levels of DNMTs in migraine patients with TT and CC genotypes. We found high positive correlation between levels of DNMT1, DNMT3a and DNMT3b and *MTHFR* C677T genotypes ($r = 0.96$). TT individuals had lower levels of 3 DNMTs compared to CC individuals ($p < 0.05$).

Discussion and conclusions. DNA methyltransferases (DNMTs), responsible for the transfer of a methyl group from the universal methyl donor, *S*-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA, are essential for mammalian development [17]. There are four members of the DNMT family, including DNMT1, DNMT3A, DNMT3B and DNMT3L. *DNMT1* encodes the maintenance methyltransferase and *DNMT3A/DNMT3B* encode the *de novo* methyltransferases required to establish

and maintain genomic methylation [18]. DNMTs play an important role in genomic integrity, disruption of which may result in chromosome instability and tumor progression [19].

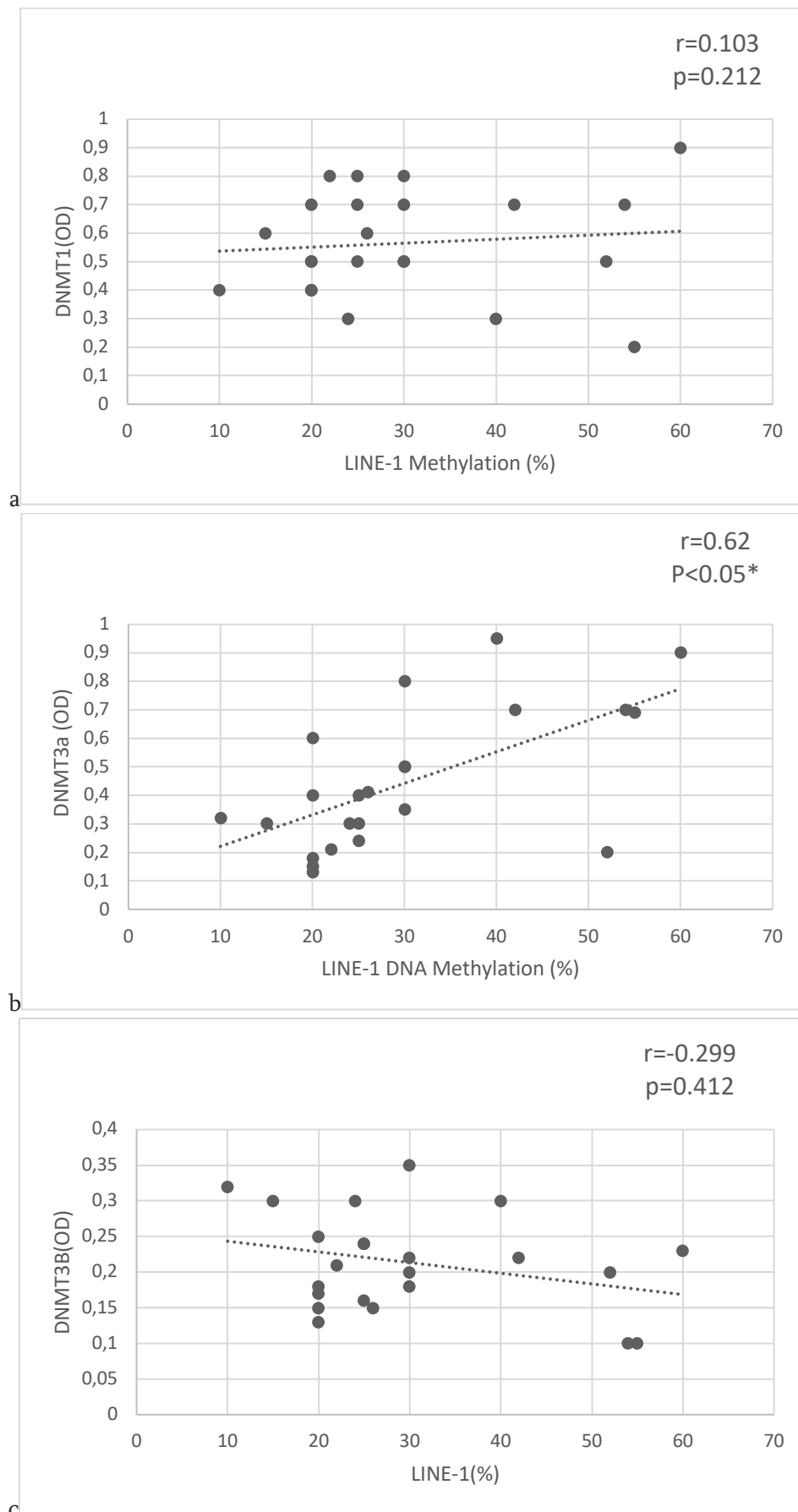


Fig. 1. Correlation between the levels of DNMT1 (a), DNMT3A (b), DNMT3B (c) and methylation status of LINE-1 in Migraine patients. Statistically significant values are marked with an asterisk.

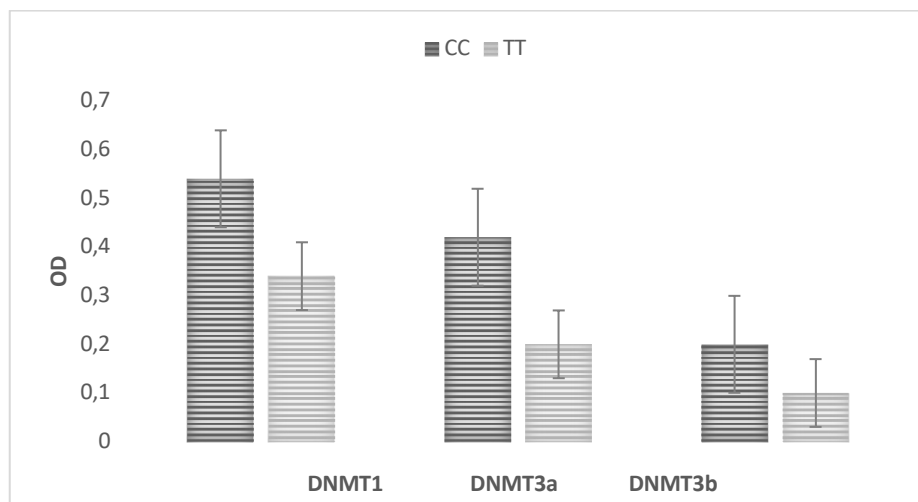


Fig. 2. Relationship between levels of DNMTs and TT and CC genotypes of *MTHFR* gene rs1801133 polymorphism.

In the present study we analyzed the expression of DNMT1, DNMT3a, and DNMT3b in patients with migraine compared with healthy controls as well as the association between the levels of DNMTs and the LINE-1 methylation status in migraine patients with *MTHFR* C677T variant. LINE-1 and Alu represents a family of retrotransposons that are interspersed throughout genomic DNA. Several studies have suggested that hypomethylation of LINE-1 and Alu are the causes for global DNA hypomethylation and genomic instability in many malignancies and inflammatory diseases [20]. We found that levels of DNMT3a in migraine group positively correlated with LINE-1 methylation level. In addition, migraine patients with TT genotype of *MTHFR* rs1801133 polymorphism expressed higher levels of DNMT1, DNMT3a, and DNMT3b compared with individuals with CC genotype.

In conclusion, altered levels of DNA methyltransferases and DNA hypomethylation is one of the possible epigenetic mechanisms associated with the complex etiology of migraine [21]. The decreased expression of DNMT1 and DNMT3a and DNMT3b in patients with TT genotypes of *MTHFR* gene rs1801133 polymorphism may play an important role in the pathogenesis of migraine. Further investigation of enzymes involved in DNA methylation with larger number of samples may provide important insight into the development of novel treatments for episodic and/or chronic migraine.

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¹Tbilisi State Medical University, Department of Molecular and Medical Genetics, Tbilisi, Georgia;

²Tbilisi State Medical University, V. Bakhutashvili Institute of Medical Biotechnology, Tbilisi, Georgia;

³Tbilisi Institute of Medicine, Tbilisi, Georgia

SUMMARY

Migraine is a common, disabling disorder characterized by attacks of 4 to 72 h of severe headache and associated autonomic and neurological symptoms. In the present study we analyzed the expression of DNMT1, DNMT3a, and DNMT3b in patients with migraine compared with healthy controls as well as the association between the levels of DNMTs and the LINE-1 methylation status in migraine patients with MTHFR C677T variant. **Materials and Method:** A total 48 individuals were enrolled in this study (24 patients with migraine and 24 age-matched healthy controls). The study protocol was approved by the Ethics Committee of Tbilisi State Medical University.

Results: We observed that methylation of LINE-1 were correlated with the levels of DNMT3a in migraine. In addition, levels of DNMT1, DNMT3a and DNMT3b in patients with TT genotypes of MTHFR gene rs1801133 polymorphism were significantly lower compared with individuals with CC genotypes. **Conclusions:** Differential levels DNMTs may play an important role in the pathogenesis of migraine. Further investigation of enzymes involved in DNA methylation with larger number of samples may provide important insight into the development of novel treatments for episodic and/or chronic migraine.

Keywords: DNA, Migraine, Methylation, DNMT, correlation