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Searching for Predictors of Migraine Chronification: a Pilot Study of 1911A>G Polymorphism of *TRPV1* Gene in Episodic Versus Chronic Migraine

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Abstract

Transient receptor potential vanilloid type 1 (TRPV1) receptors activated by heat and capsaicin are expressed in trigeminal nociceptive neurons and implicated in the generation of migraine pain. Genetic studies suggested that single-nucleotide polymorphism (SNP) 1911A>G (rs8065080), leading to amino acid substitution Ile585Val, in the *TRPV1* gene affects functional activity of TRPV1 receptors and is involved in different pain conditions. However, this polymorphism has not been tested in migraine patients. The objective of this pilot study was to investigate genetic factors of migraine susceptibility. We evaluated frequency distribution of AA, AG, and GG variants of SNP 1911A>G in the *TRPV1* gene in patients with episodic and chronic migraine compared with healthy individuals. The study included 46 patients diagnosed with migraine (27 episodic and 19 chronic) and 50 healthy individuals as a control group. DNA from peripheral blood was used to test *TRPV1* SNP using allele-specific PCR combined with gel electrophoresis. The genotype frequency distribution in episodic migraine was comparable with that in controls (AA 33%, AG 56%, GG 11% and AA 34%, AG 46%, GG 20%, respectively). On the contrary, in chronic migraine, the distribution differed significantly ($p < 0.05$) (AA 68%, AG 32%, GG 0%). This are first indications for a distinctive genotype frequency distribution of *TRPV1* 1911A>G in chronic migraine patients compared with episodic migraine patients and controls. Our data confirm a different predisposition to chronic pain in migraine and give a prerequisite for a new look at the nature of chronification of migraine, proposing that the absence of GG genotype may be considered as possible risk biomarker of episodic migraine evolution to chronic form.

Keywords Migraine · TRPV1 · Single-nucleotide polymorphism · rs8065080 · Chronification · Pain sensitivity

Introduction

Migraine is a common primary headache disorder, which according to the frequency of attacks can be classified into two main subtypes: chronic migraine (CM) and episodic migraine (EM). CM is defined as a headache occurring on 15 or more days per month for more than 3 months, of which migraine headache occurs on at least 8 days per month, whereas EM is associated with less-frequent headaches (Headache Classification Committee of the International Headache Society 2018).

Because of high prevalence and the burden of attacks, it is of great importance to improve diagnostic tools for patient stratification and personalized treatment of migraine. In this regard, CM is of special interest as it can be regarded "an evolution" of EM (Buse et al. 2019), although the factors predisposing to these forms of migraine could be different. In light of this, it is of interest to investigate the contribution of transient receptor potential vanilloid type 1 (TRPV1) receptors, as they are expressed in trigeminal nociceptors, in particular in meningeal tissue, the supposed site where migraine pain originates from (Zakharov et al. 2015).

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TRPV1 receptors are non-selective cation channels that are activated by capsaicin, protons, noxious heat (> 43 °C), endogenous lipids, and certain inflammatory mediators (Szallasi and Blumberg 1999; Tominaga et al. 1998; Tominaga and Caterina 2004). TRPV1 receptors contribute to the sensitization of peripheral nociceptors in neuropathic pain (Cortright and Szallasi 2009; Stucky et al. 2009). Activation of TRPV1 receptors results in release of calcitonin gene–related peptide (CGRP), which is one of the main migraine pain mediators (Edvinsson et al. 1990; Goadsby and Edvinsson 1993; Jansen-Olesen et al. 1996; Bernardini et al. 2004), and gives long-lasting activation meningeal afferents (Zakharov et al. 2015). Recent studies suggest that the non-synonymous *TRPV1* single-nucleotide polymorphism (SNP) 1911A>G (rs8065080), resulting to amino acid substitution Ile585Val, co-determines functional activity of these receptors (Forstenpointner et al. 2007; Okamoto et al. 2018).

There is a controversy to what extent the amino acid change at position 585 affects the functionality of TRPV1 receptors. Whereas one in vitro study of the functional responses to capsaicin, pH, and temperature in whole-cell patch clamp and calcium imaging experiments in HEK293 cells did not show a difference for both SNP variants on receptor structure and function (Hayes et al. 2000), another study in HeLa cells claimed a decreased channel function for the protein expressing the valine at position 585 (Cantero-Recasens et al. 2010). At the in vivo level, the effect of the polymorphism is also not clear. Whereas one study reported no association on cold/heat pain sensitivity in European Americans (Kim et al. 2006), other studies showed that homozygous carriers of the G variant exhibited cold hypoalgesia (Kim et al. 2004; Binder et al. 2011). In comparison to GG carriers who showed normal sensitivity to tactile sensation, heat, and mechanical pain detection, carriers of the two other genotypes were reported to have heat hyperalgesia, mechanical (pinprick) hyperalgesia, and mechanical hypaesthesia to non-painful mechanical stimuli (Binder et al. 2011). In that study, no difference in genotype distribution was observed for neuropathic pain patients compared to healthy volunteers suggesting that the variant had no role in chronic neuropathic pain. In a study by Forstenpointner et al. (2007), the GG genotype was associated with less warm hypoesthesia and less heat pain sensitivity; in addition, that genotype may prevent from peripheral and secondary central sensitization in patients with chronic neuropathic pain (Binder et al. 2011). Previously, Carreno et al. (2012) already showed some evidence for association of the 2841C>T (rs222741) SNP of *TRPV1* gene with migraine, but it was different from 1911A>G SNP and that study was performed in overall migraine group in Spanish population, without subdivision on certain disease forms. Thus, it is of special interest to test *TRPV1* 1911A>G SNP in migraine patients and its possible contribution to evolution of episodic migraine to chronic.

The aim of the current study was to investigate the genotype frequency distribution of *TRPV1* 1911A>G SNP in chronic and episodic migraine and compare those with healthy controls to clarify whether genotype frequencies are associated with any of these migraine subtypes. Our results give first evidence that the genotype distribution of *TRPV1* SNP 1911A>G is different between CM compared with the EM and healthy groups. Of note, in the CM group, the AA genotype is nearly doubled, whereas the GG genotype was not present. It is tempting to speculate, but has to be shown in replication in larger migraine cohorts, whether the GG genotype may serve as a potential marker of migraine chronification protection.

Material and Methods

Study Subjects

The Ethics Committee of the Kazan State Medical University approved the study (permission protocol number 7 from 25.09.2018), which was performed according to the Declaration of Helsinki. Blood samples of 96 subjects were examined. The study participants consisted of 19 patients with CM (17 females, 2 males), 27 patients with EM (22 females, 5 males), and 50 healthy volunteers (27 females, 23 males), all were Caucasians from Russian population.

The diagnosis of migraine was established by experienced neurologists according to the International Classification of Headache Disorder (ICHD), (Headache Classification Committee of the International Headache Society 2018). Patients meeting the following criteria were included in the study: previously diagnosed episodic or chronic migraine as defined in ICHD and age from 18 to 55 years. Exclusion criteria included probable migraine and familial or sporadic hemiplegic migraine according to the ICHD, chronic diseases in stage of decompensation, oncology, pregnancy, and breast feeding. Criteria for inclusion in the control group were no present or former history of migraine or other types of chronic headaches and age from 18 to 55. Written informed consent was obtained from all participants prior to the sampling and clinical testing. Data on European population genotypes from the 1000 Genomes Project (Ensembl 98, 2020) was used as population control.

Genetic Analysis

Peripheral venous blood (2 mL) was collected from each subject into a vacuum tube containing an ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Total genomic DNA was extracted from samples from each participant by an isolation kit (Litech, Russia) according to the manufacturer's instructions. Measurement of DNA concentration in samples was performed using a NanoDrop2000 (Thermo Fisher

Scientific Inc., USA). Extracted DNA samples were stored at − 20 °C. The *TRPV1* 1911A>G polymorphism was analyzed using an allele-specific polymerase chain reaction (AS-PCR) assay. *TRPV1* gene sequence from database GenBank (accession number NG_029716) and PrimerSelect program from Lasergene software package (DNASTAR, USA) were used for primer design. The SNP was detected by PCR using the following primers: 5′-TGTGCCGTTTCATGTTTGTC TTCG-3′ (forward), 5′-CTGTGCCGTTTCATGTTTGT CTTCA-3′ (forward), and 5′-TGAGGTAGGAGAAT TGCTTGAACC-3′ (reverse). Primers were ordered from Eurogen (Russia).

The total volume of AS-PCR reaction solution was 10 μL, including 1.0 μL buffer solution 10X TaqPol, dNTP mix (25 mM) 0.4 μL, MgCl2 (25 mM) 0.5 μL, forward primer (100 μM) 0.1 μL, reverse primer (100 μM) 0.1 μL, TaqPol polymerase, 5 U/μL (Eurogen, Russia) 0.2 μL, 0.5 μL of 10–100 ng of DNA extract, and nuclease-free water to adjust the volume. Amplification was performed in a Thermal Cycler C1000 (BioRad, USA) under the following conditions: (i) initial denaturation at 94 °C for 5 min; (ii) 40 cycles including denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 25 s; (iii) final extension for 5 min at 72 °C. Each reaction was repeated at least twice for each DNA sample at different times. Amplified PCR products were separated by gel electrophoresis using 1% agarose gel. After electrophoresis, the gel was stained by ethidium bromide and visualized using GelDOC XR+ Imagine system (BioRad, USA) (Fig.1).

Samples were genotyped in a randomized order and all labtechnical procedures were performed blind to any phenotype information.

Statistical Analysis

We organized the data of this observational study into 3 × 2 (number of variants × number of groups) contingency tables and analyzed differences between the groups in genotype distributions using Fisher's exact test. Fisher's exact test is more accurate than the chi-square test when the sample size is small. It has been recommended that this test is used when the total sample size is smaller than 1000 (McDonald 2014) or always with 3 × 2 tables (Kroonenberg and Verbeek 2018). For computations, we used the on-line software (Kirkman 1996) implementing the algorithm by Clarkson et al. (1993) when the values in all cells were smaller than 100, or myfisher23 Matlab-function (Cardillo 2007) when a cell value exceeded 100. The Hardy-Weinberg equilibrium was calculated for both cases and controls. A p value smaller than 0.05 was considered as significant.

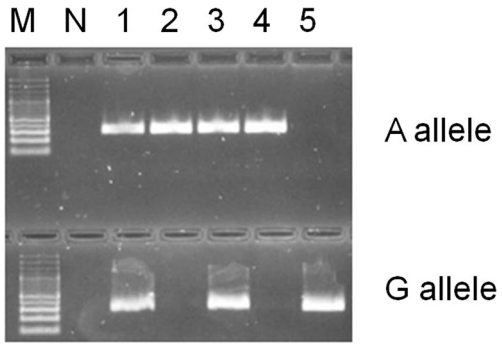

Fig. 1 Allele-specific PCR analysis of 1911A>G genotypes. Column M: the 100 bp marker DNA ladder. Column N: negative control. Columns 1 and 3 show a sample heterozygote AG. Columns 2 and 4 show a sample homozygote of the wild-type AA. Column 5 shows a sample homozygote of GG variant

Results

We checked the accordance of *TRPV1* 1911A>G genotyping results in the studied healthy control group with the European population data from the 1000 Genomes Project (Ensembl 98, 2020). Our data matched those data as SNP's distribution was nearly identical ($p = 0.907$) in these two cohorts (Table 1).

Next, we compared the genotype frequencies in male and female data from 1000 Genomes Project (Ensembl 98, 2020) and their distributions did not differ significantly ($p = 0.342$). In addition, there were no marked differences between male and female genotypes for the studied groups of CM, EM, and control group (Table 2). Based on these findings, we included the data from both genders to the main analysis.

Among the participants that were analyzed for the *TRPV1* 1911A>G SNP in this study, obtained distribution of genotypes was the following: in the EM group: AA wild type 33%, AG heterozygote 56%, GG variant 11%; in the CM group: AA 68%, AG 32%, GG 0%; in the control group: AA 34%, AG 46%, GG 20% (Table 3; Fig. 2). The distribution of the investigated genotypes was in Hardy-Weinberg equilibrium.

The genotype distribution of CM patients differed significantly from the control ($p = 0.015$) and from the EM ($p = 0.042$) groups. The EM group did not differ from the control group ($p = 0.594$).

Table 1 Distribution of *TRPV1* 1911A>G genotypes in the controls and 1000 Genomes Project

Genotype	Control % (n)	1000 Genomes Project % (n)	p value
AA	34 (17)	37 (187)	0.907
AG	46 (23)	44 (221)	
GG	20 (10)	19 (95)	

%, percentage from total number of samples in each group; n, number of each genotype samples; p value calculated by Fisher's exact test

Table 2 Genotype gender distribution of *TRPV1* 1911A>G SNP in studied groups and the 1000 Genomes Project

	Control, *n*		Episodic, *n*		Chronic, *n*		1000 Genomes Project, *n*	
	Male	Female	Male	Female	Male	Female	Male	Female
AA	8	9	1	8	1	12	82	105
AG	12	11	3	12	1	5	113	108
GG	3	7	1	2	0	0	45	50
p value	0.538		0.797		1.0		0.342	

n, number of each genotype samples in males and females; *p* value calculated by Fisher's exact test

Discussion

In this study, we analyzed, for the first time, the *TRPV1* 1911A>G genetic polymorphism in two subtypes of migraine based on attack frequency, i.e., episodic and chronic migraine. The most valuable finding is that genotype distribution in CM markedly differs from healthy group and less from EM. In the CM group, there is even a complete absence of the GG genotype. Moreover, chronic migraineurs demonstrated a double increase in AA genotype.

Apart from rare familial forms of migraine, there is a strong evidence that even the common types of migraine to large extend are determined by genetic factors (Piane et al. 2007; Chasman et al. 2011; van den Maagdenberg et al. 2019). This view supports a continuous interest to genetic studies in migraine in order to find out predictive tools that could serve as an approach towards personalized medicine. Variety of different genes and their SNPs working separately or in synergy and resulting in certain types of migraine have been reported (Kara et al. 2003). According to the possible role of TRPV1 receptors in migraine pathogenesis (Hoffmann et al. 2012; Meents et al. 2012; Chatchaisak et al. 2012; Zakharov et al. 2015) revealing in their activation and further release of CGRP (Akerman et al. 2003; Nicoletti et al. 2008), TRPV1 receptor gene is one of the primary candidates for study in light of migraine. However, despite a number of studies on the role of TRPV1 receptor and its polymorphisms in different pain conditions, the *TRPV1* 1911A>G SNP was not studied in a migraine yet.

In the current study, we found that the control group was presented by AA, AG, and GG genotypes with the prevalence of AG genotype and more or less equal presence of AA and GG variants. EM patients did not differ significantly from the control group but the contribution of still presenting GG variant was relatively reduced. However, distribution of genotypes in CM patients was markedly different: the AA genotype nearly doubly increased, whereas the GG variant was completely absent. The latter finding of the complete absence of the GG variant in CM was the most surprising, what could be interpreted that this genotype is associated with adaptive protective mechanism against migraine chronification, as far as the prevailing view suggests that CM is a progression of EM (Buse et al. 2019). Thus, considering the absence of GG variant in CM, we can suppose that the individuals having EM with the GG variant should have smaller or have not at all a probability to be progressed to CM.

The prevalence of AA variant in CM is in line with data that the AA genotype is associated with higher pain sensitivity, particularly with a lower threshold for heat and mechanical stimuli (Jansen-Olesen et al. 1996). Therefore, it can be assumed that patients with CM should have higher pain sensitivity. Furthermore, Binder et al. (2011) showed that patients with neuropathic pain carrying GG variants were significantly protected from heat hyperalgesia (Binder et al. 2011). Likewise, other studies concluded that GG is a loss-of-function genotype, resulting in a heat sensitivity decrease (Wang et al. 2016), significant cold hypoalgesia (Kim et al. 2006; Binder et al. 2011), low sensitivity to tactile sensation, mechanical stimuli, and heat pain (Forstenpointner et al. 2007; Binder et al. 2011).

It is worth noting that people with *TRPV1* 1911A>G polymorphism are also differently sensitive to capsaicin

Table 3 *TRPV1* 1911A>G genotype and allele distribution in the control group and EM and CM patients

Genotype	Control % (*n*)	Episodic migraine % (*n*)	Chronic migraine % (*n*)
AA	34 (17)	33 (9)	68 (13)
AG	46 (23)	56 (15)	32 (6)
GG	20 (10)	11 (3)	0 (0)
A allele	57 (57)	61 (33)	84 (32)
G allele	43 (43)	39 (21)	16 (6)

%, percentage from total number of samples in each group; *n*, number of each genotype samples

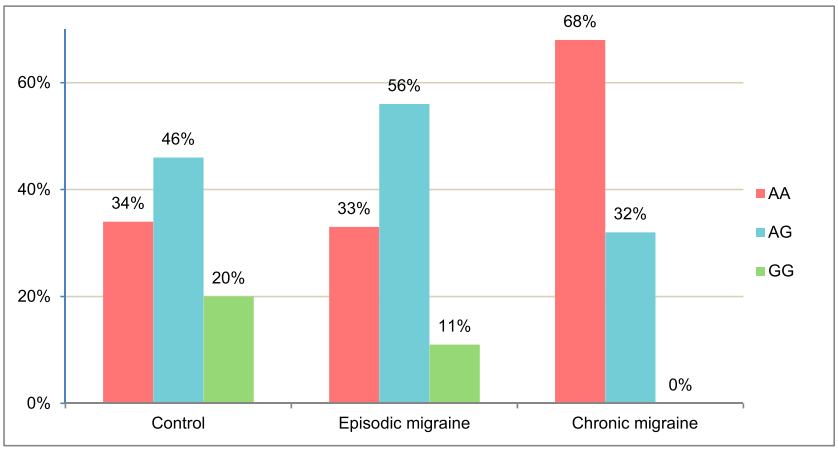

Fig. 2 *TRPV1* 1911A>G SNP distribution in the control group and EM and CM patients. CM group revealed high frequency of AA and missing GG genotype

(Forstenpointner et al. 2007; Okamoto et al. 2018), which is the agonist of TRPV1 receptors (Caterina et al. 1997). Previously found highly heterogeneous reaction of migraine patients to stimulation of TRPV1 receptors by capsaicin (Kamshilin et al. 2018) also may be explained by different *TRPV1* 1911A>G polymorphism in EM and CM patients' cohorts.

Taken together, the available data suggest that different variants of SNP are associated with different properties of pain sensitivity, thus forming the specific phenotype of CM and EM patients. We may assume that there is different genetic determinism of episodic and chronic forms of migraine based on differences in *TRPV1* 1911A>G genotype distribution. Therefore, the possible role of TRPV1 receptors in the heterogeneity of migraine and the risk of chronification may be suggested. At the same time, it should be noted that the genetic background might be just a predisposing element, whereas epigenetic mechanisms can be even more effective in chronification triggered by the stressful environmental factors.

The main strength of this study is the discovery of complete absence of GG variant in patients with CM. However, some study limitations should be mentioned. In this study, EM and CM groups of patients were mostly females in contrast to the control group, which formally limits our conclusions so far to this gender. Although, according to the World Health Organization data (World Health Organization 2011), this reflects a general trend of migraine prevalence in females, including both episodic and chronic migraine (Özge et al. 2015), it is worth noting that despite a prevalence of females in both EM and CM groups they revealed significantly different distribution of genotypes, what probably reflects a general trend. In addition, our results are limited only by subjects of European descent. Besides, the distribution of SNP in the control group matches very well the similar distribution in much bigger cohort from the 1000 Genomes Project (Ensembl 98, 2020); number of patients in the studied groups was not numerous and the obtained data should be confirmed in larger cohorts.

In summary, if our results are proven in further studies, *TRPV1* 1911A>G SNP genotyping may be used as a prognostic factor and clinical biomarker for predicting severity of migraine and choosing a timely prophylactic treatment strategy.

Conclusion

In conclusion, this pilot study revealed the genotype difference of the CM group from the EM and healthy control groups. The main finding is that *TRPV1* 1911A>G SNP is differently associated with CM and EM, what assumes different genetic predisposition to these migraine forms. Potentially, detection of GG variant of *TRPV1* 1911A>G polymorphism can serve as a marker of protection against progression of EM to CM (migraine chronification) and could be a step to personalized treatments of migraine patients.

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Code Availability Not applicable.

Authors' Contributions A.Y. performed genotyping experiments, analyzed data, and wrote the manuscript. Y.D. designed and performed the experiment and represented it in the manuscript. J.T. performed statistical analysis of data and represented it in the manuscript. A.Y., O.K., I.K., and D.N. contributed to patients' enrolment and sample collection. A.K. and R.G. conceived the idea of the study. R.G. and A.R. discussed the results and revised the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval The study was conducted in Kazan in accordance with ethical standards presented in the Declaration of Helsinki. The Ethics Committees of the Kazan State Medical University prior the research approved the protocol of this study (permission protocol number 7 from 25.09.2018).

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Not applicable.

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