RESEARCH ARTICLE

Analysis of Genetic Variants in *SCN1A*, *SCN2A*, *KCNK18*, *TRPA1* and *STX1A* as a Possible Marker of Migraine

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Abstract: ***Background*:** Migraine is a polygenetic disease, considered as a channelopathy. The dysregulation of ion functioning due to genetic changes may activate the trigeminovascular system and induce migraine attack both migraine with aura (MA) and without aura (MO).

***Objectives*:** The aim of the study was to analyze the following variants of genes encoding ion channels and associated protein: c.3199G>A *SCN1A*, c.56G>A *SCN2A*, c.28A>G and c.328T>C *KCNK18*, c.3053A>G *TRPA1*, c.31-1811C>T *STX1A* in migraine patients.

***Patients and Methods*:** The study included 170 migraine patients and 173 controls. HRMA and Sanger sequencing were used for genotyping. Meta-analysis was performed for c.28A>G, c.328T>C *KCNK18,* and c.31-1811C>T *STX1A*.

***Results*:** AA genotype of c.56G>A *SCN2A* was found only in migraine patients. Patients with c.328T>C *KCNK18* mutation had an increased risk of developing migraine before the age of 18. Moreover, individuals with AA/TC haplotype of *KCNK18* had higher attack frequency than those with AA/TT ($p<0.05$). T allele of c.31-1811C>T *STX1A* was more frequent in MA patients than MO ($p<0.05$). The c.3053A>G *TRPA1* polymorphism was more common in patients with migraine onset before the age of 15 ($p<0.05$), while c.31-1811C>T *STX1A* and c.3199G>A *SCN1A* before the age of 10 ($p<0.01$). Meta-analysis showed a significant association of c.31-1811C>T *STX1A* polymorphism with migraine overall (OR=1.22, $p=0.0086$), MA, and MO. No association was found for c.28A>G *KCNK18*, c.328T>C *KCNK18,* and migraine overall.

***Conclusion*:** Changes in genes encoding ion channels or proteins regulating their functioning may increase the risk of migraines and correlate with clinical features of disease, *e.g.* age of onset and attack frequency.

1. INTRODUCTION

Migraine is a common neurological disorder affecting about 11% of the adult population. It is diagnosed according to the criteria of the 3rd edition of International Classification of Headache Disorders (ICHD-3), which was updated in 2018. The disease occurs in two main clinical subtypes: migraine with aura (MA) and migraine without aura (MO), the more common one. Migraine can also be divided into episodic and chronic form [1, 2]. Although the prevalence of migraine is high, some problems in its diagnosis and pharmacotherapy still occur. Almost half of the migraine patients

are not under the care of a physician to treat this disorder [3]. Another problem is an inefficient treatment or the overuse of abortive medications, mainly the overuse of the nonsteroidal anti-inflammatory drugs (NSAID) [4]. Interestingly, triptans the specific migraine abortive drugs are underused [5]. The explanation may be that approximately 60% of migraine patients use the over-the-counter drug (OTC) without consulting treatment with a specialist [6].

These problems may be solved by finding a biomarker that would indicate the risk of migraine development, treatment response, or the clinical feature of migraine. No specific diagnostic tests in migraine, either biochemical and genetic, which would be helpful in a fast diagnosis and proper treatment, is available. The best-studied biochemical factors in migraine are serotonin (5-HT) and calcitonin gene-related peptide (CGRP), but they are not used in routine clinical

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practice due to the inconsistency of results [7]. The genetic tests are performed only in familial hemiplegic migraine (FHM), a rare, monogenic type of MA or if the migraine is considered as a symptom of a monogenic disease, such as CADASIL or mitochondrial diseases.

The genetic studies in FHM gave basis to the theory that migraine or at least aura is the channelopathy. There are three types of FHM (FHM1-3). Type 3 (FHM3) is caused by a mutation in the *SCN1A* gene, encoding the α subunit of the neuronal voltage-gated sodium channel Nav1.1. So far, only ten mutations were found in *SCN1A* to cause the FHM3 [8]. Polymorphisms in *SCN1A* were also found in patients with sporadic migraine, as well as epilepsy [9, 10]. Another gene associated with the sodium channel is *SCN2A* encoding α subunit of the $Na_v1.2$ channel. Changes in *SCN2A* were found in epileptic patients and in multiple drug resistance in the course of treating epilepsy [11], which is important for patients using the antiepileptic drugs as a migraine treatment. Analysis of *SCN1A* and *SCN2A* polymorphisms may be helpful in estimating the antiepileptic drug response in migraine patients. However, genetic variants in *SCN2A* have never been studied in migraine before.

Another gene associated with ion channel in migraine is *KCNK18* (potassium channel subfamily K member 18). It encodes the TWIK-related spinal cord potassium channel (TRESK). This channel is also important in migraine therapy, as the activation of the TRESK channel was proposed to be a potential target for pain treatment [12]. Moreover, a frameshift mutation F139WfsX24 in *KCNK18*, leading to the loss of TRESK function, was found in a multigenerational family with MA [13]. Genetic changes in the *KCNK18* may result in the hyperexcitability of trigeminal nerve neurons and an increase in the susceptibility of migraine headaches [14].

The migraine headache may also be caused by the stimulation of the afferent trigeminal fibers of the transient receptor potential ankyrin 1 (TRPA1) channels by exogenous irritants [15]. Many of the migraine triggers are the agonist of TRPA1. In turn, TRPA1 channel inhibitors may have antimigraine properties, *e.g.*, parthenolide isolated from *Tanacetum parthenium* [16, 17]. Interestingly**,** no studies have been performed on the association between polymorphisms in the *TRPA1* gene and migraine.

The functioning of the aforementioned ion channels may be regulated by syntaxin 1A, a presynaptic membrane protein, encoded by *STX1A*. Syntaxin 1A is also involved in the regulation of neurotransmitters such as γ-aminobutyric acid (GABA) and 5-HT [7], whose concentration is reduced in patients in a migraine-free period [18]. Changes in *STX1A* are putative risk factors for migraine development, but so far, they have not been studied in Polish patients. It is the first correlation of *STX1A* polymorphism with pharmacotherapy response in migraine patients as well as with other changes in genes encoding ion channels.

The aim of the study was to analyze the polymorphisms c.3199G>A (p.A1067T, rs2298771) in *SCN1A,* c.56G>A (p.R19K, rs17183814) in *SCN2A*, c.28A>G (p.R10G, rs67346047) and mutation c.328T>C (p.C110R, rs140325 655) in *KCNK18* gene, as well as the polymorphisms c.3053A>G (p.H1018R, rs959976) in *TRPA1* gene and c.31-1811C>T (rs941298) in *STX1A*, in migraine patients and controls. The correlation of genetic variants with the clinical features of migraine and applied pharmacotherapy was also performed. The combined data analyses were performed for c.28A>G, c.328T>C *KCNK18* variants, and c.31-1811C>T *STX1A* polymorphism.

2. PATIENTS AND METHODS

2.1. Subjects

The study included 343 individuals, selected from a group of 420. A total of 170 migraine patients (149 females, 21 males; mean age ± SD: 39 ± 15 years) were recruited for the study from the Department of Neurology at Poznan University of Medical Sciences (PUMS) and the Neurological Outpatient Clinic between 2015 and 2018. The diagnosis of migraine was performed according to the ICHD-3 criteria [1, 2] by an experienced neurologist, and 67 patients fulfilled the diagnostic criteria for MA (57 females, 10 males; mean age ± SD: 39 ± 14 years), and 103 for MO (92 females, 11 males; mean age ± SD: 39 ± 15 years). 125 patients declared a positive family history. To analyze the association between genetic variants and the clinical features of migraine, patients were divided into smaller groups according to the age of onset, frequency of attacks, and duration of the headache. Patients were treated with abortive or preventive drugs, according to the European Federation of Neurological Societies guidelines on migraine treatment [19].

The control group consisted of 173, sex and age-matched healthy subjects (147 females, 26 males, mean age ± SD: 37 ± 14 years).

All individuals (both patients and controls) were of a Polish Caucasian population. None of them were suffering from psychiatric or any other neurological disorders. The blood was collected in headache-free periods. All samples were blinded to avoid bias. Genotyping was performed on all individuals.

The study was approved by the Local Bioethical Committee of PUMS (no. 931/14 with an extension no. 993/17) and signed, informed consent was obtained from all subjects.

2.2. Genetic Analyses

Genomic DNA was isolated using Genomic Micro AXD Blood Gravity isolation kit (A&A Biotechnology, Poland) and stored at -80°C.

The genetic variants were analyzed by high-resolution melting analyses (HRMA) using CFX Connect™ Real-Time System (Bio-Rad, USA). The primers for HRMA were designed in online Primer3Plus software based on the published genome sequence of the chosen gene. The primers sequences are presented in Table **1**.

Briefly, the 15 ng of genomic DNA was used for Real-Time PCR with the EvaGreen (SsoFast™ EvaGreen® Supermix, Bio-Rad, USA) as an intercalating dye and 250 nM of each primer. The melting analysis was performed, and the data was analyzed by Melting Analysis software (Bio-Rad, USA). The cycling conditions of HRMA will be provided upon request. The results of HRMA were confirmed by

Table 1.   Sequences of the primers used for HRMA of analyzed genetic variants.

Genetic Variant	Primers Sequence	Annealing Temperature	Product Size
SCN1A c.3199G>A	Forward: 5'-CCAAATTGCTGTGGATAGGATGC-3' Reverse: 5'-TGCCAGTTCCTATACCACTTGT-3'	59°C	233 bp
SCN2A c.56G>A	Forward: 5'-CCTCCCTGTTTCTGTAGCACTT-3' Reverse: 5'-GTCACTGTTTGGCTTTGGGC-3'	59°C	233 bp
KCNK18 c.28A>G	Forward: 5'-TCTTCCACCAGCTCCTCTTG-3' Reverse: 5'-CAATGGCAGAGAAGACCACA-3'	62°C	173 bp
KCNK18 c.328T>C	Forward: 5'-CCAGCAGAACCTTTTCCTCA-3' Reverse: 5'-TGTGGCTTTGCACTTACCC-3'	61°C	191 bp
TRPA1 c.3053A>G	Forward: 5'-TTGTCTTATTTCCCCAGTGCAA-3' Reverse: 5'-TGGCACATGCTTAAGAAAAAGTTC-3'	60°C	111 bp
STX1A c.31-1811C>T	Forward: 5'-CAGTCCATCCATCAATCAAACA-3' Reverse: 5'-TCATCAGGCTGGGCCTTAAC-3'	59°C	141 bp

HRMA – high-resolution melting analyses, *SCN1A* – sodium voltage-gated channel alpha subunit 1 gene, *SCN2A* – sodium voltage-gated channel alpha subunit 2 gene, *KCNK18* – potassium channel subfamily K member 18 gene, *TRPA1* – transient receptor potential ankyrin 1 gene, *STX1A* – syntaxin 1A gene.

Sanger sequencing in the forward and reverse directions using the 3130xl Genetic Analyzer (Applied Biosystems HITACHI, USA) in an independent unit. The reads were aligned to the human reference genome with BioEdit Software (Tom Hall Ibis Biosciences, Canada) separately by two investigators.

The functional consequences of analyzed polymorphism were predicted using *in silico* bioinformatics tools: Polyphen2 and MutationTester.

2.3. Meta-analysis

To answer if obtained result are just a random variance, a confirmatory dataset was tested. The relevant studies published until December 2019 was searched using electronic databases (PubMed, ClinicalKey®, Science Citation Index, ScienceDirect, Cochrane Library) and keywords such as "*SCN1A* AND migraine", "*SCN2A* AND migraine", "*KCNK18* AND migraine", "*TRPA1* AND migraine", "*STX1A* AND migraine", "rs2298771 AND migraine", "rs17183814 AND migraine", "rs67346047 AND migraine", "rs140325655 AND migraine", "rs959976 AND migraine", "rs941298 AND migraine". All case-control studies with extractable data were included. All studies were published as full-length articles or letters in peer-reviewed journals. The diagnosis of MA or MO was made according to ICHD-3 criteria [1].

Case-control study by Domitrz *et al.* [10] in migraine-related genes analyzed the c.3199G>A *SCN1A* polymorphism. However, the study was excluded due to the small sample size and lack of information if the polymorphism was present in controls (62 healthy subjects). Among 60 migraine patients, there were subjects with familial hemiplegic migraine and sporadic hemiplegic migraine (not consider in our study), as well as 11 MA and 23 MO patients. The GA genotype of c.3199G>A *SCN1A* was present only in 4 MA and 7 MO patients.

The present study analyzed the polymorphisms c.56G>A in *SCN2A* and c.3053A>G in *TRPA1* for the first time in migraine patients, thus looking at another cohort was impossible.

For the combined analyses of c.28A>G and c.328T>C variants in *KCNK18*, a total sample of 1164 migraine patients and 975 controls was used. It was obtained from our case-control group (170 migraine patients and 173 controls) with the prior case-control studies: the two case-control studies of multicenter collaboration by Lafrenière *et al.* [13] (A: 110 migraine patients among which 13 of French-Canadian origin, 44 of Canadian, and 53 of Portuguese origin and 80 controls of Canadian origin; B: 459 Australian migraine patients and 475 Australian controls) and the Italian study by Rainero *et al.* [20] (425 migraine patients and 247 controls).

For the meta-analysis of c.31-1811C>T polymorphism in *STX1A*, an overall sample of 725 migraine patients and 893 unrelated controls was used. It was obtained by combining our case-control group (170 migraine patients and 173 controls) with the three prior case-control studies: the Spanish study by Corominas *et al.* [21] (188 migraine patients and 210 controls, 22 migraine patients with FHM were not included), the Portuguese study by Lemos *et al.* [22] (188 migraine patients and 287 controls) and the Austrian study by Tropeano *et al.* [23] (179 migraine patients and 223 controls).

2.4. Statistics

When planning the studies, we determined the minimum sample size in every case, taking into account the calculations performed with the statistical package Statistica 13 with Medical Kit (StatSoft Inc., USA) and GraphPad for Windows, assuming 0.9 as the test power and an alpha (α) error equal to 0.05 (*i.e.* values frequently used in medical research). Genetic data were compared between groups using Fisher's exact tests (with OR – Odds Ratio and 95% CI) for

categorical measures. Hardy-Weinberg equilibrium was verified for all variants in both MA, MO and controls. In the analysis, the nominal significance threshold was set at $p<0.05$ and lowered to $p<0.016$ after the multiple comparison correction of Bonferroni.

To combine individual study results, a meta-analysis was performed. Data were analyzed using a fixed-effects model. The results of the meta-analysis were visualized using a forest plot, which illustrates the results of the individual studies and the summary effect. Cochran's Q test was used for testing homogeneity.

3. RESULTS

3.1. Comparison of Clinical Features of Migraine and Applied Pharmacotherapy

In the present study, the frequency of the following variants: c.3199G>A *SCN1A*, c.56G>A *SCN2A*, c.28A>G and c.328T>C *KCNK18*, c.3053A>G *TRPA1*, c.31-1811C>T *STX1A* in studied subjects and the clinical features of migraine were analyzed. The MA and MO patients differed in the clinical features of migraine, such as the age of onset, attack frequency and duration of the attack (Table **2**). The

majority of MA patients had milder migraine attacks (less frequent $p=0.0016$ and shorter $p=0.0032$) than MO patients. The MO more often started after the age of 15, while in MA in more than half cases, attacks started before that age ($p=0.0082$). The length of migraine history (MA+MO) correlates with the attack duration ($p=0.0250$, $R=+0.175$) and frequency ($p=0.0400$, $R=+0.160$). Older patients had longer attacks ($p=0.0070$, $R=+0.280$). Most of MA and MO patients had a family history of migraine. However, no differences in clinical features were found between patients with and without a family history.

MA and MO patients used different group of painkillers (Table **3**). Among MA patients the most popular were NSAID, while among MO patients both triptans and NSAID were more popular.

3.2. Allele and Genotype Frequencies and their Correlation with Clinical Features of Migraine

Table **4** shows the frequencies of analyzed genetic variants in controls and migraine patients. The genotype frequencies of c.56G>A *SCN2A* polymorphism differed between MA patients and controls ($p=0.0468$), however, not

Table 2. Comparison of clinical features of migraine in MA and MO patients.

Clinical Feature of Migraine		Migraine With Aura (MA)	Migraine Without Aura (MO)	P-value
Age of onset	≤15 years	52.3 %	31.3 %	**0.0082**
	>15 years	47.7 %	68.7 %	
Attack frequency	≤2/mth	79.1 %	54.5 %	**0.0016**
	>2/mth	20.9 %	45.5 %	
Duration of attack	≤48 h	75.4 %	52.0 %	**0.0032**
	>48 h	24.6 %	48.0 %	
Family history of migraine	Yes	69.2 %	77.7 %	**0.2763**
	No	30.8 %	22.3 %	

Statistical significance at $p<0.05$, Fisher's exact test.

Table 3. Comparison of applied pharmacotherapy in MA and MO patients.

-	Migraine With Aura (MA)	Migraine Without Aura (MO)
Triptans	12.5 %	26.6 %
Nonsteroidal anti-inflammatory drugs	34.4 %	25.0 %
Non-anti-inflammatory analgesics	6.3 %	14.1 %
Divascan (iprazochrome)	6.3 %	1.6 %
Antiepileptic drugs	15.6 %	6.3 %
Antidepressant drugs	6.3 %	1.6 %
Calcium channel blocker	0.0 %	1.6 %
Mix	9.4 %	20.3 %
Nothing	9.4 %	3.1 %

Table 4. Allele and genotype frequencies of analyzed genetic variants in migraine patients.

-	Controls	MA	MO	Migraine Patients (MA+MO)	p		
					MA *vs* C	MO *vs* C	M *vs* C
SCN1A c.3199G>A							
Genotypes: GG (%) GA (%) AA (%)	23 (13.3) 71 (41.0) 79 (45.7)	7 (10.6) 27 (40.9) 32 (48.5)	7 (6.8) 56 (54.4) 40 (38.8)	14 (8.3) 83 (49.1) 72 (42.6)	0.8365[2]	0.0579[2]	0.1825[2]
Alleles: G (%) A (%)	117 (33.8) 229 (66.2)	41 (31.1) 91 (68.9)	70 (34.0) 136 (66.0)	111 (32.8) 227 (67.2)	0.5884[1]	1.0000[1]	0.8080[1]
SCN2A c.56G>A							
Genotypes: GG (%) GA (%) AA (%)	152 (87.9) 21 (12.1) 0 (0.0)	54 (80.6) 11 (16.4) 2 (3.0)	90 (87.4) 11 (10.7) 2 (1.9)	144 (84.7) 22 (12.9) 4 (2.4)	**0.0468[2]**	0.1756[2]	0.1216[2]
Alleles: G (%) A (%)	325 (93.9) 21 (6.1)	119 (88.8) 15 (11.2)	191 (92.7) 15 (7.3)	310 (91.2) 30 (8.8)	0.0800[1]	0.5959[1]	0.1912[1]
KCNK18 c.28A>G							
Genotypes: AA (%) AG (%) GG (%)	152 (87.9) 21 (12.1) 0 (0.0)	61 (91.0) 6 (9.0) 0 (0.0)	95 (92.2) 8 (7.8) 0 (0.0)	156 (91.8) 14 (8.2) 0 (0.0)	0.6495[1]	0.3124[1]	0.2851[1]
Alleles: A (%) G (%)	325 (93.9) 21 (6.1)	128 (95.5) 6 (4.5)	198 (96.1) 8 (3.9)	326 (95.9) 14 (4.1)	0.6595[1]	0.3263[1]	0.2985[1]
KCNK18 c.328T>C							
Genotypes: TT (%) TC (%) CC (%)	168 (97.1) 5 (2.9) 0 (0.0)	66 (98.5) 1 (1.5) 0 (0.0)	100 (97.1) 3 (2.9) 0 (0.0)	166 (97.6) 4 (2.4) 0 (0.0)	1.0000[1]	1.0000[1]	1.0000[1]
Alleles: T (%) C (%)	341 (98.6) 5 (1.4)	133 (99.3) 1 (0.7)	203 (98.5) 3 (1.5)	336 (98.8) 4 (1.2)	1.0000[1]	1.0000[1]	1.0000[1]
TRPA1 c.3053A>G							
Genotypes: AA (%) AG (%) GG (%)	108 (62.4) 59 (34.1) 6 (3.5)	45 (67.2) 20 (29.9) 2 (3.0)	74 (71.8) 25 (24.3) 4 (3.9)	119 (70.0) 45 (26.5) 6 (3.5)	0.7909[2]	0.2288[2]	0.3025[2]
Alleles: A (%) G (%)	275 (79.5) 71 (20.5)	110 (82.1) 24 (17.9)	173 (84.0) 33 (16.0)	283 (83.2) 57 (16.8)	0.6097[1]	0.2162[1]	0.2395[1]
STX1A c.31-1811C>T							
Genotypes: CC (%) CT (%) TT (%)	53 (30.6) 92 (53.2) 28 (16.2)	16 (23.9) 33 (49.2) 18 (26.9)	35 (34.0) 54 (52.4) 14 (13.6)	51 (30.0) 87 (51.2) 32 (18.8)	0.1508[2]	0.7716[2]	0.8112[2]
Alleles: C (%) T (%)	198 (57.3) 148 (42.8)	65 (48.5) 69 (51.5)	124 (60.2) 82 (39.8)	189 (55.6) 151 (44.4)	0.1018[1]	0.5324[1]	0.7004[1]

SCN1A – sodium voltage-gated channel alpha subunit 1 gene, *SCN2A* – sodium voltage-gated channel alpha subunit 2 gene, *KCNK18* – potassium channel subfamily K member 18 gene, *TRPA1* – transient receptor potential ankyrin 1 gene, *STX1A* – syntaxin 1A gene.

Statistical significance at p<0.05 for the analyses of alleles and p<0.016 (after the multiple comparison correction of Bonferroni) for the analyses of genotypes, [1]Fisher's exact test, [2]Chi-square test.

statistically significant after Bonferroni correction. The AA genotype was found only in migraine patients (both MA and MO). When we consider the family history of migraine, the A allele of c.56G>A *SCN2A* polymorphism occurred more frequently in MO patients without a family history of migraine than in MO patients with a family history (OR=3.410, 95% CI=1.165-9.981, p=0.0464) or controls (OR=2.778, 95% CI=1.109-6.955, p=0.0334).

The c.31-1811C>T polymorphism in the *STX1A* gene was found more often in MA patients than in MO. The T allele was found to be a risk factor for MA as compared to MO (OR=1.6053, 95% CI= 1.0350-2.4897, p=0.0442). However, the difference was not significant if we compare MA patients to controls. The TT genotype was more common in the MA group than MO (OR=2.3353, 95% CI= 1.0698-5.0976, p=0.0437), but the result was not significant after Bonferroni correction.

No statistical differences were found in frequencies of c.28A>G and c.328T>C *KCNK18*, c.3199G>A *SCN1A* and c.3053A>G *TRPA1*.

Changes in the *KCNK18* gene were found both in migraine patients and controls. Interestingly, the c.328T>C *KCNK18* mutation in both MA and MO group was present only in patients with a positive family history. Migraine pa-

tients with TC c.328T>C *KCNK18* genotype had an increased risk of developing migraines before the age of 18 than those without the mutation (p=0.0169). Moreover, individuals with AA/TC haplotype (c.28A>G and c.328T>C) of *KCNK18* had higher attack frequency than those with AA/TT haplotype (p=0.0464).

We found that three of the analyzed polymorphisms were associated with the age of migraine onset (Table **5**). The G allele of c.3053A>G *TRPA1* (OR=1.883, 95% CI=1.130-3.138, p=0.0199) and the T allele of c.31-1811C>T *STX1A* (OR=2.309, 95% CI=1.1183-3.034 p=0.0076) were more common in patients whose migraine started before the age of 15, while the G allele of c.3199G>A *SCN1A* (OR=3.659, 95% CI=1.673-8.003, p=0.0011) was more common in individuals with migraine onset before the age of 10.

None of the analyzed variants was associated with the duration of the migraine attack.

The applied pharmacotherapy in MA and MO patients according to genetic variants was analyzed. Interestingly, all MA patients with c.328T>C *KCNK18* mutation used NSAID. The highest variation of treatment was observed in MA and MO patients with c.31-1811C>T *STX1A* polymorphism, only a quarter of patients used drugs acting on the serotoninergic system (triptans, Divascan – the 5-HT recep-

Table 5.   Allelic distribution of analyzed genetic variants in migraine patients according to age of onset.

Age of Onset	< 10 Compare to ≥ 10 Years Old			< 15 Compare to ≥ 15 Years Old		
-	**p**	**OR**	**95% CI**	**p**	**OR**	**95% CI**
SCN1A **c.3199G>A** G *vs*. A	**0.0011**	**3.659**	**1.673-8.003**	0.1448	0.6993	0.4348-1.125
SCN2A **c.56G>A** A *vs*. G	0.5036	0.6353	0.2058-1.962	0.4381	0.7319	0.3440-1.557
KCNK18 **c.28A>G** G *vs*. A	0.3459	0.5480	0.1156-2.598	0.3867	0.5517	0.1810-1.681
KCNK18 **c.328T>C** C *vs*. T	0.3028	0.2151	0.01136-4.071	0.6512	0.6563	0.09122-4.721
TRPA1 **c.3053A>G** G *vs*. A	0.0738	0.4476	0.1930-1.038	**0.0199**	**1.883**	**1.130-3.138**
STX1A **c.31-1811C>T** T *vs*. C	1.0000	1.046	0.4826-2.267	**0.0076**	**1.895**	**1.183-3.034**

SCN1A – sodium voltage-gated channel alpha subunit 1 gene, *SCN2A* – sodium voltage-gated channel alpha subunit 2 gene, *KCNK18* – potassium channel subfamily K member 18 gene, *TRPA1* – transient receptor potential ankyrin 1 gene, *STX1A* – syntaxin 1A gene.

Statistical significance at p<0.05, Fisher's exact.

tor antagonist and antidepressant drugs). 14.3% of MA patients with c.56G>A *SCN2A* and none of MO patients with this polymorphism used antiepileptic drugs probably because of the possible multiple drug resistance caused by this variant.

3.3. Meta-analysis

In order to provide a more comprehensive evaluation of the association between c.31-1811C>T *STX1A* and c.28A>G, c.328T>C *KCNK18* variants and migraine, we performed the combined analyses of the case-control data from the present study and studies published previously.

The T allele of c.31-1811C>T *STX1A* polymorphism showed a significant association with the migraine overall (OR=1.22, 95% CI 1.05-1.41, p=0.0086, power of test=0.83), as it is shown in Fig. (**1**). Cochran's Q statistical test for heterogeneity was not significant (p=0.4499). Subdividing the migraine group into MA and MO, the association remained significant in both MA (OR=1.29, 95% CI 1.05-1.58, p=0.0151, power of test=0.91) and MO (OR=1.19, 95% CI 1.00-1.41, p=0.0440, power of test=0.88) groups.

The allelic forest plot for the migraine group is shown in Fig. (**2**). No association has been found between c.28A>G *KCNK18* polymorphism (OR=0.80, 95% CI 0.60-1.06, p=0.1226, power of test=0.98) as well as c.328T>C *KCNK18* mutation (OR=0.72, 95% CI 0.36-1.43, p=0.3425, power of test=0.99) and migraine overall. Cochran's Q statistical test for heterogeneity was not significant (p=0.7505 and p=0.5194, respectively). The allelic forest plots for the migraine group are shown in Figs. (**2** and **3**).
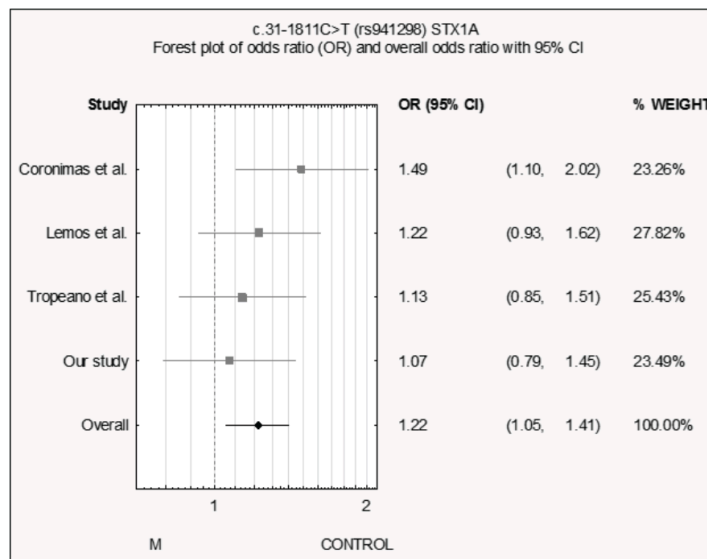
Fig. (1). A forest plot of odds ratio (OR) and overall OR with 95% CI for the T allele of the c.31-1811C>T *STX1A* polymorphism in migraine group using the fixed-effects model. CI - confidence interval.
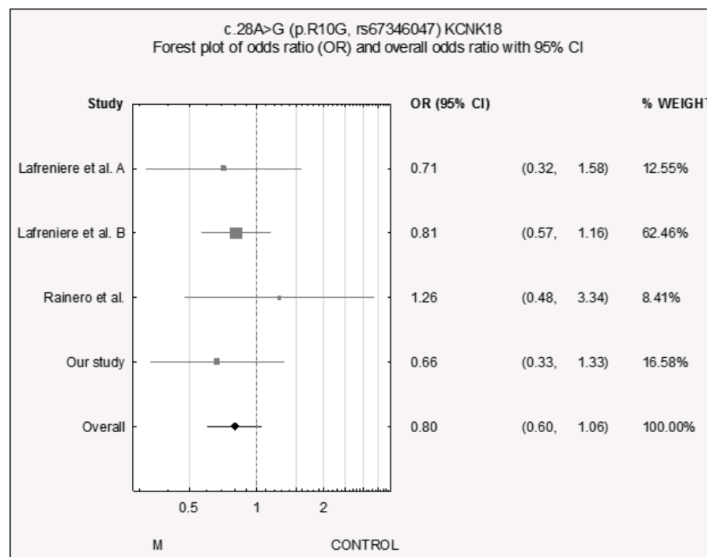
Fig. (2). A forest plot of odds ratio (OR) and overall OR with 95% CI for the c.28A>G *KCNK18* polymorphism in migraine group using the fixed-effects model. CI - confidence interval.
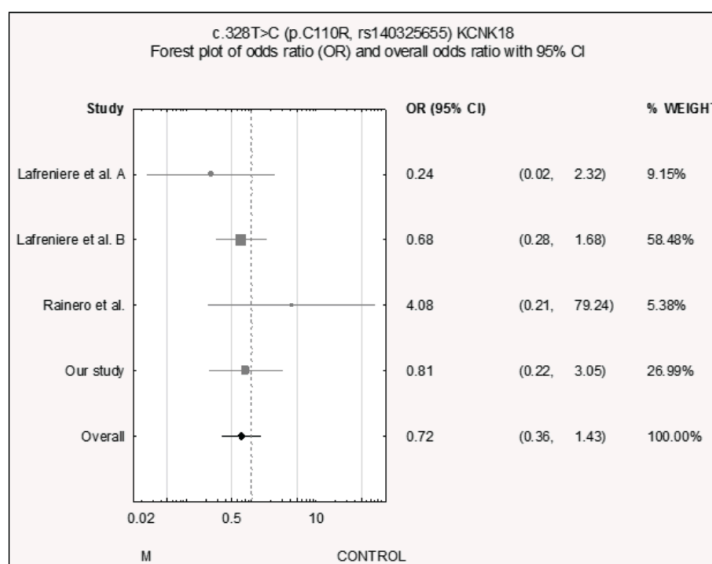
Fig. (3). A forest plot of odds ratio (OR) and overall OR with 95% CI for the c.328T>C *KCNK18* mutation in migraine group using the fixed-effects model. CI - confidence interval.

4. DISCUSSION

No specific biomarker of migraine, either diagnostic or therapeutic, has been found so far. One of the possible explanations is a broad spectrum of migraine phenotypes making identification of genotype-phenotype correlations complicated. The heterogeneity of clinical manifestation includes the variable attack frequency and severity, multiple attack triggers, different accompanying symptoms, and the relation with comorbid disorders [24, 25]. Moreover, migraine is a polygenic disease with the contribution of multiple genetic variants, each of them having a relatively small effect. It is believed that migraine development is a result of the complex interaction of numerous gene variants and epigenetic mechanisms with both environmental and lifestyle factors [24, 26, 27].

The searching of potential disease-causing variants was made easier due to the rapid development of next-generation sequencing (NGS) coupled with high-throughput bioinformatics analysis. So far, the single published NGS study in migraine patients used only a family approach [28]. The main limitation of that study was a small group size consisted of four cases (father and three children) and four unrelated controls. Additional, yet not published in the print report, describes two large families with MA and MO screened for mutation using NGS by Cutrer *et al.* [29]. Their unpublished data indicated five candidate genes in one MA family and a single variant in the second family. Moreover, the designed migraine NGS panels were focused only on FHM [30]. Unfortunately, no NGS studies have been performed in non-familial cases of migraine. However, Hansen *et al.* [31] suggest that family-based NGS approach can be used to find rare high-risk variants for migraine as it was possible in case of other common complex diseases, *e.g.* bipolar disorder, schizophrenia and late-onset Alzheimer's disease. Another method which expanded knowledge of genetic factors in migraine is a genome-wide association studies (GWAS) using large case-control cohorts [27]. The recent meta-analysis

of GWAS in migraine reported new genes associated with MA and MO, most of which encode ion channels or proteins responsible for ion homeostasis [32]. It is proposed that migraine headaches may be a result of deregulated nerve excitation due to ion channels pathology as the trigeminovascular system (TGVS) is strictly regulated by surrounding ion channels. According to neurovascular theory, the activation of TGVS, also by the cortical spreading depression (CSD), is a main mechanism involved in the pathomechanism of migraine [33].

The genes we have chosen in the present study encode ion channels (*KCNK18* encoding TRESK, *SCN1A* and *SCN2A* encoding Nav1.1, Nav1.2 channels, *TRPA1* encoding TRPA1 channel) and protein controlling their functions (*STX1A* encodes syntaxin 1A). Fig. (**4**) shows the relation between the aforementioned proteins in migraine. Stimulation of TRPA1 channels depolarizes the peripheral nerve terminal, activates the $Na_v1.1$, $Na_v1.2$ channels, and leads to the generation of the action potentials which transmit the information to central terminals. Overexpression of *TRPA1* increases the concentration of CGRP and substance P contributing to neurogenic inflammation [34], while the mutations in *SCN1A* increase the extracellular potassium ions and glutamate concentration [35]. Release of glutamate and potassium ions initiate the CSD involved in MA pathomechanism. CSD activates the TGVS, which induces the chemical cascade of vasoactive neuropeptides, such as the previously mentioned CGRP and substance P.

The $Na_v1.1$, $Na_v1.2$ channels may be inhibited by the activation of TRESK channels. The underexpression of *KCNK18* reduces the TRESK activity and leads to hyperexcitation of primary afferent neurons and pain [36]. TRESK channels may be regulated indirectly by syntaxin 1A *via* cytoplasmic calcium signaling. The syntaxin 1A regulates synaptic plasticity, serotoninergic, and glutamatergic transmission, as well as the functioning of sodium and potassium channels.
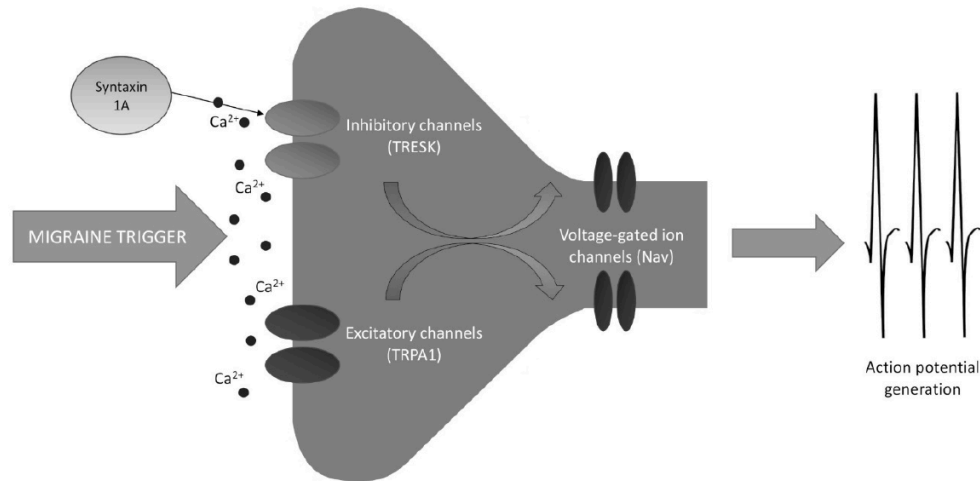
Fig. (4). Interaction between syntaxin 1A, TRESK, TRPA1, and Na$_v$ channels in neurotransmission. The migraine trigger activates the excitatory (inhibitory) channels in primary sensory neurons leading to depolarization (hyperpolarization) of the peripheral nerve terminal and activation (inhibition) of the voltage-gated channels and generation (no generation) of action potentials. The signal (*e.g.* pain signal) is (is not) then transmitted to the CNS.

In the present study, we found that polymorphism c.28A>G, as well as mutation c.328T>C of *KCNK18* gene, occurred with similar frequency in the control group and migraine patients. It corresponds with previous studies of c.28A>G [13, 20] and c.328T>C [13, 37] variants. Whereas, Rainero *et al.* [20] found the mutation only in migraine patients, both MA and MO. It was also previously found in Polish patients with MA [10]. Performed meta-analysis showed no association between both variants of *KCNK18* and migraine. Interestingly, all our patients with c.328T>C *KCNK18* had a family history of migraine. This mutation also increased the risk of migraine onset before the age of 18, and increased the attack frequency. According to the literature data, the lower age of onset among adults is associated with a family history of migraine [38]. The functional analyses of c.28A>G and c.328T>C variant of *KCNK18* were performed previously. The c.28A>G polymorphism does not influence the protein activity (TRESK currents almost indistinguishable from WT) [37]. The c.328T>C *KCNK18* variant is a dominant negative TRESK mutation that does not increase sensory neuron excitability [36, 39]. However, this variant reduces the TRESK currents leading to a loss of TRESK channel function [37]. According to the functional analysis TRESK channel can heterodimerize with other potassium channels: TREK1 and TREK2. The difference between c.328T>C mutation and frameshift mutation F139WfsX24 of *KCNK18* is that first variant inhibits only TRESK activity on dimerization, while second also inhibit the activity of heterodimerization [40]. It may explain why the c.328T>C *KCNK18* is probably an insufficient factor for migraine development and may require the presence of other variants as migraine is a multifactorial disease. Our patients with c.328T>C mutation of *KCNK18* had at least one other variant analyzed: c.3199G>A *SCN1A* and/or c.31-1811C>T *STX1A*.

We found that c.3199G>A *SCN1A* was more common in patients with migraine onset before the age of 10. According to the *in silico* analysis, it is a neutral polymorphism that might affect protein features and splice site. The c.3199G>A *SCN1A* was found by Domitrz *et al.* [10] also in FHM and epilepsy patients. In addition, this variant is more frequent in epilepsy patients but does not contribute to the coexistence of epilepsy and primary headaches, or migraine phenotype [9]. Another polymorphism widely studied in epileptic patients is c.56G>A *SCN2A*, which is linked to multiple drug resistance, and thus may be responsible for unsuccessful migraine treatment with antiepileptic drugs. *In silico* prediction, classified c.56G>A *SCN2A* variant as a benign change that might affect protein features and splice sites. This polymorphism has not been studied previously in migraine patients. In the present study, the AA genotype of c.56G>A *SCN2A* was found only in migraine patients, two with MA, and two with MO. The age of onset in three of them was 15.

The age at migraine onset (before 15 years) may also be determined by both c.31-1811C>T *STX1A* and c.3053A>G *TRPA1* polymorphisms. We showed that the TT genotype of c.31-1811C>T *STX1A* might be a risk factor for MA as compared to MO, similar to Corominas *et al.* [21]. It corresponds with the thesis that MA and MO have different genetic backgrounds and migraine studies should distinguish both subtypes as independent groups [41]. However, according to Lemos *et al.* [22] and Tropeano *et al.* [23], this polymorphism is associated with MO. The inconsistency of data may arise from the small size of analyzed groups. Moreover, after Bonferroni correction, our results, as well as mentioned results of Corominas *et al.* [21] and Lemos *et al.* [22], lost the significance. The meta-analysis provides more comprehensive evaluation of the association and, according to Tropeano *et al.* [23] and our results the T allele of c.31-1811C>T *STX1A* polymorphism may be a risk factor for migraine. After subdividing migraine group into MA and MO, Tropeano *et al.* [23] showed the association remained significant in the MO patients, while in the MA group, there was only a trend toward significance. Meta-analysis with our data showed a significant association both for MO and MA patients. Additionally, it is suggested that c.31-1811C>T *STX1A* polymor-

phism may change the expression of syntaxin 1A by altering the transcription factor binding sites [42].

On the other hand, little is known about another polymorphism, c.3053A>G *TRPA1*. In the present study, this variant was associated with migraines occurring before the age of 15. According to Zhu [43] the GG genotype of c.3053A>G *TRPA1*, increases the pain sensitivity. However, it has not been studied in migraine, so far. *In silico* analysis showed that this benign variant might change protein features and splice site. Blocking the TRPA1 channel was suggested as the new target in migraine therapy, as it plays a role in pain generation and neurogenic inflammation.

Another potential target for migraine therapy may be syntaxin 1A as it is involved in serotoninergic system regulation by affecting the subcellular localization and expression of 5-HT transporter (5-HTT) [44]. This may be important for migraine therapy, as 5-HT receptors and transporter are targets for many antimigraine drugs, such as triptans (selective 5-HT receptor agonists) and antidepressants (*e.g.* selective 5-HT reuptake inhibitor).

The limitation of the study is a relatively small sample size, cross-sectional study design, and lack of functional studies of analyzed polymorphisms. However, to overcome the power limitations due to the relatively small sample size, we performed a combined data analysis which confirmed no association between c.28A>G and c.328T>C *KCNK18* and migraine. Meta-analysis showed the association between c.31-1811C>T *STX1A* and migraine. The future studies will concern functional analysis of c.56G>A *SCN2A* as it seems to be the most promising variant. The question is how this polymorphism alters the protein function and why it is associated with multiple drug resistant. The pharmacokinetic analysis of antiepileptic drugs level can also be performed in migraine patients with and without c.56G>A *SCN2A* polymorphism. Understanding this relationship could explain the inefficiency of antiepileptic drugs in some patients.

CONCLUSION

We showed that AA genotype of c.56G>A *SCN2A* occurred only in migraine patients. This polymorphism has not been studied in migraine patients before. Unlike other analyzed variants, it does not influence the clinical features of migraine. The genetic changes such as c.3199G>A *SCN1A*, c.328T>C *KCNK18*, c.3053A>G *TRPA1* and c.31-1811C>T *STX1A* were associated with age of migraine onset. Moreover, the T allele of c.31-1811C>T *STX1A* was more common in MA than MO patients. However, according to the meta-analysis, the T allele of c.31-1811C>T *STX1A* was a risk factor both for MA and MO.

The knowledge about the relationship between gene polymorphism or mutations and drug response may be used in personalized medicine. It seems that the perfect example is the c.56G>A *SCN2A* polymorphism. It is associated with multiple drug resistance and may be responsible for unsuccessful treatment in carriers taking antiepileptic drugs, both in the course of epilepsy and migraine. Additionally, understanding the complex role of ion channels in migraine pathomechanism may contribute to developing new antimigraine therapy. The TRESK and TRPA1 channels were

previously proposed as an anti-migraine therapeutic target, however, no such suggestion concerned the syntaxin 1A. It seems that changing the expression of *STX1A* may indirectly affect the serotoninergic system and improve the efficiency of drugs associated with 5-HT.

According to our study, polymorphisms and mutations in genes encoding ion channels may be risk factors for migraine or probably therapeutic target and may correlate with clinical features of migraine. However, the possible role of analyzed genetic variants as a biomarker of migraine requires further studies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Local Bioethical Committee of Poznan University of Medical Sciences, Poland (no. 931/14 with an extension no. 993/17).

HUMAN AND ANIMAL RIGHTS

No animals were used in this study, All reported experiments on human were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Signed, informed consent was obtained from all subjects.

AVAILABILITY OF DATA AND MATERIALS

The data sets analyzed during the current study are available from the corresponding author (J.D.) upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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