Analysis of association between epilepsy related genes and vertigo within the Polish population

Association between epilepsy related genes and vertigo

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Abstract

Considering possible common genetic background of vertigo and epilepsy, we have genotyped an affected group of individuals with vertigo and an unaffected group by studying 26 single nucleotide polymorphisms (SNPs) in 14 genes which were previously reported to be of particular importance for epilepsy. The significant differences were found between the patients and the control group ($\chi 2 = 38,3$, df = 3 p=1.6×10-7) for the frequencies of haplotypes consisting of 2 SNPs located in chromosome 11 (rs1939012 and rs1783901 within genes *MMP8* and *SCN3B*, respectively). The haplotype rs1939012:C-rs1783901:A consisting of the minor frequency alleles was found to be associated with a higher risk of vertigo (OR = 5.0143, 95% CI = 1.6991 – 14.7980, p = 0.0035). In contrast, the haplotype rs1939012:T-rs1783901:A, showed significant association with a decreased risk of the disease (OR = 0.0597, 95% CI = 0.0136 – 0.2620, p = 0.0002). Our results suggest that SNPs rs1939012 and rs1783901 may implicate a potential role of gene regulation and/or epistasis in a complex etiology of vertigo.

Keywords: epilepsy, *MMP8*, *SCN3B,* SNP, vertigo

Introduction

Epilepsy is among the most common neurological disorders in humans: in the United States, 3 million American adults reported active epilepsy (NHISs 2013-2015), whereas in Poland, the condition affects 400 000 of the whole population. There are two age intervals of increased incidence: until 5 and after 65 years old. The background of epilepsy is heterogeneous. Perinatal complications, head injuries, infections, vascular diseases of central nervous system and inheritance (nearly 200 genes have been implicated in epilepsy) are main risk factors.

Vertigo and epilepsy have been believed to have a common background for a very long time. It is described that focal seizures may lead to hyperactivity in vestibular cortical areas associated with vestibular dysfunction followed by vestibular symptoms [Hewett and Bartolomei, 2013].

Advances have been made by the combination of intracranial stimulation studies in epilepsy patients and modern electroencephalography (EEG) techniques, structural and functional imaging. Although the results suggest that epilepsy may play a role in generating vestibular pathology [Hewett and Bartolomei, 2013], "vestibular epilepsy" as the cause of vertigo is difficult to prove [Tarnutzer et al., 2015]. Vestibular seizures are defined as sensory seizures in which the overriding symptom is vertigo [Young, 1994]. When a seizure activity involves the projection of the vestibular system, heterogeneous vestibular symptoms such as vertigo, dizziness and disequilibrium may be the components of seizures, or can occur prior to and between episodes of seizures, like syncopal sensations. It is reported that 20% of patients with temporal lobe epilepsy, and 1% of all seizure patients demonstrate "vestibular seizures"; chronic epilepsy is accompanied by objective vestibular dysfunction in 46.6% of patients [Kogeorgos et al., 1981; Hamed et al., 2017]. The family history of epilepsy, previous head injuries and vestibular symptoms intertwined with other epileptiform symptoms may indicate this specific form of epilepsy [Hewett and Bartolomei, 2013]. Since there is no specific test confirming the "vestibular epilepsy", it seems that genetic research may give credence to this diagnosis [Pawlak-Osinska et al., 1999]. Moreover, there are also many other types of dizziness, the origin of which is unclear. Vertigo lasting seconds is often classified as benign paroxysmal positional vertigo. However, such recognition seems to be overused, since in many cases tests confirming the diagnosis are controversial and the causes of dizziness remain unknown, or at least uncertain. The situation is similar with regard to dizziness that lasts for minutes, hours (without hearing impairment) and often repeats itself. These symptoms require a long diagnostic process involving many specialists. Vestibular hyperactivity observed during caloric stimulation of the labyrinths is sometimes the only pathological vestibular finding, in such case "channelopathy" may be one of or the only suspected cause of dizziness [Monzani et al., 2015].

It is known that genetic factors play an important role in the etiology of epilepsy. The main causes are mutations in genes encoding subunits of ion channels and receptors for neurotransmitters, but they do not explain all the cases. In generalized epilepsy with febrile seizure (GEFs +) mutations have been identified in the genes encoding subunits of sodium channels (*SCN1A, SCN1B* and *SCN2*) and GABA receptor subunit (*GABRG2* and *GABRD*), but overall these mutations are responsible for only about 10% of GEFs + [Poduri and Lowenstein, 2011]. In rare cases, association studies indicated a significant effect of mutations in the genes encoding subunits of the GABA receptor (*GABRG2* and *GABRA1*) and chloride (*CLCN2*) and calcium channels (*CACNA1H*), as well as in the gene encoding EFHC1 calcium-binding protein. Nevertheless, most epilepsy cases are determined by many genes and the phenotypic effect is a result of coexistence of many common sequence variants [Helbig et al., 2008]. Recently conducted genome-wide association studies and meta-analyses allowed to identify new loci associated with the risk of epilepsy, both in the genes encoding ion channels and the genes unrelated to "channelopathies" [Epilepsies ILAECoC, 2014; Steffens et al., 2012]. Considering a possible link between the epilepsy and vertigo, we analyzed single nucleotide polymorphisms related to epilepsy in our sample comprising the patients diagnosed with vertigo. In our genetic analysis we were interested in how many patients among the group with vertigo of various origin, but more or less related with chanellopathy or genetic etiology, could be selected as genetically profiled. Moreover, among the selected genes that seemed to be responsible for epileptic coding, we hoped to specify the uncertain etiology of vertigo called “paroxysmal”.

Materials and Methods

Selection of SNPs

26 single nucleotide polymorphisms (SNPs) located in 14 different genes were selected for the study. The 16 SNPs associated with epilepsy were selected from the database The Epilepsy

Genetic Association Database; p-value < 0,05 in European populations was used as a criterion [epiGAD TEGAD http://www.epigad.org]. The selected SNP are located within genes which encode sodium ion channel *SCN1A* (rs1685381, rs8191987), *SCN8A* (rs303778), *SCN3B* (rs1783901), potassium ion channel *KCNQ2* (rs1801545), *KCNAB1* (rs992353), and glutamate receptor *GRM4* (rs4711374, rs1466650, rs937039, rs2499697, rs2451357, rs9380405, rs745501, rs11753413, rs2451334, rs2029461). Furthermore, additional 10 loci included *VRK2* (rs13026414), *COPZ2* (rs72823592), *ZEB2* (rs10496964), *CHRM3* (rs12059546), *SCN1A* (rs11890028), *PCDH7* (rs28498976, rs1044352), *GOLIM4* (rs111577701), *GABRA2* (rs535066) and *MMP8* (rs1939012) which were found to be associated with epilepsy in genome-wide association analyses (GWAS), were included in the study [Epilepsies ILAECoC, 2014; Steffens et al., 2012].

Material and clinical methods

96 patients suffering from vertigo (age range 20-68, mean 45.2; women 59, men 37) and 101 healthy people (age range 22-57, mean 38.6; women 66, men 35) without any otoneurological symptoms were enrolled in the study. Patients were not treated for epilepsy and were not on medications. The samples were collected at the Department of Otolaryngology and Oncology, Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Torun. All participants were of Polish origin (Caucasians). All subjects signed a written informed consent form and the study was approved by the University Bioethics Committee.

Every participant underwent a complex otoneurological examination including: a detailed anamnesis (extended Claussen's questionnaire), videonystagmography with the analysis of vesibular-oculomotor and vestibular-visual reflexes together with searching for benign paroxysmal positional vertigo (BPPV) and posturography (vestibular- spinal reflexes). To exclude otoneurological symptoms in control individuals, no history of vertigo or dizziness was stated, no pathological signs on videonystagmography were observed. Postural reflexes

measured in static and dynamic posturography had to be normal. It should be mentioned that it cannot be excluded that a small number of individuals in the control group may be presently asymptomatic for vertigo, but could develop it later in life.

Because a single seizure may be a physiological reactive brain response to alcohol, sedative withdrawal, hyponatremia, hypocalcemia, hepatic diseases with toximetabolic complications, hypoxia and sleep deprivation, all these occurrences were excluded.

Searching for the origin of vertigo, a thorough clinical work-up was performed. Every patient underwent: videonystagmography (spontaneous, positional, gaze nystagmus, optokinetic nystagmus, saccadic movements, smooth pursuit, cervical test, caloric examination), Dix-Hallpike and roll maneuvers, vestibular evoked potentials of cervical origin, somatosensory evoked potentials, visual evoked potentials, posturography and audiological examination. Among 96 patients, 35 were diagnosed as having central type of vertigo, additionally, every patient had the familiar history of vertigo (89 patients) or head injuries (32 cases) or concomitant epileptiform symptoms (29 individuals); sometimes the history was multithreaded. 12 patients had subjective dizziness without any pathological findings during otoneurological objective study performed. 4 patients in our group were diagnosed as having vestibular neuritis that after some time revealed as the beginning of multiple sclerosis (MS). In every case, a familiar appearance of MS was present; these were 3 first and 1 second degree relatives. Only 18 patients had vertigo of pure labyrinth origin (majority of them demonstrated Meniere's disease with familiar appearance). Peripheral vestibular disorders does not exclude vestibular epilepsy- they may exist simultaneously or may be originated from the same etiologic factors [Young, 1994]. 27 patients had vertigo as the sign of general diseases like hypertension, arrhythmia or migraine, which is suspected to increase susceptibility to seizures.

47 patients (those with central and subjective dizziness) noted paroxysmal type of symptoms lasting from seconds to minutes with a high frequency, i.e. every day, or many times a week. They were patients whose vertigo was paroxysmal like in epilepsy (48.9% of the tested group).

In otoneurology they represent the most problematic group - the etiological diagnosis in majority of such patients remained uncertain. The syndrome called "vestibular epilepsy" is often used for such cases. Hyperactivity in caloric test is sometimes, but not always, observed (in 21 out of our 47 paroxysmal cases).

Genetic analysis

DNA was extracted from buccal swabs using *GeneMATRIX Bio-Trace DNA Purification Kit* (EURx, Gdańsk, Poland) and quantified spectrophotometrically. Genotyping of the selected SNPs was performed by allelic discrimination method based on real-time PCR, using *TaqMan SNP Genotyping Assay* and *ViiA™ 7 Real-Time PCR System* (Thermo Fisher Scientific, Carlsbad, CA USA) according to the manufacturer's instructions. The set of *TaqMan SNP Genotyping Assay*s used for the genotyping is shown in Table 1. The *Custom TaqMan Genotyping Assay* service was used to obtained primers and probes for genotyping rs1783901 (forward: 5'-CATCCCACCCCCACATTCTG-3', reverse: 5'-GTGCATGGACAGGGAAGAGA-3', VIC-TCCCGGTGACATTGT-NFQ, FAM-CCCGGTGGCATTGT-NFQ) and rs111577701 (forward: 5'-GAACTGCCTGAGACTGGGTAATTTA-3', reverse: 5'-CCTCGGCCTCCCAAAGTG-3', VIC-TGAGCCACCGTGCCTG-NFQ, FAM-AGCCACCATGCCTG-NFQ. The PCR reaction contained 1ng/µl of template DNA in a final volume of 10 µl.

Table 1 The set of *TaqMan SNP Genotyping Assays* used for the genotyping of selected SNPs.

Genetic Risk Score Computation

To test the aggregate effect of the epilepsy risk SNPs a Genetic Risk Score (GRS) for each individual was calculated. These loci included *SCN1A* (rs8191987), *SCN8A* (rs303778), *KCNQ2* (rs1801545), *KCNAB1* (rs992353), *GRM4* (rs9380405), *VRK2* (rs13026414), *COPZ2*

(rs72823592), *ZEB2* (rs10496964), *CHRM3* (rs12059546), *PCDH7* (rs1044352), *GOLIM4 (*rs111577701), *GABRA2* (rs535066) and *MMP8* (rs1939012). Among SNPs in linkage disequilibrium, only the SNP with the most significant main effect in our study was included in the score. Two methods were used to create the GRS: a simple count method (count GRS) and a weighted method (weighted GRS). Both methods assumed each SNP to be independently associated with risk. We assumed an additive genetic model for each SNP, applying a linear weighting of 0, 1, and 2 to genotypes containing 0, 1, or 2 risk alleles, respectively. The count method assumes that each SNP in the panel contributes equally to the risk for disease and was calculated by summing the values for each of the SNPs, producing a score out of 26 (the total number of risk alleles). Only persons with complete data were included to count the GRS. For the weighted GRS, each SNP was weighted by their effect on epilepsy risk (OR - odds ratio). The weighted GRS was calculated by multiplying each OR by the number of corresponding risk alleles (0, 1, or 2) and then summing the products.

Statistical analysis

The chi-squared test (adjusted by Yates correction where necessary) was used to compare case and control groups for possible associations between allele, genotype and haplotype frequencies and disease state. The Arlequin software version 3.1 was used to determine the linkage disequilibrium (LD) and estimate haplotype frequencies. The logistic regression was used to evaluate vertigo risk depending on gender, age (in years) and GRS. The association between phenotypes and haplotypes was expressed by odds ratios (ORs) with 95% confidence intervals. We applied Bonferroni correction for multiple tests ($p < 0.004$ was considered as statistically significant). Power of test was calculated with *correction for continuity* for each SNP using Z-test. The statistical calculations were performed using *Statistica package v.12.5* (StatSoft Polska Sp. z o.o., Kraków, Polska).

Results

The data for genotype/allele frequencies found in the patients and the controls are shown in Table 2. No statistically significant differences were observed for any of the selected SNPs between the patients and the healthy individuals. Further adjustment for conventional risk factors, including sex and age, showed that only age significantly impacted vertigo risk (p = 0,00016).

Table 2 Genotype and allele frequencies in the patients suffering from vertigo and the controls.

While none of the selected SNPs were directly associated with disease state, one cannot exclude that co-existence of many variants may affect phenotype. Therefore we combined the variants by computing weighted and unweighted GRSs, which congregates information from multiple genetic variants. The GRS was not associated with increased vertigo risk (case: median = 13; mean = 13.53; SD = 2.017; control: median = 13; mean = 13.29; SD = 2.178; p = 0.5629). Similarly the weighted GRS was not associated with increased vertigo risk (case: median = 19.6; mean = 19.88; SD = 2.788; control: median = 19.6; mean = 19.47; SD = 3,052; p = 0.4774). Furthermore we performed haplotype analysis for SNPs for which significant pair-wise LD were found (see Supplemental Digital Content, Tables S1-S4). The results of haplotype reconstruction and frequency estimations are shown in Tables 3-6.

Table 3 The frequencies of haplotypes consisting of 10 SNPs located in the *GRM4* gene.

Table 4 The frequencies of haplotypes consisting of 3 SNPs located in the *SCN1A* gene.

Table 5 The frequencies of haplotypes consisting of 2 SNPs located in the *PCDH7* gene.

Table 6 The frequencies of haplotypes consisting of 2 SNPs located in chromosome 11.

Highly significant difference between the patients and the control group was found for the frequencies of haplotypes consisting of 2 SNPs (rs1939012 and rs1783901 within genes *MMP8* and *SCN3B*, respectively) located in chromosome 11 ($\chi^2 = 38.3$, df = 3, $p = 1.6\times10^{-7}$). The haplotype rs1939012:C-rs1783901:A consisting of the minor alleles of the markers was significantly associated with a higher vertigo risk (OR = 5.0143, 95% CI = 1.6991 – 14.7980, p = 0.0035). In contrast, the haplotype rs1939012:T-rs1783901:A, showed highly significant association with decreased risk of the disease (OR = 0.0597, 95% CI = 0.0136 – 0.2620, p = 0.0002).

Discussion

Disorders of electrical activity of the cerebral cortex featuring tinnitus are related to physiological abnormalities characteristic of epilepsy. Considering this, we aimed at searching for genetic polymorphisms predisposing to equilibrioception disorders in the genes that were previously reported to be of particular importance in epilepsy. A total of 26 SNPs which showed associations with the condition in both gene-based and genome-wide studies (GWAS) were selected for this study and tested in affected individuals with vertigo, and in an unaffected control group.

By treating each SNP individually, we have not observed statistically significant differences between the cases and controls. However, it cannot be excluded that vestibular disorders are dependent on a small effect size of many SNP markers, each of which determines variation of phenotype to a very small extent. This appears to be confirmed by the low values of the odds ratio (OR) for each locus analyzed separately (Table 2). To combine information from multiple

genetic variants we counted weighted and unweighted genetic risk score (GRSs). We observed no significant association between GRS (weighted and unweighted) and increased risk of vertigo. Given the limitations of our study like the small sample size, lack of adequate coverage of the selected genes and/or heterogeneity of the phenotype, we could not precisely estimate the predictive power of the GRS.

After testing for pairwise linkage disequlibrium (LD), we proceeded to analyze haplotype frequency distributions in both groups of studied individuals. Frequencies of haplotypes that include two intronic SNPs within chromosome 11 (rs1939012 and rs1783901 within genes *MMP8* and *SCN3B*, respectively) showed statistically significant differences ($p=1,6\times10^{-7}$) between the group of patients with vertigo and the control group. Both SNPs are in strong LD (see Supplemental Digital Content, Table S4). Haplotype rs1939012:C-rs1783901:A constituted by minor frequency alleles was found to be associated with a higher risk of vertigo. Conversely, rs1939012:T-rs1783901:A haplotype was suggestive of protective value against the condition (Table 6). *MMP8* rs1939012 was recently found to be associated with genetic generalized epilepsy on a genome-wide level [Epilepsies ILAECoC, 2014], while rs1783901 in *SCN3B* was also implicated in association with non-syndromic oral clefts [Park et al., 2006]. Considering that lack of association of these SNPs individually could be due the small number of individuals in our study, we compared patient SNP MAFs of the Polish groups to European population frequencies included in databases gnomAD and the 1000 Genomes Project. Likewise no significant differences were found (p= 0,5421 and p= 0,5317 for rs1939012; p = 0,8883 and p = 0,4396 for rs1783901, respectively). However, it is worth noting that the samples in the databases are anonymous and have no associated medical or phenotype data, (information about donors' ethnicity and gender are only available).

Although functional significance of rs1939012 and rs1783901 is difficult to assess at this point in time, their intronic localization and implication in different medical conditions suggest a potential role in regulation of gene expression and/or possible epistatic interactions [Hube and

Francastel, 2015]. The changes that cause generation or loss of new CpG sites might influence methylation and by extrapolation gene expression and regulation. Since a single loss of a CpG usually does not cause a change in gene expression and, in general methylation is a cooperative process, meaning that not a single site but average methylation of multiple CpGs in a region acts as a switch we took a closer look on CpG content/distribution in the close neighborhood of both SNPs. Analysis in USCS Genome Browser database showed no CpGs around both SNPs. The alterations in DNA methylation of *MMP8* gene were found in Preeclampsia, the disease in which dizziness is one of the symptoms. Mousa et al shoved that DNA hypomethylation significantly increased MMP-8 expression. The promoter regions of the *MMP1* and *MMP8* genes have reduced methylation in omental arteries of preeclamptic women compared with those of normal pregnant women [Mousa et al., 2012].

Most association studies (both gene-based and genome-wide) show that identification of mechanisms underlying intronic variants are complex [Walsh et al., 2016]. Therefore, a possible regulatory role of rs1939012 and rs1783901 needs to be further elucidated by transcriptome and other gene expression analyses.

Except for the *MMP8* and *SCN3B* haplotypes, we have not found any other associations between the studied markers and vertigo, neither on individual SNP nor on haplotype level. In particular, we have not observed any statistically significant differences between frequencies of alleles and haplotypes of *SCN1A* gene in the studied groups. It is worth noting that *SCN1A* was previously indicated as the gene with the highest number of known mutations related to epilepsy, including rs11890028, rs8191987, rs16851381 which were proved to be associated with epilepsy on a genome-wide level [Steffens et al., 2012; Escayg and Goldin, 2010]. This may suggest different mechanisms underlying genetic susceptibility to epilepsy and vertigo. Alternatively, the lack of associations in our study may be due to small sample sizes of both case and control groups. Indeed, our analysis had only 4-28% power to detect the association between vertigo and individual SNPs, the actual value being dependent on particular SNP

(Table 2). Moreover, one cannot exclude possible hidden population substructure affecting the results of association analysis. These observations suggest the need for extending current research and replicating the results of this study in larger cohorts of a defined biogeographic ancestry. Importantly, in future studies concerning potential genetic background of vertigo, candidate gene strategy and genome-wide analysis should be complemented by massively parallel sequencing (MPS) approaches, including exome and complete genome analyses. The latter were proved to be efficient tools in identifying both common and rare genetic variants associated with a variety of complex clinical phenotypes [Auer and Lettre, 2015].

Conclusions

Our results suggest that SNPs rs1939012 and rs1783901 located in intronic regions in chromosome 11 may implicate a potential role of gene regulation and/or epistasis in a complex etiology of vertigo.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics approval

This study was approved by the Ethics Committees of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland (KB 346/2015).

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List of Supplemental Digital Content

Supplemental Digital Content. Tables:

Table S1 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within gene *GRM4*.

Table S2 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 2.

Table S3 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 4.

Table S4 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 11.

Table 1 The set of *TaqMan SNP Genotyping Assays* used for the genotyping of selected SNPs.

SNP	Gene	Position	Assay ID
rs12059546	*CHRM3*	Chr.1: 239806797	*C__31713436_10*
rs10496964	*ZEB2*	Chr.2: 144602342	*C__30543741_20*
rs16851381	*SCN1A*	Chr.2: 166056929	*C__34802969_10*
rs8191987	*SCN1A*	Chr.2: 166058504	*C__25954838_20*
rs11890028	*SCN1A*	Chr.2: 166086767	*C__32279520_10*
rs13026414	*VRK2*	Chr.2: 57706920	*C__31843236_10*
rs992353	*KCNAB1*	Chr.3: 156538672	*C__11245410_10*
rs111577701	*GOLIM4*	Chr.3: 168143620	*Custom TaqMan Genotyping Assay*
rs1044352	*PCDH7*	Chr.4: 31146252	*C___8283257_10*
rs28498976	*PCDH7*	Chr.4: 31149735	*C__58252851_10*
rs535066	*GABRA2*	Chr.4: 46238270	*C___7537086_10*
rs4711374	*GRM4*	Chr.6: 34047988	*C__29315792_10*
rs1466650	*GRM4*	Chr.6: 34062519	*C___7513892_10*
rs937039	*GRM4*	Chr.6: 34075875	*C__11544635_10*
rs2499697	*GRM4*	Chr.6: 34077141	*C__16029558_20*
rs2451357	*GRM4*	Chr.6: 34086616	*C__16014002_10*
rs9380405	*GRM4*	Chr.6: 34093090	*C__29636698_10*
rs745501	*GRM4*	Chr.6: 34100942	*C_____5808_10*
rs11753413	*GRM4*	Chr.6: 34105630	*C__31859290_10*
rs2451334	*GRM4*	Chr.6: 34135885	*C__16013973_20*
rs2029461	*GRM4*	Chr.6: 34138013	*C__12027758_10*
rs1939012	*MMP8*	Chr.11: 102724404	*C__11484592_10*
rs1783901	*SCN3B*	Chr.11: 123642798	*Custom TaqMan Genotyping Assay*
rs303778	*SCN8A*	Chr.12: 51750944	*C____956043_10*
rs72823592	*COPZ2*	Chr.17: 48045642	*C__98000051_10*
rs1801545	*KCNQ2*	Chr.20: 63414925	*C__12084036_10*

Base pair position refers to GRCh38

Table 2 Genotype and allele frequencies in the patients suffering from vertigo and the controls.

Gene	SNP ID	G	Cases (N)	Control (N)	p-value	Major allele	Minor allele	MAF Control (N)	MAF Cases (N)	OR (95% CI)	p-value	Power
CHRM3	rs12059546	AA AG GG	58 (89) 28 3	70 (96) 22 4	0.5331	A	G*	0.16 (192)	0.19 (178)	1.2750 (0.7431 – 2.1876)	0.3771	0.1140
COPZ2	rs72823592	AA AG GG	12 (88) 26 50	2 (82) 31 49	0.0541	G*	A	0.21 (164)	0.28 (176)	1.4626 (0.8899 – 2.4038)	0.1326	0.2795
GABRA2	rs535066	GG GT TT	16 (90) 42 32	19 (97) 44 34	0.9503	T	G*	0.42 (194)	0.41 (180)	0.9535 (0.6319 – 1.4389)	0.8206	0.0440
GOLIM4	rs111577701	AA AG GG	2 (82) 22 58	2 (85) 27 56	0.7804	G*	A	0.18 (170)	0.16 (164)	0.8448 (0.4768 – 1.4968)	0.5630	0.0675
GRM4	rs2499697	AC CC AA	10 (85) 74 1	12 (82) 70 0	0.9560	C	A*	0.07 (164)	0.07 (170)	1.0395 (0.4530 – 2.3852)	0.9272	0.0306
GRM4	rs4711374	CC CT TT	64 (85) 19 2	60 (82) 19 3	0.9955	C*	T	0.15 (164)	0.14 (170)	0.8699 (0.4718 – 1.6041)	0.6552	0.0527
GRM4	rs1466650	AA AT TT	4 (85) 27 54	4 (82) 26 52	0.9432	T	A*	0.21 (164)	0.21 (170)	0.9913 (0.5836 – 1.6839)	0.9742	0.0363
GRM4	rs937039	AA AG GG	17 (85) 51 17	17 (82) 47 18	0.9333	G*	A	0.49 (164)	0.50 (170)	1.0247 (0.6672 – 1.5738)	0.9113	0.0397
GRM4	rs2451357	AA AG GG	1 (85) 11 73	1 (82) 16 65	0.5071	G*	A	0.11 (164)	0.08 (170)	0.6716 (0.3178 – 1.4192)	0.2946	0.1366
GRM4	rs9380405	CC CT TT	13 (85) 49 23	17 (82) 48 17	0.4990	T*	C	0.50 (164)	0.44 (170)	0.7895 (0.5133 – 1.2143)	0.2816	0.1607
GRM4	rs745501	AA AT TT	26 (85) 48 11	32 (82) 41 9	0.5174	A*	T	0.36 (164)	0.41 (170)	1.2458 (0.8010 – 1.9374)	0.3291	0.1371
GRM4	rs11753413	CC CT TT	14 (85) 42 29	10 (82) 47 25	0.5516	G	C*	0.41 (164)	0.41 (170)	1.0134 (0.6552 – 1.5675)	0.9522	0.0387
GRM4	rs2451334	AA AG	6 (85) 42	4 (82) 40	0.9541	G	A*	0.29 (164)	0.32 (170)	1.1250 (0.7058 – 1.7933)	0.6205	0.0614

		GG	37	38								
GRM4	rs2029461	CC CT TT	11 (85) 44 30	9 (82) 44 29	0.9217	T*	C	0.38 (164)	0.39 (170)	1.0440 (0.6715 – 1.6232)	0.8482	0.0417
KCNAB1	rs992353	CC CT	58 (85) 27	54 (81) 27	0.8302	C*	T	0.17 (162)	0.16 (170)	0.9441 (0.5270 – 1.6912)	0.8465	0.0385
KCNQ2	rs1801545	CG GG CC	9 (96) 86 1	14 (101) 87 0	0.7630	G*	C	0.07 (202)	0.06 (192)	0.8161 (0.3610 – 1.8449)	0.6253	0.0704
MMP8	rs1939012	CC CT TT	22 (92) 52 18	19 (69) 30 20	0.2228	T*	C	0.49 (138)	0.52 (184)	0.8905 (0.5726 – 1.3849)	0.6066	0.0643
PCDH7	rs28498976	AA AG GG	8 (89) 45 36	10 (80) 39 31	0.7611	G	A*	0.37 (160)	0.34 (178)	0.8925 (0.5713 – 1.3942)	0.6172	0.0630
PCDH7	rs1044352	GG GT TT	34 (86) 43 9	32 (84) 40 12	0.7505	G	T*	0.38 (168)	0.35 (172)	0.8930 (0.5745 – 1.3881)	0.6151	0.0632
SCN1A	rs16851381	AA AG GG	62 (87) 24 1	67 (84) 15 2	0.4321	A	G*	0.11 (168)	0.15 (174)	1.3777 (0.7310 – 2.5965)	0.3204	0.1299
SCN1A	rs8191987	AA AG GG	63 (91) 27 1	72 (89) 15 2	0.1910	A	G*	0.11 (178)	0.16 (182)	1.5862 (0.8536 – 2.9475)	0.1422	0.2580
SCN1A	rs11890028	GG GT TT	9 (89) 40 40	7 (95) 39 49	0.6132	T*	G	0.28 (190)	0.33 (178)	1.2494 (0.8000 – 1.9513)	0.3273	0.1382
SCN3B	rs1783901	AA AG GG	0 (84) 28 56	1 (80) 26 53	0.9970	G	A*	0.18 (160)	0.17 (168)	0.9429 (0.5304 – 1.6760)	0.8411	0.0389
SCN8A	rs303778	AA AG GG	70 (87) 17 0	68 (84) 15 1	0.9955	A	G*	0.10 (168)	0.10 (174)	0.9618 (0.4736 – 1.9532)	0.9141	0.0334
VRK2	rs13026414	AA AG GG	33 (94) 50 11	44 (101) 46 11	0.4750	A	G*	0.34 (202)	0.38 (188)	1.2231 (0.8083 – 1.8509)	0.3404	0.1345
ZEB2	rs10496964	CC CT	67 (88) 21	81 (97) 16	0.2108	C*	T	0.08 (194)	0.12 (176)	1.5073 (0.7597 – 2.9904)	0.2381	0.1717

G= genotype; MAF = minor allele frequency; SNP = single nucleotide polymorphism. * = The epilepsy risk allele. Power of test calculated with correction for continuity for each SNP.

Table 3 The frequencies of haplotypes consisting of 10 SNPs located in the *GRM4* gene.

Haplotype	Cases (N=170)	Controls (N=164)	OR (95% CI)	p
A A A G G C C C T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A A G G C T T T T	0.02	0.00	6.8746 (0.3523 - 134.1393)	0.2034
A A A G G C T T T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A T G G C C T C T	0.01	0.04	0.2670 (0.0546 - 1.3047)	0.1028
A A T A A C C C T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G A G G C T T C T	0.06	0.04	1.4018 (0.5205 - 3.7751)	0.504
A A T G G C C C T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G T G G C C T C T	0.31	0.37	0.7649 (0.4860 - 1.2037)	0.2466
A A T G G C C C T T	0.02	0.04	0.4029 (0.1024 - 1.5855)	0.1934
A A T G G C C T C C	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
A A T G G C C T T T	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
A G A G G C C T C T	0.01	0.04	0.2670 (0.0546 - 1.3047)	0.1028
A G A G G C T T T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G T A G C C T C C	0.01	0.02	0.6389 (0.1054 - 3.8737)	0.6261
A G T G A C C C T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G T G G C C C T C	0.03	0.02	1.6263 (0.3823 - 6.9174)	0.5103
T A T A G C C C T T	0.02	0.00	6.8746 (0.3523 - 134.1393)	0.2034
A A A G G C C T T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A A G G C T C T T	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
T A T A G C C C T C	0.20	0.18	1.1167 (0.6469 - 1.9275)	0.6919
T A T G G C C T C T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A A G G C C C T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A T A G A C T C C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T A A G A A C T T T	0.04	0.02	2.3047 (0.5857 - 9.0694)	0.2323

A G T G G C T T C T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T A A G G C C C T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T A T G G C C T T T	0.03	0.01	2.4545 (0.4695 - 12.8335)	0.2873
T A T A A A C C T C	0.01	0.02	0.6389 (0.1054 - 3.8737)	0.6261
T A T A A A C T T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G T G G C C C T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A G T G G C C C C T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T A T G G C C C T C	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T G A G G C T C T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T G T A G C C C T C	0.02	0.02	0.9641 (0.1918 - 4.8468)	0.9646
T G T G G C C T T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
T A A A G C C C T C	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
T A A A G C T C T C	0.01	0.02	0.6389 (0.1054 - 3.8737)	0.6261
T A T G A A C T T T	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
T A T G G C C T T C	0.01	0.01	0.9643 (0.1342 - 6.9274)	0.9712
T G T G G C C C T T	0.01	0.00	4.8813 (0.2326 - 102.4548)	0.3073
A A A G G C T C T C	0.00	0.02	0.1353 (0.0069 - 2.6403)	0.187
A A A G A C T C T C	0.00	0.02	0.1353 (0.0069 - 2.6403)	0.187
A G T G G C C C C C	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859
A A T G A C C C T C	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859
T A A G A C C T T T	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859
T A A A G T T C T T	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859
T A T A G A C C T C	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859
T A T G G C C C T T	0.00	0.02	0.1353 (0.0069 - 2.6403)	0.187
T A A A A A C T T C	0.00	0.01	0.1906 (0.0091 - 4.0009)	0.2859

rs745501, rs937039, rs1466650, rs2451334, rs2451357, rs2499697, rs4711374, rs11753413, rs9380405 and rs2029461 respectively.

Table 4 The frequencies of haplotypes consisting of 3 SNPs located in the *SCN1A* gene.

Haplotype	Cases (N=166)	Controls (N=160)	OR (95% CI)	p
GAA	0.34	0.28	1.3010 (0.8119 - 2.0847)	0.274
TAA	0.51	0.61	0.6639 (0.4275 - 1.0311)	0.0682
TGG	0.16	0.11	1.5324 (0.8075 - 2.9079)	0.1916

rs11890028, rs8191987 and rs16851381 respectively

Table 5 The frequencies of haplotypes consisting of 2 SNPs located in the *PCDH7* gene.

Haplotype	Cases (N=172)	Controls (N=160)	OR (95% CI)	p
AT	0.34	0.37	0.8709 (0.5550 - 1.3668)	0.5479
GG	0.65	0.62	1.1502 (0.7353 - 1.7990)	0.5399
GT	0.01	0.01	0.9294 (0.1294 - 6.6774)	0.942

rs28498976 and rs1044352 respectively

Table 6 The frequencies of haplotypes consisting of 2 SNPs located in chromosome 11.

Haplotype	Cases (N=166)	Controls (N=112)	OR (95% CI)	p
C G	0.34	0.47	0.5821 (0.3566 - 0.9504)	0.0305
C A	0.16	0.04	5.0143 (1.6991 - 14.7980)	0.0035
T G	0.49	0.32	2.0118 (1.2203 - 3.3164)	0.0061
T A	0.01	0.17	0.0597 (0.0136 - 0.2620)	0.0002

rs1939012 and rs1783901 respectively

Supplemental Digital Content

Analysis of association between epilepsy related genes and vertigo within the Polish population

Table S1 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within gene *GRM4*.

A)

	0	1	2	3	4	5	6	7	8	9
0	*	+	−	+	+	+	+	+	+	+
1	+	*	−	+	−	+	−	+	+	+
2	−	−	*	+	−	−	+	−	−	+
3	+	+	+	*	−	−	−	+	+	+
4	+	−	−	−	*	+	−	−	+	−
5	+	+	−	−	+	*	−	−	+	−
6	+	−	+	−	−	−	*	−	−	−
7	+	+	−	+	−	−	−	*	+	+
8	+	+	−	+	+	+	−	+	*	+
9	+	+	+	+	−	−	−	+	+	*

B)

	0	1	2	3	4	5	6	7	8	9
0	*	+	−	+	+	+	−	+	+	+
1	+	*	−	+	+	+	−	+	+	+
2	−	−	*	−	+	−	+	−	−	−
3	+	+	−	*	−	−	−	+	+	+
4	+	+	+	−	*	+	−	−	+	−
5	+	+	−	−	+	*	+	−	+	−
6	−	−	+	−	−	+	*	−	−	−
7	+	+	−	+	−	−	−	*	+	+
8	+	+	−	+	+	+	−	+	*	+
9	+	+	−	+	−	−	−	+	+	*

(0) rs745501, (1) rs937039, (2) rs1466650, (3) rs2451334, (4) rs2451357, (5) rs2499697, (6) rs4711374, (7) rs11753413, (8) rs9380405, (9) rs2029461. In the group of patients with vertigo (A) and in the control group (B). Values of $p < 0.05$ was designated +.

Table S2 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 2.

A)

	0	1	2	3	4
0	*	+	+	-	-
1	+	*	+	-	-
2	+	+	*	-	-
3	-	-	-	*	-
4	-	-	-	-	*

B)

	0	1	2	3	4
0	*	+	+	-	-
1	+	*	+	-	-
2	+	+	*	-	-
3	-	-	-	*	-
4	-	-	-	-	*

(0) rs11890028, (1) rs16851381, (2) rs8191987, (3) rs13026414, (4) rs10496964. In the group of patients with vertigo (A) and in the control group (B). Values of $p < 0.05$ was designated +.

Table S3 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 4.

A)	0	1	2
0	*	+	-
1	+	*	-
2	-	-	*

B)	0	1	2
0	*	+	-
1	+	*	-
2	-	-	*

(0) rs1044352, (1) rs28498976, (2) rs535066. In the group of patients with vertigo (A) and in the control group (B). Values of $p < 0.05$ was designated +.

Table S4 The matrix showing the results of analysis of linkage disequilibrium (LD) between the analyzed loci within chromosome 11.

A)	0	1
0	*	+
1	+	*

B)	0	1
0	*	-
1	-	*

(0) rs1939012, (1) rs1783901. In the group of patients with vertigo (A) and in the control group (B). Values of p <0.05 was designated +.