ARTICLE

A Synonymous Variant, *GABRG2* rs211037 Might Be a Predictive Genetic Marker of migraine: A Case Control Study from Pakistan

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Abstract

Background: *Migraine is a severe neurovascular disease with some temporary symptoms like unilateral headache attacks associated with sensory and autonomic disturbances. It affects 12% of the general population worldwide. Females are more susceptible to migraine than males. The genetic and environmental factors contribute as a causative agent to its symptomatology. Gamma-aminobutyric acid (GABA) neurotransmitter plays a potential role in migraine pathophysiology that prompted us to evaluate the association between gamma-aminobutyric acid type a receptor gamma two subunit gene (GABRG2) polymorphisms and the risk of a migraine attack.*

Methods *The present case-control study included 220 subjects (100 control; 120 patients). DNA was isolated from all the blood samples drawn from participants. The selected SNPs (rs211037, rs121909672, and T813C) of exons 5, 7, and 8 of the GABRG2 gene were genotyped for cases and controls.*

Results: *A silent polymorphism was found at the rs211037 polymorphic site, while no variation was found on other targeted sites either in the case or control population. Statistical analysis indicated significant differences in genotypic (p=<0.05) and allelic frequencies (p = <0.001; OR 2.039; 95% CI 1.346-3.089) and for dominant model (p = <0.001; OR 2.836; 95% CI 1.618-4.970).*

Conclusion: *The result of our study showed that rs211037 polymorphism of the GABRG2 gene was significantly associated with migraines in the Pakistani population.*

Key words: *GABA-A receptor; GABRG2 gene variants; Migraine; Polymorphism; Silent Mutation.*

Introduction

Migraine, a chronic, intermittent, and disabling neurovascular condition is typified by paroxysms of intense headaches associated with sensory, autonomic, and cognitive perturbations (Bertisch, et al., 2020). Migraine is found to be the second-largest disabling disease among all neurological diseases (GBD 2015 Maternal Mortality Collaborators, 2016) and affects about 12% of global population (Lipton, et al., 2007). Migraine attacks are often accompanied by transient neurological symptoms, including unilateral pain, aggravation, fatigue, nausea, photophobia, and Phonophobia (Goadsby, et al., 2002). The migraine prevalence is age and gender related, with highest occurrence in puberty, with slight early onset in males compared to females (Breslau & Rasmussen, 2001). Females are more likely to suffer from migraines, with an average prevalence of 18 to 20%, followed by 6% in males and 4 to 8% in children (Krymchantowski & Moreira Filho, 1999).

Two common forms of this condition that have been categorized according to the International Headache Society (IHS) are migraine with aura (MA), occurring in ~25% of migraineurs and migraine without aura (MO) which arises in ~70–75% of migraineurs (HCC, 2018). In (MA), the

cerebral blood flow of the affected area decreases while regional cerebral blood flow is increased or remains normal in the case of MO (Gervil, et al., 1998). The etiology of migraine indicates the environmental and genetic factors responsible for its polygenic and multifactorial nature. Indeed, stress, weather changes, hormonal changes, and high caffeine consumption are principal triggers. Alcohol consumption and insufficient sleep can increase attack frequency (Karli, et al., 2005, Spierings, et al., 2001). Heterogeneity of diagnostic criteria, familial predisposition, and lack of specific diagnostic markers for migraine are complicating factors in the epidemiological and genetic study of migraine (Mulder, et al., 2003).

Heritability research has verified that genetic factors play a major role in migraine. Genes contribute about 40-60% to the susceptibility of migraine (Mulder, et al., 2003). Migraine is known predominantly as a familial autosomal dominant disease with variable penetration (Rezapour, et al., 2018).

Several pathophysiological mechanisms have been attributed as causative agents to heterogeneous symptomatology and frequency, including activation of a trigeminovascular system (TVS), which results in the central transmission of pain followed by a cascade of biochemical events especially the release of neuropeptides (Lambert & Zagami, 2009). This leads to central sensitization, neuronal depolarization, chronic neurogenic inflammation, persistent cortical spreading depression, hypothalamic dysfunction, or a combination of these (Parsons & Strijbos, 2003, Gervil, et al., 1998).

Migraine pathogenesis is related to different neurotransmitters and their associated receptors (D'Andrea & Leon, 2010). Gamma-amino-butyric acid (GABA) is the most common inhibitor of brain neurotransmitters involved in cortical spread depression (CSD), and the activation of the TVS, both of these are responsible for aura symptomatology and migraine pain respectively (García-Martín, et al., 2018). The action of GABA is carried out by activating two subclasses of GABA receptors (GABRs) classed as GABAA and GABAB. The hetero-pentameric GABAA are ligand-gated chloride channels consisting of two alpha (α) subunits, two beta (β) subunits, and one gamma (γ) or (δ) subunits, which work as inhibitory receptors in mammalian brain. The central nervous system has widespread localization (Olsen & Sieghart, 2008). The chloride channels function as a chloride efflux pathway, participating in transmembrane chloride movements associated with GABAergic synaptic transmission and playing a necessary role in an inhibitory response of γ-aminobutyric acids. The *GABRG2* gene encodes the γ2 subunit of the receptor γ2-aminobutyric acid (GABA). This gene resides on the short arm (q31.1-q33.1) of chromosome 5 with nine exons (Kananura, et al., 2002). Mutations in the GABAergic receptors have been found to cause defective ion channel gating, decreased mRNA stability, altered subunit folding, and abnormal glycosylation resulting in impaired assembly and trafficking of receptors. These mutations reflect the depolarization of chloride channels at low voltages, resulting in the accumulation of intracellular chloride ions, and a reduction in the inhibitory response of γ-aminobutyric acid. The hyper-excitability results in nociceptive transmission by activation of the TVS (Wallace, et al., 2001). While Genome-Wide Association studies (GWAS) did not include GABR genes among the 38 migraine susceptibility loci, the potential role of GABA in migraine pathophysiology makes it appropriate to evaluate the possible potential relation between GABR gene polymorphisms and the risk of a migraine attack (Gormley, et al., 2016). In the Australian population, rs211037; a single nucleotide polymorphism of the *GABRG2* gene has been genotyped for migraines (Chen, et al., 2012). *GABRG2* mutations were also found associated with other neurological disorders like febrile seizures and epilepsy. Genetic connections between migraine and epilepsy were especially apparent in the case of hemiplegic migraine, where several mutations in the same gene could cause migraine, epilepsy, or both (Rogawski, 2008). This gene was selected to evaluate its possible role in migraine onset due to symptomatic similarities in migraine and epilepsy.

Methods

Sampling

The current study was certified for research on human subjects by the bioethics committee of University of the Punjab, Lahore, Pakistan. All the subjects were clinically diagnosed with migraines by neurophysiologists according to HIS criteria and gave informed consent prior to participation. All participants were interviewed and completed a detailed proforma on demographic attributes and family history. The study was conducted on 220 individuals: 120 migraine patients and 100 controls. Migraineurs with other neurological disorders like stroke or epilepsy were eliminated from the study. Controls were healthy volunteers with no history of any neurological disorder. The case and control populations were age and sex-matched from the same geographical location to avoid potential biases. Blood sample from each subject was collected in EDTA coated tubes.

Genotyping

Modified organic method was used to extract the genomic DNA from leukocytes (Sambrook et al., 1989) which was later quantified using Nanodrop Tm Spectrophotometer. Exon 5, 7, and 8 of the *GABRG2* gene were selected for mutational analysis. Amplification of targeted SNPs was done by polymerase chain reaction using previously reported primers as shown in Figure 1 (Haerian, et al., 2016, Baulac, et al., 2001). To make 25 µl of a reaction mixture, 4 µl MgCl2 (25 mM), 4 µl10x PCR buffer, 3 µl dNTP mix (2.0 mM), 0.5 µl Taq Polymerase (500 U; Thermo Fisher Scientific), 7.5 µl DEPC water, 3 µl of genomic DNA and 1.5 µl of each PCR primer was mixed in microcentrifuge tubes. The cycling conditions for PCR were initial denaturation at 95˚C for 5 min, followed by 30 cycles of denaturation at 95˚C for 30 sec,45sec of annealing at 60.5˚C, 47˚C and 57˚C for exon 5,7 and 8 respectively, and extension at72˚C for 45sec.Gelelectrophoresis was performed to check the amplification of targeted sequences. To detect the change in DNA, Sanger's sequencing was done from a commercial source.

Sequence and statistical analysis

The sequences were visualized on BioEdit software as shown in Figure 2, and BLAST was also performed for each sequence. The genotypic distribution was checked using Hardy Weinberg equilibrium through the Chi-square test. Categorical and continuous variables are presented in form of number

(percentage) and mean ± standard deviation respectively. Dominant model was used to check the rs211037 polymorphism association with migraine. The dominant model for allele T is CC versus CT+TT. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the risk factor for mutant allele carriers. Mega 6 software was used to evaluate consequent changes in amino acid sequence as shown in Figure 3.

Results

The 220 subjects were enrolled in this study and were grouped as migraine group having 120 subjects and control group having 100 subjects. The mean age of the 120 migraineurs, containing 92 women and 28 men was 24.85±5.19 while the mean age of 100 controls having 19 men and 81 women were 27.45±5.19 years. The age differences between the case and control groups were statistically non-significant (p = 0.23). In terms of gender, the difference was statistically significant (p=0.018) as most of the cases were women (case and control groups had women 76.6 and 81%, respectively). The demographic and clinical attributes of the case and control group are listed in Table 1.

Table 1: Demographic attributes of migraine and control group.

Characters	Cases (N)	Percentage
Sex		
Male	28	23.34
Female	92	76.66
Age (Years)	24.85±5.19	
Frequency of attack		
Low (1 time per week)	28	23.34
Medium (2-3 times per week)	42	35.00
High (>3 times per week)	50	41.66
Trigger factors		
Change in sleep hours	82	68.33
Emotional stress	69	57.50
Weather changes	28	23.33
Smell	56	46.66
Family history		
First degree relatives	53	44.16
Second degree relatives	29	24.16

* Only yes responses are included for Trigger factors and Family history

No variant was detected in exon 7 and 8 of the *GABRG2* gene in both case and control population, while c.588C>T polymorphism was observed profusely both in cases and controls. The chi-square test was used to check the HWE for rs211037and it was not violated. For *GABRG2* gene rs211037 polymorphism a statistically significant difference at both allelic (p = <0.001; OR 2.039; 95% CI 1.346-3.089) and dominant model (p = <0.001; OR 2.836; 95% CI 1.618-4.970) was found. Higher genotypic frequency for both CT and TT (50 vs. 28% and 17 vs. 10% respectively) was observed in cases compared to controls. At the allelic level the higher percentage for minor allele T was observed in migraineurs than in healthy controls (39.16 vs. 24%). At the genotypic level statistically significant difference at p<0.05 was found by logistic regression. This association indicated a significant risk factor of migraine for T allele in patients as OR 2.039 as shown in Table 2.

Discussion

The present study investigated the effect of genetic variants of the *GABRG2*gene cluster on chromosome 5 in migraineurs. In the context of genetics, a link between *GABRG2* gene polymorphism and migraine were the first time evaluated in the

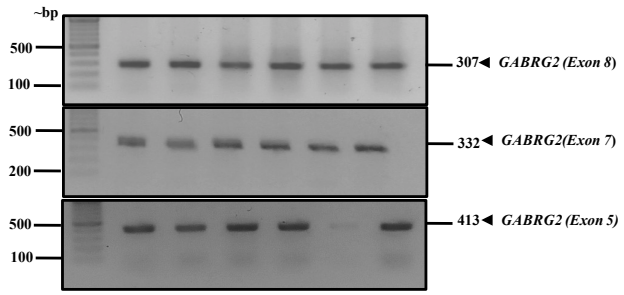

Figure 1: PCR results showing targeted amplification of selected exons of GABRG2 gene

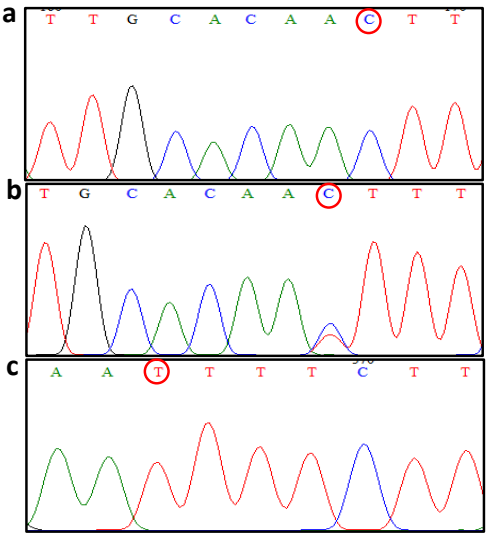

Figure 2: Electropherograms of rs211037 polymorphism; (a) CC Genotype (b) CT Genotype (c) TT Genotype

Pakistani population. Migraine and epilepsy are overlapping in clinical symptomatology, especially with reference to consciousness changes, sensory and other visual disturbances, and pain. A variety of medications have also been found to be effective for epilepsy and migraine, again pointing to the overlapping and commonality of the two illnesses (Tsai, et al., 2008).

Both epilepsy and migraine are deemed to be disorders of neuronal hyper-excitability. The leading cause of hyper-excitability includes a low concentration of GABA and the improper binding of GABA receptors with the neurotransmitter. Due to the substantial contribution of the γ2 subunit of GABA to epilepsy and migraine, it is possible that *GABRG2* associated SNPs indirectly influence the function of the *GABRG2* protein at transcriptional, post-transcriptional or translational levels. Pre- and post-synaptic GABAergic inhibitory response to neuronal excitation decreases due to abnormal protein activity that makes a person more prone to migraine and epilepsy (Haerian, et al., 2016). Based on these genetic links between these two disorders, previously evaluated polymorphic sites (rs211037, rs121909672, and T813C) for epilepsy were studied

Table 2: Genotypic and allelic frequencies of cases and control group.

SNP	Model	Allele/ Genotype	Migraine N=120 %	Control N=100 %	Crude Analysis COR (95% CI)	P value	Adjusted Analysis AOR (95% CI)	P value
rs211037	Allele	C	146 (60.84)	152 (76)	2.039(1.346-3.089)	<0.001***		
		T	94 (39.16)	48 (24)				
	Genotype	CC	43 (35.83)	62 (62)				
		CT vs. CC	60 (50)	28 (28)	3.090(1.706-5.595)	<0.001***	2.987(1.631-5.471)	<0.001***
		TT vs. CC	17 (14.16)	10 (10)	2.451(1.024-5.866)	0.044*	2.406(0.984-5.884)	0.054
	Dominant	CC	43 (35.83)	62 (62)				
		CT+TT	77 (64.16)	38 (38)	2.922(1.686-5.063)	<0.001***	2.836(1.618-4.970)	<0.001***

Data presented as number (N) and percentage (%); COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval; *p < 0.05, **p < 0.01, ***p < 0.001 statistically significant; Adjusted for age and sex

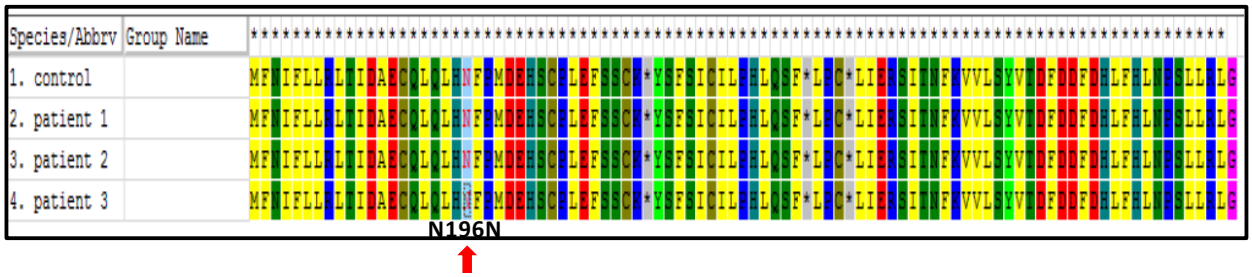

Figure 3: Amino acid alignment of rs211037 polymorphism

to check its possible association with migraine. It has been reported that rs211037 and the risk of epilepsy are strongly related (Spencer, et al., 2006). Consequently, it is assumed that rs211037 could be used as an effective genetic marker for predicting migraine susceptibility

This study shows a positive association between *GABRG2* gene polymorphism and migraine susceptibility for the studied population. It further revealed that CT and TT genotypes were present both in the case and control group, but the difference between case and control is statistically significant. Our results are inconsistent with a study conducted on the Australian population that reported the absence of a significant association between rs211037 and migraine (Chen, et al., 2012). This variation at C588T led to a silent change (Asn196Asn), that did not alter the encoded protein sequence. An earlier study had reported that rs211037 causes silent change at 588 positions (C/T) in exon 5 of the *GABRG2* gene in patients of childhood absence epilepsy (Chen, et al., 2012). Silent SNPs affect the mRNA splicing and protein folding. Silent mutations change the rate of translation, which could potentially alter the folding patterns of the protein (Tsai, et al., 2008). The cells having many misfolded proteins are in the condition of stress. These cells stimulate unfolded protein responses that can change the biogenesis of GABA receptors and thus compromise the GABAergic inhibition (Macdonald & Kang, 2009).

The SNP rs211037 is in an area of genes with high levels of recombination. A range of neurological disorders such as epilepsy and migraine has been associated with rearrangements in such DNA regions. The alteration of this SNP by recombination events is likely to influence the structure of the chromatin, DNA topology, or chromosome domain organization, thus changing the expression of the *GABRG2* gene (Kang, et al., 2009).

To the best of our knowledge, it was the first attempt worldwide that had been made to check polymorphic sites rs121909672 and T813C association with migraine, previously evaluated for epilepsy in Chinese and Indian populations (Haerian, et al., 2016, Baulac, et al., 2001).

No variation at polymorphic sites, rs121909672, and T813C (N1) were found. It could be the result of environmental factors, or multiple genes could be involved in the polygenic inheritance of migraine, and one single gene could have very little effect on the disease. The extent to which this finding can be extended to other populations remained to be determined, as the association will be limited to those ethnic groups with a particular genetic background that interacts. Further studies are required to understand the association of migraine and other single nucleotide polymorphism within the *GABRG2* gene in different populations and the relationship between genotype and phenotype in migraineurs

Conclusion

In conclusion, rs211037 may be the contributing risk factor for migraine. A broad population-based study is required to predict the potential role of *GABRG2* gene polymorphisms in migraine. The current study focused on variants present in three different exons of this gene, while six exons and regulatory regions of this gene still need to be checked for hidden variants. Further studies are required to unearth all the variants of the *GABRG2* gene with respect to migraine subjects so that baseline data can be generated, and population-specific markers can be developed for prior diagnosis and treatment of the disease.

Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: NS, TS, HM, RZ; data collection: TS, HM, MI, SKS; analysis and interpretation of results: TS, HM,

SKS; draft manuscript preparation: TS, HM, SKS, RZ: Overall supervision of the study; TS, NS. All authors reviewed the results and approved the final version of the manuscript.

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References

Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'homme, J. F., Baulac, M., Brice, A., Bruzzone, R., & LeGuern, E. (2001). First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. *Nature Genetics, 28*(1), 46–48. https://doi.org/10.1038/ng0501-46

Bertisch, S. M., Li, W., Buettner, C., Mostofsky, E., Rueschman, M., Kaplan, E. R., Fung, J., Huntington, S., Murphy, T., Stead, C., Burstein, R., Redline, S., & Mittleman, M. A. (2020). Nightly sleep duration, fragmentation, and quality and daily risk of migraine. *Neurology, 94*(5), e489–e496. https://doi.org/10.1212/WNL.0000000000008740

Breslau, N., & Rasmussen, B. K. (2001). The impact of migraine: Epidemiology, risk factors, and co-morbidities. *Neurology, 56*(6 Suppl 1), S4–S12. https://doi.org/10.1212/wnl.56.suppl_1.s4

Chen, T., Murrell, M., Fowdar, J., Roy, B., Grealy, R., & Griffiths, L. R. (2012). Investigation of the role of the GABRG2 gene variant in migraine. *Journal of the Neurological Sciences, 318*(1-2), 112–114. https://doi.org/10.1016/j.jns.2012.03.014

D'Andrea, G., & Leon, A. (2010). Pathogenesis of migraine: from neurotransmitters to neuromodulators and beyond. *Neurological Sciences, 31*(Suppl 1), S1–S7. https://doi.org/10.1007/s10072-010-0267-8

García-Martín, E., Esguevillas, G., Serrador, M., Alonso-Navarro, H., Navacerrada, F., Amo, G., García-Albea, E., Agúndez, J., & Jiménez-Jiménez, F. J. (2018). Gamma-aminobutyric acid (GABA) receptors GABRA4, GABRE, and GABRQ gene polymorphisms and risk for migraine. *Journal of Neural Transmission, 125*(4), 689–698. https://doi.org/10.1007/s00702-017-1834-4

Gervil, M., Ulrich, V., Olesen, J., & Russell, M. B. (1998). Screening for migraine in the general population: validation of a simple questionnaire. *Cephalalgia*, *18*(6), 342–348. https://doi.org/10.1046/j.1468-2982.1998.1806342.x

Goadsby, P. J., Lipton, R. B., & Ferrari, M. D. (2002). Migraine--current understanding and treatment. *The New England Journal of Medicine, 346*(4), 257–270. https://doi.org/10.1056/NEJMra010917

Gormley, P., Anttila, V., Winsvold, B. S., Palta, P., Esko, T., Pers, T. H., Farh, K. H., Cuenca-Leon, E., Muona, M., Furlotte, N. A., Kurth, T., Ingason, A., McMahon, G., Ligthart, L., Terwindt, G. M., Kallela, M., Freilinger, T. M., Ran, C., Gordon, S. G., Stam, A. H., … Palotie, A. (2016). Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nature Genetics, 48*(8), 856–866. https://doi.org/10.1038/ng.3598

Haerian, B. S., Baum, L., Kwan, P., Cherny, S. S., Shin, J. G., Kim, S. E., Han, B. G., Tan, H. J., Raymond, A. A., Tan, C. T., & Mohamed, Z. (2016). Contribution of GABRG2 Polymorphisms to Risk of Epilepsy and Febrile Seizure: a Multicenter Cohort Study and Meta-analysis. *Molecular Neurobiology, 53*(8), 5457–5467. https://doi.org/10.1007/s12035-015-9457-y

Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. (2018). Cephalalgia : an international *Journal of Headache, 38*(1), 1–211. https://doi.org/10.1177/0333102417738202

Kananura, C., Haug, K., Sander, T., Runge, U., Gu, W., Hallmann, K., Rebstock, J., Heils, A., & Steinlein, O. K. (2002). A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Archives of Neurology, 59*(7), 1137–1141. https://doi.org/10.1001/archneur.59.7.1137

Kang, J. Q., Shen, W., & Macdonald, R. L. (2009). The *GABRG2* mutation, Q351X, associated with generalized epilepsy with febrile seizures plus, has both loss of function and dominant-negative suppression. *The Journal of Neuroscience, 29*(9), 2845–2856. https://doi.org/10.1523/JNEUROSCI.4772-08.2009

Karli, N., Zarifoglu, M., Calisir, N., & Akgoz, S. (2005). Comparison of pre-headache phases and trigger factors of migraine and episodic tension-type headache: do they share similar clinical pathophysiology?. *Cephalalgia, 25*(6), 444–451. https://doi.org/10.1111/j.1468-2982.2005.00880.x

GBD 2015 Maternal Mortality Collaborators (2016). Global, regional, and national levels of maternal mortality, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet (London, England), 388*(10053), 1775–1812. https://doi.org/10.1016/S0140-6736(16)31470-2

Krymchantowski, A. V., & Moreira Filho, P. F. (1999). Atualização no tratamento profilático das enxaquecas [Update on migraine prophylactic treatment]. *Arquivos de Neuro-Psiquiatria, 57*(2B), 513–519. https://doi.org/10.1590/s0004-282x1999000300027

Lambert, G. A., & Zagami, A. S. (2009). The mode of action of migraine triggers: a hypothesis. *Headache, 49*(2), 253–275. https://doi.org/10.1111/j.1526-4610.2008.01230.x

Lipton, R. B., Bigal, M. E., Diamond, M., Freitag, F., Reed, M. L., Stewart, W. F., & AMPP Advisory Group (2007). Migraine prevalence, disease burden, and the need for preventive therapy. *Neurology, 68*(5), 343–349. https://doi.org/10.1212/01.wnl.0000252808.97649.21

Macdonald, R. L., & Kang, J. Q. (2009). Molecular pathology of genetic epilepsies associated with GABAA receptor subunit mutations. *Epilepsy Currents, 9*(1), 18–23. https://doi.org/10.1111/j.1535-7511.2008.01278.x

Mulder, E. J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D. A., Nyholt, D. R., Martin, N. G., MacGregor, A. J., Cherkas, L. F., Boomsma, D. I., & Palotie, A. (2003). Genetic and environmental influences on migraine: a twin study across

six countries. Twin Research, 6(5), 422–431. https://doi.org/10.1375/136905203770326420

Olsen, R. W., & Sieghart, W. (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacological Reviews, 60(3), 243–260. https://doi.org/10.1124/pr.108.00505

Parsons, A. A., & Strijbos, P. J. (2003). The neuronal versus vascular hypothesis of migraine and cortical spreading depression. Current Opinion in Pharmacology, 3(1), 73–77. https://doi.org/10.1016/s1471-4892(02)00016-4

Rezapour, P., Borjali, A., Hatamian, H., Shafaei, F., Janalipour, K. (2018). Effectiveness of Acceptance and Commitment Therapy in Pain Control in Multiple Sclerotic Women. Caspian Journal of Neurological Sciences, 4 (2), 64-70. http://cjns.gums.ac.ir/article-1-153-en.html

Rogawski M. A. (2008). Common pathophysiologic mechanisms in migraine and epilepsy. Archives of Neurology, 65(6), 709–714. https://doi.org/10.1001/archneur.65.6.709

Sambrook, J., Fritsch, E. R., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor Laboratory Press.

Spencer, C. C., Deloukas, P., Hunt, S., Mullikin, J., Myers, S., Silverman, B., Donnelly, P., Bentley, D., & McVean, G. (2006). The influence of recombination on human genetic diversity. *Plos Genetics, 2*(9), e148. https://doi.org/10.1371/journal.pgen.0020148

Spierings, E. L., Ranke, A. H., & Honkoop, P. C. (2001). Precipitating and aggravating factors of migraine versus tension-type headache. *Headache, 41*(6), 554–558. https://doi.org/10.1046/j.1526-4610.2001.041006554.x

Tsai, C. J., Sauna, Z. E., Kimchi-Sarfaty, C., Ambudkar, S. V., Gottesman, M. M., & Nussinov, R. (2008). Synonymous mutations and ribosome stalling can lead to altered folding pathways and distinct minima. *Journal of Molecular Biology, 383*(2), 281–291. https://doi.org/10.1016/j.jmb.2008.08.012

Wallace, R. H., Marini, C., Petrou, S., Harkin, L. A., Bowser, D. N., Panchal, R. G., Williams, D. A., Sutherland, G. R., Mulley, J. C., Scheffer, I. E., & Berkovic, S. F. (2001). Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nature Genetics, 28*(1), 49–52. https://doi.org/10.1038/ng0501-49