



A Study on Association of Single Nucleotide Polymorphisms on CACNA1A Gene with Migraine in South Indian Population

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Abstract

Mechanism based migraine therapy gained steam in early 1990s and has led to an extensive research for improved understanding of genetics of classical and common migraine, as each has its own hereditary characteristics. This has steered the researchers to associate single nucleotide polymorphisms (SNPs) to migraine. rs16023 of CACNA1A gene is one among the SNPs that are localized for classical migraine. The study was conducted with cohort of 28 volunteers of South Indian origin comprising of 20 volunteers reporting migraine and 8 without migraine. Also in this cohort 16 were common migraineurs and 4 were classical migraineurs. The results of this study indicated that all the 3 female classical migraineurs out of the 4 classical migraineurs exhibited A to T polymorphism which points to the fact that, females are more prone to migraine and also females of South Indian origin show association of rs16023 SNP to classical migraine.

Keywords: Migraine, CACNA1A Gene, Classical migraine, Association of SNP rs16023,

Introduction

Recent advances in neurogenetics and technology has rendered it easier for the researchers to explore the DNA sequence of an individual in depth to solve a mystery related to a disease or disorder. One such mystical disorder is migraine, a most common cause of headaches, yet with no particular cause or cure. Migraine is a ubiquitous familial disorder characterized by periodic, commonly unilateral, often pulsatile headaches which begin in childhood, adolescence or early adult life and recur with diminishing frequency during advancing years (1). Migraine headaches can sometimes be very debilitating.

Recently, it has been reported that migraine is hereditary (1). The genetics of classical and common migraine are not same. Early research and epidemiology studies have suggested that aura migraines and migraines without aura each have their own hereditary characteristics. Advancing neuroscience has enhanced our understanding of the migraine mechanisms and its polygenic inheritance. This has led researchers to dig deeper to associate

single nucleotide polymorphisms to migraine. rs16023 of CACNA1A gene is one among many SNPs that are localized for classical migraine(2). The prevalence of migraine in Indian population is now getting increasingly recognized and the disability of migraine is the same as it is in other major diseases. Lack of a single well defined cause for migraine has hampered efforts in finding a cure so far. Since migraine is hereditary, genetic studies which may lead to localization and association of a particular SNP with migraine may lend support to efforts aimed at a better understanding of the disease.

Materials and Methods

The cohort consisting of 28 volunteers with a South Indian origin sphering through the age of 15 to 50 years comprising 20 females and 8 males were interviewed and taken up for the study. Herein, 20 subjects reported migraine and 8 did not. Out of the 20 migraineurs, 4 reported migraine with aura and 16 without aura. The patient profile is presented in Table 1.

5ml of venous blood samples were collected from this cohort in sterile EDTA vials and stored at -20°C till further molecular analysis. Genomic DNA was extracted from blood using rapid non-enzymatic method (Bunce method - standard protocol of BioAxis DNA Research Centre, Hyderabad). To confirm the presence of DNA from the sample, agarose gel electrophoresis has been employed. The gene CACNA1A was identified and the FASTA sequence of CACNA1A gene was retrieved from NCBI database (3) and a region of approximately 200bp containing the SNP was selected from the gene for amplification. The SNP database of the NCBI was referred for the selection of the SNP rs16023 of CACNA1A gene. Primer was designed using Primer3 software for the SNP. All the DNA samples were subjected to PCR amplification using the forward primer 5'GAGGGTCCGGAGGACAAG3' and the reverse primer 5'CCTCGTACGTGGCTGGAG3'. The annealing temperature was 58°C. The amplicons were then sequenced by outsourcing them to Bioserve India, Hyderabad and the DNA sequence for the amplified product was obtained using ABI Sequencer. The obtained sequences were aligned using the multiple sequence alignment tool

CLUSTALW and were compared for the selected SNP.

Table 1: Clinical phenotype and rs16023 polymorphism in CACNA1A in the study group

SAMPLE / CONTROL	GEN DER	AGE	MA/ MO	CACNA 1A
				rs16023
				A to T
S1	F	40	MO	NO
S2	F	53	MO	NO
S3	F	21	MO	NO
S4	F	47	MO	NO
S5	F	35	MA	YES
S6	F	45	MO	NO
S7	M	34	MO	NO
S8	M	23	MA	NO
S9	F	30	MO	NO
S10	F	34	MA	YES
S11	F	21	MO	NO
S12	F	21	MO	NO
S13	F	21	MO	NO
S14	F	50	MA	YES
S15	M	51	MO	NO
S16	F	16	MO	NO
S17	F	40	MO	NO
S18	M	35	MO	NO
S19	F	43	MO	NO
S20	M	23	MO	NO
C1	M	21	-	NO
C2	F	48	-	NO
C3	M	36	-	NO
C4	M	32	-	NO
C5	F	23	-	NO
C6	F	21	-	NO
C7	F	23	-	NO
C8	F	19	-	NO

S – Sample, C – Control, MO- Migraine without Aura, MA – Migraine with Aura, M – Male, F – Female

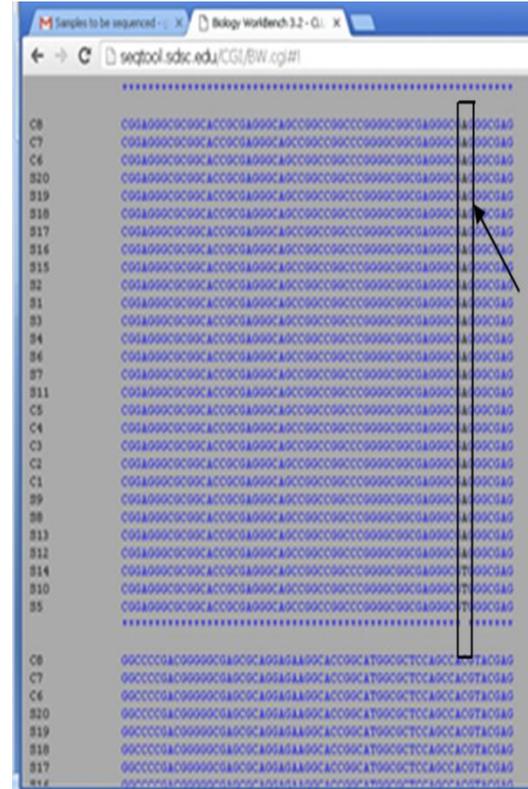
Results and Discussion

From the volunteers’ profile, out of the 20 migraine individuals, there were 15 females and 5 males. The control cohort comprised of 5 females and 3 males. The incidence of migraine is found to be 3 times more in females than males, thereby suggesting that females are more prone to migraine than males. Only 4 individuals out of 20 reported as classical migraineurs. This indicates that migraine with aura is much rarer than migraine without aura. The classical migraineurs reported visual impairment and vomiting. They added that skipping meals or sleepless nights are triggers and they are also

sensitive to bright light. These headaches are also the cause for keeping them awake in nights.

The blood samples collected from these volunteers was subjected to DNA extraction and agarose gel electrophoresis confirmed the presence of DNA. The results involving rs16023 of CACNA1A gene (Fig 1) indicates that out of 20 samples only in 3 samples A to T polymorphism is observed.

Fig 1: CLUSTALW Result for CACNA1A Gene – A to T Mutation



D'Onofrio *et al.* (2009) have reported 32/117 association of the SNP rs16023 of CACNA1A and Bashir *et al.* (2014), have reported no mutation of this SNP in Indian migraineurs. The CACNA1A gene is generally been associated with migraine with aura. Therefore in considering the symptoms and segregating the migraineurs as classical migraineurs and common migraineurs it was found that 4 are classical migraineurs and the rest 16 are common migraineurs. Out of these 4 classical migraineurs, all the 3 female samples showed A to T polymorphism. This indicates that 3 out of 4 samples are associated with classical migraine in South Indian origin. (chi square statistic = 8; p value is 0.004678). This greater extent of association indicates the possible involvement of CACNA1A in migraine with aura as suggested by Ophoff *et al.* (1996), Estevez and Gardner (2003). The limitation of this research is its small sample size. The probable mechanism of pain phase of migraine attack is attributed to the inhibition of P/Q calcium channels and Na⁺ /Ca²⁺

homeostasis leading to decreased threshold for CSD and increased susceptibility to the spontaneous activation of brainstem output mechanism. In P/Q type Ca^{2+} channel alpha 1 subunit is encoded by *CACNA1A* which forms the pore for the calcium channel(7). Mutations in *CACNA1A* gene gives rise to abnormal channel dynamics (8) and all these mutations affect the pore or the voltage sensor part of the ion channel. The voltage gated Na^{+} ion channels acts as propagators of electrical signal and the voltage gated Ca^{2+} channels play an important role in pain neurotransmitter release leading to pain signalling(9).

Conclusion

In our study, the *CACNA1A* gene is found to be associated to female patients with classical migraine but not with the common migraineurs of South Indian origin. The females are found to be more prone to migraine than males probably due to the hormonal changes occurring within their system. It could be concluded that though there might be ethnic effects for migraine, still there could be a common link that should be explored which might be the cause for mutation in different ethnic groups. Due its polygenic inheritance, extensive research on influence of lineage ethnicity and gender based studies on genes and SNP association to migraine could lead to a better understanding the mechanism of pain phase in migraine and probably lead to a cure or prevention.

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