

Mutation analysis of *CACNA1A* gene in Iranian migrainous and review literatures

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Background: There are contrary results about the role of *CACNA1A* gene in the causation of common migraine in different populations. However, migraine may be genetically heterogeneous and more studies in different families and populations are required for a definite conclusion. The aim of this study was to surveyed leukocyte genomic DNA mutation of *CACNA1A* in Iranian migraine patients with [MA] and without aura [MO] who has family history of migraine and we performed a narrative review of all studies that evaluated *CACNA1A* gene, non-hemiplegic migraine [MA and MO] and FHM [familial hemiplegic migraine]. **Materials and Methods:** The 30 patients with family history of migraine were selected for mutations analysis for *CACNA1A* gene by PCR method. For review, we searched MEDLINE-PUBMED, ISI, Scopus and Cochrane databases up to December 2012. **Results:** Mutation analysis of the 4 exons of the *CACNA1A* gene in these patients revealed no mutations in this gene. Direct sequencing revealed a polymorphism previously reported G to A transition in the exon 16 [nt2369, G→A] in 9 patients. In review, the correlation of FHM loci [*CACNA1A* gene] with MA and MO has been showed in different population and only small population from Caucasians presented this correlation. **Conclusion:** *CACNA1A* is most likely not a major susceptibility gene for common migraine in Iranian migrainous. It's essential to study more on larger series and covering all 47 exons of the *CACNA1A* gene to confirm this hypothesis.

Key words: *CACNA1A* gene, linkage, migraine, mutation

INTRODUCTION

Migraine is a prevalent neurological disturbance with the incidence of 16% of general population and impact roughly 20% of adults,^[1-4] that involves women more than men by the percent of up to 12% of men and 24% of women in the general population.^[5] Migraine without aura [MO] and migraine with aura [MA] in which headache is started with supplementary neurological symptoms, are two principal pattern of migraine disease.^[6,7]

Studies on twin and family have substantiated that migraine comprises a main genetic component that MA has more than MO. The disease is genetically elaborate because many genes and environmental factors play a part in this disorder^[5,7] and half of the patients have an affected first degree relative that it manifests the strong familial aggregation.^[6]

Although some clinical heterogeneity are shown in the MA and MO subtypes, Mochi *et al.*^[8] with segregation analysis proposed that there may be a common genetic etiology for MA and MO.^[9]

Familial hemiplegic migraine [FHM] is one of the most powerful congenital migraine sort of headache which

is a rare autosomal dominantly inherited subtype of migraine with aura^[5] that many studies revealed association this kind of migraine with mutations in the *CACNA1A* gene in different ethnic groups. Ophoff *et al.*^[10] characterized the *CACNA1A* gene in preparation for a mutation search in neurologic disorders that map to 19p13. They found that the gene covers 300 kb with 47 exons.^[11]

On the other hand, several reports^[5,12,13] suggest the possible involvement of *CACNA1A* in non-hemiplegic migraine, but the other studies show contradictory results.^[5,8,14,15]

Another studies showed the role of *CACNA1A* gene in the causation of common migraine,^[12,13] however, some others in East Asia^[16] did not show any correlation role. There is no study from Middle East and west of Asia to show the prevalence and correlation of this gene.

On the other hand migraine may genetically heterogeneous,^[4] and more studies in different families and populations are required for a definite conclusion.

Therefore, the aim of this study was to surveyed leukocyte genomic DNA mutation of *CACNA1A* in

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Iranian migraine patients with and without aura who have family history of migraine.

MATERIALS AND METHODS

Patient selection

MA and MO subtype of migraine were detected by expert clinical neurologist, according to the International Headache Society criteria [Headache Classification Committee of the International Headache Society [IHS] [MA = criteria 1.2.1 and MO = criteria 1.1].^[17]

Mutation analysis

Then turning to genetic studies [found mutated in FHM^[10]] were studied in 30 patients who had family history of migraine with or without aura in first degree relatives. Genomic DNA was isolated from peripheral EDTA-treated blood cells by Qiagen DNA Mini kit [cat No: 51304]. DNA of each patient was subjected to PCR amplification of the Exon 5, 16, 32 and 36 of *CACNA1A* gene. Our study focused on 4 of the 47 exons of the *CACNA1A* gene. Exons 5, 16, 32 and 36 were studied because missense mutations [S218L, T666M, R1667W, L1682P, W1683R and I1811L, 2] have been found in FHM families.^[10,18-20]

Twenty five-μL PCR reactions were carried out using 50 μL containing 100-200 ng total DNA from the patient, 10 pmol of each primers [Table 1], 2.5 mM MgCl₂, 200 mM each of dNTP and 1 U Taq DNA polymerase [Roche Diagnostics, Mannheim, Germany]. The reaction mixture was amplified under following condition: 94°C for 30s, 60°C for 30s and 72°C for 45s for 35 cycles followed by one cycle of 72°C for 7 minutes, after initial denaturation 95°C for 6 minutes. The amplification products were detected on 2% agarose gel, run in 0.5X TBE at 110 V for 50 minutes and visualized under UV upon staining with 0.002 mg/mL ethidium bromide. Good quality PCR products were sequenced using the Big Dye Terminator sequencing kit [Applied Biosystems] and an ABI 3130 Genetic Analyzer [Applied Biosystems]. Sequence results were compared with the published sequence [GenBank no.X99897] by using Chromas and DNAMAN software. The primers sequences have been reported in Table 1.

Review

Additionally, we performed a narrative review of all studies that evaluated *CACNA1A* gene, non-hemiplegic migraine [MA and MO] and FHM. Key words were classic migraine, migraine with aura, migraine without aura, common migraine and familial hemiplegic migraine and *CACNA1A* gene, gene mapping, gene screening and polymorphism. We searched MEDLINE-PUBMED, ISI, Scopus and Cochrane databases up to December 2012. We searched references lists from articles identified by search as well as a key review article to identify additional articles. We identified case control, case series.

RESULT

Mutation analysis of the 4 exons of the *CACNA1A* gene in these patients revealed one polymorphism, but no mutations were identified in this gene. Direct sequencing revealed a polymorphism previously reported polymorphism^[10,21] G to A transition in the exon 16 [nt2369, G→A] in 9 patients. In review, the correlation of FHM loci [*CACNA1A* gene] with MA and MO has been showed in different population and only small population from Caucasians presented this correlation.

DISCUSSION

In this study we found no *CACNA1A* gene mutations in Iranian patients. In accordance with our study, many researches had been reported such result. Table 2 showed all familial hemiplegic migraine [FHM] loci previously reported as candidate loci in migraine with typical aura [MA] and migraine without aura [MO] in different population. All of studies with linkage analysis [microsatellite marker in 19p13], mutations analysis on *CACNA1A* gene and related polymorphism were included in this table. Similar to our study, most of studies in western countries^[9,14,22-30] and east of Asia^[16] showed no correlation with FHM loci and MA and MO. Only a few studies in Netherlands and Finland^[5,12] and a small subset of patients and families^[31,32] explained this correlation. It appears the correlation of FHM loci with MA and MO is not depended to specific ethnic and only small population from Caucasians showed this correlation.

Table 1: Primer pair sequences are those used by ophoff et al.^[10]

| Exon | Amino acid change | Forward primer | Reverse primer | Tan (°C) | Product size (bp) |
|------|----------------------------|-------------------------------|-------------------------------|----------|-------------------|
| 5 | S218L | ctt ggt ggc ggg ggt t | ctg cct aat cct ccc aag ag | 60 | 290 |
| 16 | T666M | tcc aca gct gca tct cca ag | acc ctg cct tga gcc cct | 60 | 270 |
| 32 | R1667W L1682P W1683R | tct gtg agt ggt gac agc tc | gtc acc tgt ctt ctc agc | 60 | 240 |
| 36 | I1811L | ttc att ccc tog gtc tct gc | ctg act gaa cct gtg aga c | 60 | 350 |

Table 2: Familial hemiplegic migraine loci previously reported as candidate loci in migraine with typical aura and migraine without aura in different population

| Country | Authors | Study design | Type of migraine | No. of families (cases/controls) | Correlation between FHM and migraine |
|------------------------------|----------------------------------|--|-----------------------------|--|--|
| Los Angeles, Calif | Jen <i>et al.</i> , 2004 | Sequencing and Mutation screen all exons in <i>CACNA1A</i> | HM/BM/MO/MA | 18 MA cases, 25 MO cases, 7 BM cases, 19 HM, 40 controls | No correlation |
| Santa Clara, California, USA | Jones <i>et al.</i> 2001 | Linkage analysis with microsatellite marker in 19p13 | MA | 16 | No correlation |
| Los Angeles, California, USA | Kim <i>et al.</i> 1998 | Mutation screen all exons in <i>CACNA1A</i> | MA+MO with episodic vertigo | 9 | No correlation Known polymorphisms, intron (10,8) (nt1723+7: C>T, nt1474-31: A>G) and exon (29,3) nt4898: T>C, nt737: C>T) |
| Canada | Noble-Topham <i>et al.</i> ,2002 | Linkage analysis and TDT1 with microsatellite markers in 19p13 | MA | 64 | No correlation |
| Germany | Wieser <i>et al.</i> , 2003 | Mutation screen exons 4,13,16, 17, 27,36 in <i>CACNA1A</i> | MA+MO | 143 cases | No correlation |
| Italy | Brugnoni <i>et al.</i> , 2002 | Sequencing and mutation screen 22 exons in <i>CACNA1A</i> | MA | 12 | No correlation A known polymorphism (5682-14:C>T) was found in exon 36 |
| Italy | Cevoli <i>et al.</i> , 1997 | Mutation screen exon 4,17,36 in <i>CACNA1A</i> | MA | 12 migraine stroke/15MA | No correlation |
| Italy | Monari <i>et al.</i> , 1997 | Linkage pedigrees analysis with Microsatellite marker in 19p13 | MA+MO | 14 | No correlation |
| Denmark | Kirchmann <i>et al.</i> , 2006 | Linkage analysis and sequencing with microsatellite marker in <i>CACNA1A</i> | MA | 174 MA/79 unaffected relatives | No correlation with MA |
| Netherlands | Terwindt <i>et al.</i> , 2001 | Linkage analysis with microsatellite marker in 19p13 | MA+MO | 36 | Correlation with MA |
| Netherlands | May <i>et al.</i> , 1995 | Linkage analysis with microsatellite marker in 19p13 | MA+MO | 28 | Correlation |
| Finland | Kaunisto <i>et al.</i> ,2005 | Linkage analysis with eight polymorphic microsatellite marker in 19p13 | MA | 72 | No correlation. Association between the <i>INSR</i> locus (in the proximity of <i>CACNA1A</i>) and MA and MO |
| Finland | Hovatta <i>et al.</i> , 1994 | Linkage analysis with microsatellite marker in 19p13 | MA+MO | 4 | Correlation |

Contd...

Table 2: Contd...

| Country | Authors | Study design | Type of migraine | No. of families (cases/controls) | Correlation between FHM and migraine |
|----------------|-------------------------------|--|------------------|--|--|
| Australia | Lea <i>et al</i> , 2005 | microsatellite marker in 19p13 | MA | 92 | No correlation |
| Australia | Lea <i>et al.</i> , 2001 | Linkage analysis, case control, TDT and sequencing with microsatellite marker in 19p13 | MA+MO | 82 | No correlation |
| Australia | Nyholt <i>et al.</i> , 1998 | Linkage analysis with microsatellite marker in 19p13 | MA+MO | 4 | One of four families that transmit MA had positive linkage to 19p13 |
| Japan (poster) | Iigaya <i>et al</i> , 2003 | Mutation analyses of the CACNA1A gene by direct nucleotide sequence analysis of 12 exons | MA | 30 cases | No correlation SNP (nt 2369:G>A) was found in 15/30 patients in exon 16 |
| Japan | Sakai <i>et al</i> , 2003 | Mutation analyses of the CACNA1A gene | MA | | No correlation |
| Japan | Chen Feng <i>et al</i> , 2006 | Mutation analyses of the CACNA1A gene by direct nucleotide sequence analysis of 3 exons | FHM, MA | 10 FHM cases, 12 relatives of the patients in 2 pedigrees, 53 MA cases, 10 control | No correlation |

FHM=Familial hemiplegic migraine; HM=Hemiplegic migraine; BM=Basilar migraine; MA=Migraine with aura; MO=Migraine without aura; TDT=Transmission disequilibrium test

Hence, CACNA1A is not probable responsible gene for types of migraine. Other genes or loci that are involved are more suitable alternative genes for MA and MO migraine,^[7] for example in a study it was found that a responsible gene for migraine was the insulin receptor gene *INSR*, which lies in a Chr19p13 region near CACNA1A. Five single-nucleotide polymorphisms within the insulin receptor gene were significantly associated with migraine. Five single-nucleotide polymorphisms within the insulin receptor gene were considerably related with migraine, but there are no practical outcomes of this polymorphism.^[33]

However, it seems, there is possible limitation for unobtaining any mutation in the CACNA1A gene. At least 18 CACNA1A gene mutations in 47 exons have been identified in people with FHM1.^[19,20] In our studies and some others^[22] only limited mutations of FHM loci in limited exons were analyzed. Therefore, more studies with larger sample size and covering all 47 exons of the CACNA1A gene are necessary to confirm this hypothesis. We found a known polymorphism (nt 2369 G A) in exon 16 in 9 patients. Many studies identified other polymorphisms in the coding region of FHM loci in the patients with migraine, a number of them with significant difference^[14,30,34] and some others without significant differences compare with control.^[35,36]

CONCLUSION

As a result, CACNA1A is most likely not a major susceptibility gene for types of migraine in Iranian migraines. It is essential to study more on larger series of MA patients and covering all 47 exons of the CACNA1A gene to surely include or exclude whether migraine and FHM share common genetic defects.

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