

Thr698Thr (nt2369) polymorphism on *CACNA1A* gene and head pain severity in familial migraine

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Background: Migraine is a common neurological disorder with a significant genetic component. Less information is known about the contribution of minor genetic variations, such as single nucleotide polymorphism (SNP) on the migraine process. In the present study, we aim to investigate the role of *CACNA1A* gene polymorphism on severity and related factors in family positive migraine patients. **Materials and Methods:** We included 74 common migraine patients consequently. Headache severity was evaluated according to Headache Impact Test (HIT6) questionnaire and quality of life of patients was investigated according to MSQ (Migraine-Specific Quality of Life Questionnaire v2.1) questionnaire. Thirty patients with positive family history of migraine were selected and sequencing analysis after DNA extraction was performed. **Results:** Direct sequencing revealed a known SNP G to A transition in the exon 16 (nt2369, G → A) in 9 patients. There was no significantly correlation between polymorphism and type of migraine, severity, frequency, duration and quality of life in family positive migraine. Evaluated migraine severity by HIT6 questioner couldn't act as a risk factor for this polymorphism (OR: 0.93, CI%95 0.82-1.06 $P = 0.3$). **Conclusion:** In Iranian population no significant association was seen between Thr698Thr (nt2369) polymorphism and head pain severity in familial migraine. Confirmation of this hypothesis needs further investigation.

Key words: Migraine, quality of life, single nucleotide polymorphism, severity

INTRODUCTION

Migraine is a mysterious neurological disorder affecting about 18% of females and 6% of males.^[1,2] The most common forms of this disorder have been classified as migraine with aura (MA) and migraine without aura (MO)^[3] that could influence quality of life of patients and their families.^[4,5] Many factors such as psychological, environmental, biochemical, neurophysiologic, and genetic factors have a main impact to trigger attacks in this disorder,^[6,7] especially genetic factor may affect treatment response.^[5] Heritability for migraine estimated as 39-58% and this explains a strong familial aggregation.^[8,9]

Familial hemiplegic migraine (FHM) is one of the most strong congenital in subtype migraine with aura in which mutations in the calcium-channel gene *CACNA1A*, located on chromosome 19 has been confirmed.^[10]

Previously, a common single nucleotide polymorphism (SNP) from the *CACNA1A* gene, the Thr698Thr (nt2369, G → A) has been reported.^[11]

Limited information about the role of minor genetic variations, such as SNPs in clinical manifestation of migraine exists. Many studies has investigated the role of different SNP on migraine process.^[12,13]

As regards, some studies presented the role of *CACNA1A* gene in the causation population of common migraine,^[14,15] the aim of our study was to investigate the role of *CACNA1A* gene Thr698Thr polymorphism on type of migraine, severity, frequency, duration and quality of life in a sample of cases with family positive migraine patients.

MATERIALS AND METHODS

Patient selection

A total of 74 individuals were selected, where MA and MO subtype of migraine were detected by questioning and survey that is managed 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)].^[3]

All of the data including sex and age, family history, aura signs and marital status, level of education, the mean duration and frequency of headache per month obtained from 74 (58 females and 16 males) patients that including 50 patients with positive familial history and 24 individuals without family history. Headache severity was evaluated according to Headache Impact Test (HIT6) questionnaire^[16] and quality of life of patients was investigated according to MSQ (Migraine-Specific Quality of Life Questionnaire v2.1) questionnaire.^[16]

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DNA samples

Then turning to genetic studies were studied in thirty patients who had family history of migraine with or without aura in first degree relatives. DNA samples were obtained from whole blood for molecular genetic analysis. Whole blood samples were collected at the time of the interview for all cases for the genetic portion of the study. Blood samples were collected in Monovettes tubes containing EDTA. All samples were labeled with a unique ID number. Genomic DNA was isolated from peripheral EDTA-treated blood cells by Qiagene DNA Mini kit (cat No: 51304).

Variant screening

To detect the presence of the Thr698Thr (nt2369, G → A; CACNA1A gene, exon16) polymorphism, DNA samples were amplified by polymerase chain reaction (PCR) using the following primers (5' to 3'): Exon 16 Forward: TCC ACA GCT GCA TCT CCA AG and reverse: ACC CTC CCT TGA GCC CCT.^[11]

PCR reactions were performed in 50 µl containing 100-200 ng total DNA from the patient, 10 pmol of each primers, 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 Bio systems) and an ABI 3130 Genetic Analyzer (Applied Bio systems). Sequence results were compared with the published sequence (GenBank no.X99897) by using Chromas and DNAMAN software.

Statistical analysis

Statistical analysis was carried out by using the SPSS version 18. The comparison of clinical characteristics of migraines subtypes, polymorphism and family history of migraine were achieved by *t*-tests, Chi-square and logistic regression.

RESULTS

We included 74 patients. The baseline characteristic, severity, frequency, duration and quality of life of migraine patients according to family history of migraine in first degree relative were shown in [Table 1]. According to MSQ scale, quality of life in migraine patients with positive family history was significantly worse than negative family history group ($P = 0.047$).

Variant screening on exon 16 on the CACNA1A gene in these patients revealed one polymorphism, G to A transition in the exon 16 (nt2369, G → A) in 9 patients. This polymorphism previously reported.^[11,17]

Table 2 showed that there were no significantly correlation between polymorphism and type of migraine, severity, frequency, duration and quality of life in family positive migraine. Evaluated migraine severity by HIT6 questioner couldn't act as a risk factor for this polymorphism (OR: 0.93, CI% 95 0.82-1.06 P value = 0.3).

DISCUSSION

We found a known polymorphism (nt 2369 G → A) in exon 16 in 9 patients. The frequency of these polymorphisms in case control study was 0.12.^[11] This polymorphism also

Table 1: Baseline characteristic, of migraine patients according to family history of migraine in first degree relative

Characteristics	Migraine		P value
	Family history*	No family history	
Age (mean±SD)	33.24±10.78	35.37±9.62	0.412
Sex (%)			
Female	40 (80.0)	18 (75.0)	0.625
Male	10 (20.0)	6 (25.0)	
Marital (%)			
Married	41 (82.0)	18 (75.0)	0.483
Non-married	9 (18.0)	6 (25.0)	
Frequency of migraine attack per month (mean±SD)	9.66±8.11	9.95±9.39	0.889
Duration of migraine attack (mean±SD)	18.08±11.49	18.83±12.11	0.796
Severity of migraine according to HIT6 ¹ (mean±SD)	62.84±7.03	61.04±6.55	0.297
Quality of life according to MSQ ² (mean±SD)	44.8±13.5	38.38±10.9	0.047

*Family history of migraine in first relatives; ¹HIT6=Headache impact test; ²MSQ = Migraine-specific quality of life questionnaire

Table 2: Correlation between polymorphism and factor related to migrainee in family positive migrainous patients

Variable	Polymorphism	No polymorphism	P value
Type of migraine			
MA (%)	3 (33.3)	6 (28.6)	
MO (%)	6 (66.7)	15 (71.4)	0.794
Severity of migraine according to HIT6 ⁽¹⁾ (mean±SD)	65.11±6.09	62.47±6.47	0.308
Quality of life according to MSQ ⁽²⁾ (mean±SD)	43.56±9.82	44.43±13.35	0.862
Frequency (mean±SD)	9.55±7.93	11.71±9.34	0.551
Duration (mean±SD)	20.88±13.38	18.28±12.55	0.614

MA=Migraine with aura; MO=Migraine without aura; ¹HIT6=Headache impact test; ²MSQ=Migraine-specific quality of life questionnaire

reported in East Asia, North America, West Africa and not specified area (www.ensembl.org). Another studies reported this polymorphism in Asian people. In one research in Japan, polymorphism was found in 15/30 patients in exon 16 (nt 2369, Thr 698) but no mutations identified in any of the 12 exons among 30 patients in Japanese families. Indeed, many studies presented other polymorphisms both significant^[18] and non-significant difference^[19,20] than control in the coding region of FHM loci in the migrainous patients.

Previous suggestion based on P/Q-type calcium channel function in migraine presented that the interaction of minor genetic variants such as SNP could influence disease process.^[21,22]

Menon *et al.* reported an association of a μ -opioid receptor gene (OPRD1) SNP in severity of head pain in migraines females.^[13] This variant also increases receptor affinity of β -endorphin that may involve pain sensitivity.^[23] Association of angiotensin converting enzyme (ACE) gene polymorphism with migraine severity could be described by effectiveness of ACE inhibitor such as captopril in reducing the frequency, duration, and severity of migraine.^[24]

In another study, Fernandez *et al.* reported that polymorphism dopamine beta hydroxylase (DBH) gene is correlated with migraine severity.^[25]

In our study, this polymorphism does not seem to correlate with disease severity, gender or age effect, clinical aura features, one possibility is that mentioned polymorphism does not have any amino acid substitution (same sense mutation), thus functionality of P/Q-type calcium channel does not alter.

The importance of our findings can be focused into main areas: 1st this is the first report of common *CACNA1A* SNP in Iranian migraineurs. Future studies will be important to understand the role of other polymorphisms of *CACNA1A* gene in our population and further investigations suggest that maybe other polymorphisms or variants involved in Iranian patients. 2nd there was no significant difference between individuals carrying this variant and type of migraine, severity, frequency, duration and quality of life in family positive migraine. However, because the number of patients studied was small, it is possible that the polymorphism may yet be found to play a minor role in increased risk of migraine or migraine susceptibility. Further studies on the genetic contribution to migraine are necessary to recognize the complex pathophysiology of this disease as regards to polygenic multifactorial characteristic of migraine disorder.

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