

## Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism (677CT) and Increased Susceptibility to Migraine in Egyptian Population: A Case-Control Study

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### Abstract

**Background:** Migraine is a recurrent neurovascular disorder which lowers the quality of life. Middle-aged women are more susceptible to migraine than men. It is either migraine with aura (MA) or migraine without aura (MO). Molecular genetic studies of migraine have investigated many polymorphisms possibly associated with migraine particularly rs4846049 and A1298C polymorphisms. Methylenetetrahydrofolate Reductase (MTHFR) gene 677CT polymorphism is one of the proposed genetic changes affecting migraine.

**Aim of Study:** This study aimed at assessing its role in migraine in Egyptian population and its relation to the clinical manifestations of the disease and to find a possible link to effectiveness or resistance to 677CT treatment protocols.

**Subjects and Methods:** This study was conducted on (180) subjects who were divided into two groups: Group I (patients, group) which included (120) adult patients with migraine recruited from Headache Clinic in Ain Shams University Hospitals and were diagnosed with migraine according to ICHD diagnostic criteria 3<sup>rd</sup> edition beta version (2013), and Group II (control group) which included (60) age- and sex-matched apparently healthy subjects. MTHFR 677CT polymorphism was assessed using Restriction Fragment Length Polymerase Chain Reaction (RFLP-PCR) technique.

**Results:** The study demonstrated a significant association between MTHFR gene polymorphism (C677T) and migraine ( $p < 0.001$ ), Odd's Ratio (OR)=6.0, 95% CI (1.84-19.59). Both heterozygous (CT) and homozygous (TT) female patients were significantly more susceptible to develop migraine than both heterozygous (CT) and homozygous (TT) males ( $\chi^2$ : 12.48,  $p < 0.05$ ). Patients with homozygous type (TT) showed positive significant risk to develop aura (50%) ( $p < 0.001$ ), OR=45.0, 95% CI (5.26-385.19) while patients with heterozygous type (CT) showed positive significant risk to have attacks of migraine associated with photophobia (58.3%) ( $p < 0.001$ ), OR=21.0, 95% CI (3.10-142.21) or phonophobia (56.5%) ( $p < 0.001$ ), OR=13.0, 95% CI (2.09-81.05) or both. Significant statistical difference was seen on comparing

between MTHFR gene polymorphism (677CT) and attack severity ( $\chi^2$ : 8.57,  $p < 0.05$ ), while no significant difference was seen between gene polymorphism and analgesic treatment dose ( $\chi^2$ : 1.86,  $p > 0.05$ ).

**Conclusion:** The study demonstrates a significant association between MTHFR gene polymorphism (677CT) and occurrence of migraine and its preceding and accompanying clinical manifestations in Egyptian population, attack severity and increased risk of disease occurrence. Unfortunately, MTHFR gene polymorphism (677CT) showed no statistical association with analgesic treatment dose.

**Key Words:** Migraine – Methylenetetrahydrofolate reductase 677CT polymorphism – Migraine – Homocysteine – PCR-RFLP.

### Introduction

**MIGRAINE** is a chronic neurological disorder with high prevalence. Females appeared to have a higher morbidity of migraine than males in developed countries [1]. It is commonly underdiagnosed and undertreated worldwide. This is partially attributable to misdiagnosis and expectations of poor treatment outcomes [2]. Migraine nearly affects 10% of the world's population specially women [3]. Children can be candidate for this disorder with (3.5%-5%) prevalence and subsequent absence from school, decreased academic level and higher rate of recurrent illnesses [4].

A number of genes have been implicated in the pathogenesis of this disease, including genes involved in regulating the vascular system which raise the possibility of being a multifactorial disease with polygenic influence with a particular interest in the methylenetetrahydrofolate reductase (MTHFR) gene and its possible role in the pathogenesis of migraine [5,6]. The MTHFR gene is located at

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chromosome 1p36.3. It is 2.2kb in length with a total of 11 exons. It is a critical enzyme in folate metabolism which is essential to the carbon transfer necessary for DNA synthesis, cell division, and tissue growth. This is through regulation of DNA methylation by conversion of 5, 10-methylenetetrahydrofolate (5, 10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF), the major methyl donor for re-methylation of homocysteine to methionine which is encoded by the MTHFR gene [7].

The clinical consequences of elevated homocysteine plasma levels include endothelial cell injury, spontaneous trigeminal cell firing, and alterations in coagulant properties of blood. Spontaneous trigeminal cell firing leading to inflammation in the meninges and dilation of cerebral vessels is thought in part to cause the pain associated with migraine [8]. Thus, homocysteine dysfunction can clearly increase patient propensity for developing play an important role in the aetiology of the disease [6,9]. Clinically, the most studied polymorphisms of the MTHFR gene are 677CT and A1298C. They both affect the enzymatic function and consequently lead to a high level of plasma homocysteine [10]. Therefore, assessing the association between MTHFR gene polymorphism (677CT) and different types of migraine and its manifestations in Egyptian patients can help improving their quality of life and open a window for targeted gene therapy goals.

## Subjects and Methods

### Subjects:

This study was conducted on (180) subjects recruited from outpatient clinics in Ain Shams University Hospitals. They were further divided into two groups: Group I (patients, group) included (120) adult patients presenting with symptoms suggestive of migraine. These patients were referred to the Headache Clinic at Ain Shams University Hospital, Cairo, Egypt, in the period from March 2015 till April 2017 for further assessment and conducting the study. Patients in this group were diagnosed with migraine according to International Classification of Headache (ICHD) diagnostic criteria 3<sup>rd</sup> edition [11]. Group II (control group) included (60) age- and sex-matched subjects with no symptoms suggestive of migraine recruited from outpatient clinics at Ain Shams University Hospitals. Based on published data, to obtain the expected association of MTHFR gene polymorphism and migraine with 95% confidence interval, the minimal sample size required was 100 subjects enrolled with 50 cases and 50 controls.

### Inclusion criteria:

- I- Age (18-69yrs) both genders.
- II- Patients diagnosed with migraine with or without aura whether episodic or chronic.

### Exclusion criteria:

- I- Probable migraine, according to ICHD 3<sup>rd</sup> edition [11].
- II- Familial or sporadic hemiplegic migraine.
- III- Other significant medical conditions that could be a cause of headache other than migraine e.g. hypertension, chronic rhinosinusitis, error of refraction and tumors.

All studied individuals were subjected to full history taking, clinical examination with special emphasis on data such as aura, photophobia, phonophobia, nausea, vomiting, awake patients from sleep or not and analgesic over use.

A verbal informed consent was obtained from all subjects before participation in the study. The procedures performed in this study were approved by the Ethical Committee of Human Experimentation of Ain Shams University, and are in accordance with the Helsinki Declaration of 1975.

### Sampling:

Under complete aseptic conditions, 5mL of venous blood were obtained by venipuncture and collected in an EDTA-containing vacutainer, then immediately transferred to the laboratory and centrifuged at (1900xg for 10 minutes). Plasma was separated into new aliquote tubes and stored at -80°C for subsequent DNA extraction and detection of MTHFR gene C677T point mutation by RFLP-PCR technique.

### Methods:

#### Analytical methods:

Detection of MTHFR gene 677CT point mutation by PCR-RFLP using hot start master mix: Ready-to-use mix for PCR supplied by (Thermo-scientific, 168 third Avenue, Waltham, MA, USA). Forward primer with sequence: 5TGAAGGA GAAGGTGTCTGCGGGA3 and reverse primer with sequence: 5AGGACGGTGCGGTGAGAG TG3 [12] kit supplied by (Invitrogen, 5791 Van Allen Wa, Carlsbad, CA, USA).

#### • Genomic DNA extraction:

DNA extraction was performed using DNA purification kit (Thermo fisher scientific, U.S.A.). Following manufacturer protocol, 100µL of sample and 400µL of the buffer AL were added to 1.5mL

microcentrifuge tube and followed by vortex for 15 seconds. Incubation was done for 10-15 minutes at room temperature, followed by vortex for 15 seconds. The mixture was transferred to spin column that was placed in a 2mL collection tube, without touching the membrane in the spin column. Centrifugation was done for 1min at 11000rpm then the collection tube was discarded and the column was put in a new collection tube. Then, 500µL of AW1 buffer is added to the spin column followed by centrifugation for 1min at 11000rpm then the collection tube was discarded and the column was put in a new collection tube. Then 200µL of AW2 buffer was added to the spin column followed by centrifugation for 1min at 11000rpm then discarded. The spin column was then placed in a 1.5ml microcentrifuge tube. 150µL of the elution buffer was then added followed by incubation at room temperature for 2 minutes, and centrifugation at 13000rpm for 1 minute. The spin column was discarded leaving 150µL of purified DNA in the DNA collection tube. For identification and confirmation of successful DNA extraction, the extracted product of DNA samples were subjected to run on 2% agarose gel for 15min at 100V, stained with ethidium bromide. The purified DNA was stored at -20 till amplification.

#### • Amplification by PCR:

In each run, the required number of PCR reaction tubes was calculated and in each tube the following reagents were added with a total volume of 25µL in each tube containing: 10µL of DNA extract, 12.5µL of the ready to use hot start master mix, 1µL of forward primer, 1µL of reverse primer, 0.5µL of RNASE free water. Sample tubes were placed in thermal cycler with the cycling program shown in (Table 1).

Table (1): Cycling program.

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95	15mins.	1
Denaturation	95	1min.	35
Annealing	60	1min.	35
Extension	72	1min.	35
Final extension	72	10mins.	1

#### • Restriction Fragment Length Polymorphism (RFLP):

The 198bp PCR amplified products were digested with restriction enzyme *HinfI* supplied by (Thermoscientific, USA) specific for 677CT polymorphism. The subject was considered:

- Homozygous mutation if: Two bands develop at 175bp and 23bp.

- Heterozygous mutation if: The three bands develop at 198bp, 175bp and 23bp.

- Wild type if: One band develop at 198bp.

All reactions were performed in a total volume of 15µL. Samples tubes were incubated at 37°C for 20 minutes then digested products were analyzed by gel electrophoresis.

#### • DNA analysis by gel electrophoresis:

Amplified product of DNA samples were subjected to run on 2% agarose gel for 60mins at 100V, stained with ethidium bromide with DNA molecular weight marker to identify the site of bands (50 base pair (bp) DNA ladder). The separated bands were visualized by ultraviolet transilluminator and photographed with polarized camera. The amplified products were at 198bp. Results for genotyping of 677CT polymorphism after *HinfI* digestion are shown in Fig. (1). Three genetic variations were detected (homozygous T/T, heterozygous C/T and wild C/C genotypes).

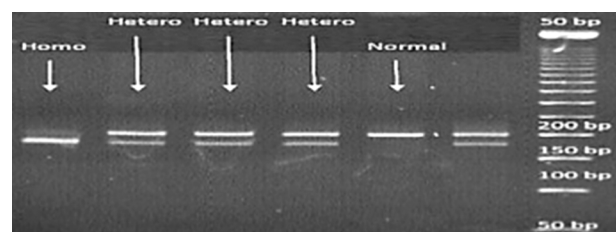


Fig. (1): PCR-RFLP results of 677CT gene polymorphism.

#### Statistical methods:

All statistical analyses were done using software Version IBM SPSS (Statistical Package for the Social Sciences) statistics (Version 25.0, IBM Corp., USA, 2017-2018). Descriptive statistics of various studied parameters were expressed as percentage for qualitative data, median (M) and Inter-Quartile Range (IQR) which extends between the 25<sup>th</sup> and 75<sup>th</sup> percentiles (Q1 and Q3, respectively) for quantitative non parametric data. Comparative statistics were done using the Wilcoxon's Rank Sum test for quantitative non parametric data and Pearson Chi Square test for qualitative non parametric data. *p*-value >0.05: Non significant; *p*-value <0.05: Significant; *p*-value <0.01 or 0.001: Highly significant.

## Results

Migraine patients (Group I) included 20 males and 100 females with age median 36 years (Q1-Q3: 30-41 years) while healthy matching controls (Group II) consisted of 28 males and 32 females with age median 45 years (Q1-Q3: 33-52 years).

Table (2) shows descriptive clinical data for Group I (migraine patients). Table (3) shows comparison between C allele and T allele frequencies in Group I and Group II. A statistical significant difference was detected between the two groups regarding allelic distribution using Wilcoxon Rank Sum test ( $Z: 2.19, p<0.05$ ).

Table (2): Descriptive clinical data of Group I (patients n=120).

Clinical data	Number (%)
Aura	24 (20%)
Photophobia	96 (80%)
Phonophobia	92 (76.7%)
Nausea	64 (53.3%)
Vomiting	44 (36.7%)
Awake patients from sleep	28 (23.3%)
Analgesic over use	100 (83.3%)
Triggers factors	64 (53.3%)
Neck pain	44 (36.7%)
Unilateral attack	60 (50%)
Severe intensity	64 (53.3%)
Attack duration more than one day	52 (43.3%)

Table (3): Comparative statistics regarding Allele frequencies of MTHFR Gene Polymorphism between Group I (patients) and Group II (controls) using Wilcoxon Rank Sum Test.

Allele	Group I (patients) n=120		Group II (control) n=60		Z	p
C allele	144	60%	88	73.33%	2.19	<0.05
T allele	96	40%	32	26.67%		

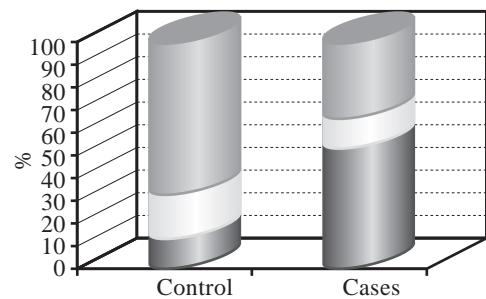
$p<0.05$ : Significant.

The MTHFR genotypes distributions between Group I and Group II are shown in (Table 4) and Fig. (2). The genotypes analysis revealed statistically significant difference between the two groups ( $\chi^2:6.79, p<0.05$ ). The observed distribution of genotype frequencies in the studied Groups I and II corresponds theoretically to the expected Hardy-Weinberg equilibrium.

Table (4): Comparative statistics regarding MTHFR Gene Polymorphism between Group I (patients) and Group II (controls) using Pearson Chi Square Test.

Parameter	Group I n=120		Group II n=60		$\chi^2$	p
	N	%	N	%		
Heterozygous (CT)	64	53.3	8	13.3	6.79	<0.05
Homozygous (TT)	16	13.3	12	20		
Wild type (CC)	40	33.3	40	66.7		

$p<0.05$ : Significant.



Genotype	Control (%)	Cases (%)
Wild type	66.7	33.3
Homozygote type	20	13.3
Heterozygote type	13.3	53.3

Fig. (2): 677CT gene polymorphism distribution in both studied groups (Group I patients, Group II controls).

Comparative statistics between Group I and Group II regarding sex showed high statistical significant difference between the two groups ( $\chi^2: 12.48, p<0.001$ ) (Table 5).

Table (5): Comparative statistics regarding sex between Group I (patients) and Group II (controls) using Pearson Chi Square Test.

Parameter	Group I n=120		Group II n=60		$\chi^2$	p
	N	%	N	%		
Female	100	83.3	32	53.3	12.48	<0.001
Male	20	16.7	28	46.7		

$p<0.001$ : Highly significant.

Table (6) shows statistically significant difference between genotypes of MTHFR gene regarding sex in Group I ( $\chi^2: 6.0, p<0.05$ ).

Table (6): Comparative statistics between MTHFR Gene Polymorphism and sex in Group I (patients) using Pearson Chi Square Test.

	Female		Male		$\chi^2$	p
	N	%	N	%		
Heterozygous (CT)	48	48	16	80	6.0	<0.05
Homozygous (TT)	12	12	4	20		
Wild type (CC)	40	40	0	0		

$p<0.05$ : Significant.

Comparative statistics between MTHFR gene polymorphism and smoking, drug abuse and family history in Group I was done. No statistically significant difference was detected among studied parameters ( $\chi^2: 1.12$  and  $1.81$  and  $0.94$  respectively and  $p>0.05$ ).

The analgesic over use for treatment of headache (no of analgesic tablets per month >15 tablet) was compared with genotypes of MTHFR gene in Group I and revealed non-significant statistical difference ( $\chi^2: 1.86, p>0.05$ ).



Comparative statistics between MTHFR gene polymorphism and location of migraine (whether unilateral or bilateral and orbital or retro-orbital) in Group I revealed no statistically significant difference either for neither unilateral or bilateral location nor orbital or retro-orbital migraine ( $\chi^2$ : 1.30 and 1.875 respectively and  $p>0.05$ ). Meanwhile, Table (7) shows significant statistical difference detected between MTHFR gene polymorphism and frontal, temporal and vertex location ( $\chi^2$ :10.11,  $p<0.05$ ).

Table (7): Comparative statistics between MTHFR Gene Polymorphism and location (Frontal, Temporal or Vertex) in Group I (patients) using Pearson Chi Square Test.

	Location						$\chi^2$	<i>p</i>
	Frontal		Temporal		Vertex			
	N	%	N	%	N	%		
Heterozygous (CT)	4	100	60	55.6	0	0	10.11	<0.05
Homozygous (TT)	0	0	16	14.8	0	0		
Wild type (CC)	0	0	32	29.6	8	100		

$p<0.05$ : Significant.

Statistical comparison was done between MTHFR gene polymorphism and types of migraine which was classified into episodic and chronic according to number of attacks (more than fifteen per month considered chronic and less than fifteen per month considered episodic) and showed non-significant difference ( $\chi^2$ :1.46,  $p>0.05$ ). Similarly, comparison between MTHFR gene polymorphism and the duration of attack of migraine showed no statistically significant difference ( $\chi^2$ :3.37,  $p>0.05$ ).

Meanwhile, as seen in (Table 8), comparison between MTHFR gene polymorphism and the severity of attack of migraine (moderate and severe) showed statistically significant difference and all homozygous genotype (TT) were severe cases ( $\chi^2$ : 8.57,  $p<0.05$ ).

Table (8): Comparative statistics between MTHFR Gene Polymorphism and disease severity (moderate and sever) in Group I (patients) using Pearson Chi Square Test.

	Severity				$\chi^2$	<i>p</i>
	Moderate		Severe			
	N	%	N	%		
Heterozygous (CT)	32	50	32	50	8.57	<0.05
Homozygous (TT)	0	0	16	100		
Wild type (CC)	24	60	16	40		

$p<0.05$ : Significant.

In (Tables 9-11) highly statistically significant difference was revealed when comparative statistics was done between genotypes of MTHFR gene and aura, photophobia and phonophobia in Group I ( $\chi^2$ : 18.90, 17.50, 14.30 respectively and  $p<0.01$ ). Patients with homozygous type (TT) showed positive significant risk to develop aura (50%),  $p<0.001$ , Odd's Ratio=45.0, 95%CI (5.26-385.19) while patients with heterozygous type (CT) showed positive significant risk to have attacks of migraine associated with photophobia (58.3%),  $p<0.001$ , Odd's Ratio=21.0, 95%CI (3.10-142.21) or phonophobia (56.5%),  $p<0.001$ , Odd's Ratio=13.0, 95%CI (2.09-81.05) or both.

No statistically significant differences were found between MTHFR genotypes and vomiting, relation to sleep (awake patient from sleep) and trigger factors of migraine ( $\chi^2$ :1.0, 3.01 and 5.78 respectively and  $p>0.05$ ).

Table (9): Comparative statistics between MTHFR Gene Polymorphism and aura in Group I (patients) using Pearson Chi Square Test.

	Aura				$\chi^2$	<i>p</i>
	No		Yes			
	N	%	N	%		
Heterozygous (CT)	60	62.5	4	16.7	18.90	<0.01
Homozygous (TT)	4	4.2	12	50		
Wild type (CC)	32	33.3	18	33.3		

$p<0.01$ : Highly significant.

Table (10): Comparative statistics between MTHFR Gene Polymorphism and Photophobia in Group I (patients) using Pearson Chi Square Test.

	Photophobia				$\chi^2$	<i>p</i>
	No		Yes			
	N	%	N	%		
Heterozygous (CT)	8	33.3	56	58.3	17.50	<0.01
Homozygous (TT)	12	50	4	4.2		
Wild type (CC)	4	16.7	36	37.5		

$p<0.01$ : Highly significant.

Table (11): Comparative statistics between MTHFR Gene Polymorphism and Phonophobia in Group I (patients) using Pearson Chi Square Test.

	Phonophobia				$\chi^2$	<i>p</i>
	No		Yes			
	N	%	N	%		
Heterozygous (CT)	12	42.9	52	56.5	14.30	<0.01
Homozygous (TT)	12	42.9	4	4.3		
Wild type (CC)	4	14.2	36	39.1		

$p<0.01$ : Highly significant.

## Discussion

Migraine is a complex disorder associated with both genetic susceptibility and environmental triggers that play a role in the pathogenesis of the disease [13]. Many genes have been considered as predisposing factors in initiating migraine attacks mainly in migraine with aura (MA) than in migraine without aura (MO) [14]. MTHFR deficiency is reported as the most common genetic cause of elevated levels of homocysteine in the plasma and subsequent hyperhomocysteinemia leading to endothelial cell injury, spontaneous trigeminal cell firing, and alterations in coagulant properties of blood. Spontaneous trigeminal cell firing leading to inflammation in the meninges and dilation of cerebral vessels is thought in part to cause the pain associated with migraine [15]. In the present study, the association between the MTHFR gene polymorphism and migraine with its different types was investigated in migraine patients.

Our study revealed that MTHFR gene polymorphism distribution in migraine patients was predominantly of heterozygous (CT) genotype (53.3%), while homozygous (TT) genotype was (13.3%) and wild (CC) genotype was (33.3%). Meanwhile, in control group, heterozygous genotype (CT) was (13.3%), homozygous genotype (TT) was (20%) and wild genotype (CC) was (66.7%). MTHFR 677CT gene polymorphism was significantly higher in our migraine group compared with control group strongly enforcing their association on a genetic background. Similarly, the study done by Bahadir et al., included 150 migraine patients with and without aura (MA and MO) and 107 non-sufferers in Turkish population revealed that the MTHFR 677CT genotype was significantly higher in their migraine group [16]. These results basically goes in agreement with the results of the study conducted by An et al., on Chinese population which included 151 migraine patients and 137 age- and sex-matched controls group [17]. Their study also showed significant difference between MTHFR gene polymorphism and migraine. Meta-analysis on 17 studies with 8903 cases and 27637 controls showed that the allele 677T was associated with a significantly increased risk of migraine in Asians [18].

A study done on the Portuguese population reported no significant difference between the frequencies of MTHFR 677CT genotypes and the T-allele among the Portuguese migraineurs when compared to the controls [19]. Interestingly, the difference in association between MTHFR 677CT genotypes in migraine and allele frequencies in

different study populations may be related to linkage disequilibrium. The non random association of alleles in linkage disequilibrium at different loci in different population may have a role in increasing or decreasing the frequency of the association other than the expected.

Results of our study revealed a significant increased T allele frequency of the 677CT polymorphism in patients Group I (40%) compared to the control Group II (26.67%) ( $p < 0.05$ ), OR=6.0, 95% CI (1.84-19.59). Supporting results on Iranian population were recently published by Salehi et al., who stated that for the 677CT polymorphism, the frequency of the T allele was significantly higher in the case group compared to their controls ( $p = 0.02$ ; OR=0.57; 95% CI, 0.34-0.94). Moreover, they reported a significant difference under dominant model observed between their studied case and control groups ( $p = 0.007$ ; OR=0.60; 95% CI, 0.41-0.87) [9].

Migraine severity may be a useful parameter to further sub-classify migraine patients into more homogeneous categories and can for example be measured by attack frequency, in addition to aura status. In our study we investigated the relation between genotypes of MTHFR 677CT polymorphism and clinical parameters of migraine; such as associated symptoms (nausea, vomiting, photophobia and phonophobia), aura, triggers of migraine, location of pain, severity and duration of attack, relation to sleep (awake patients from sleep) and types of migraine (episodic or chronic).

The highly significant difference between photophobia, phonophobia and aura and MTHFR gene polymorphism and the significant difference as regards nausea and migraine location (frontal, temporal and vertex) and attack severity seen in results of our study links MTHFR 677CT polymorphism with many of the preceding and concomitant events during migraine attacks. Heterozygous type (CT) was higher in patients who suffer from photophobia, phonophobia, nausea and frontal location (58.3%, 56.5%, 68.8% and 100%) respectively. As regards aura symptoms, homozygous type (TT) (50%) was higher in patients who suffer from aura symptoms, while heterozygous type (CT) (62.5%) was higher in patients who didn't suffer from aura.

A study conducted by Kara et al., on patients suffering from migraine with aura (MA) concluded that the homozygous mutation (TT) is associated with MA in patients, group [20]. This finding is also supported by many other studies that confirmed the significant association between the homozygous (TT) genotype and MA [6,21,22].

According to the study done by An et al., T allele frequency was significantly higher in migraine without aura (MO) than in controls [17]. Similarly to Scher et al., who stated that the TT genotype was marginally protective for MA [23].

In our study, other clinical parameters such as location of pain, duration of attack, relation to sleep (awake patients from sleep), migraine triggering factors and types of migraine (episodic or chronic) had no significant association between them and MTHFR gene polymorphism.

In contrast to our study, Azimova et al., stated that patients with the homozygous (TT) or heterozygous (CT) genotypes were significantly more sensitive to migraine attack triggers compared to wild (CC) genotype patients and that homozygous genotype (TT) had higher rates of associated symptoms [24]. In a study done by Bahadir et al., heterozygous (CT) frequency was higher in patients who suffer from compression, allodynia, fatigue, and sleeplessness [16].

Regarding age, sex and family history in our study, there was a non-significance difference between age and family history and MTHFR gene polymorphism. Meanwhile, a significant difference is seen between female sex representing 83.3% of cases enrolled in our study and MTHFR gene polymorphism. Female heterozygous (CT) genotype (48%) was more susceptible to have migraine than the homozygous (TT) and wild (CC) genotypes (12% and 40% respectively).

Bahadir et al., demonstrated that CT genotype frequency of individuals with a family history of migraine was significantly higher [16]. Liu et al., in showed that the effect of MTHFR gene polymorphism on the clinical picture of migraine was different between males and females. Male patients with the homozygous (TT) genotype developed bilateral headache more frequently compared to females and female patients with the heterozygous (CT) genotype were more prone to develop attacks of migraine with associated symptoms, such as nausea and vomiting [25].

The hypothesis of introducing treatment strategies capable of interrupting chronic disease and lead to the substitution or integration of today's predominantly symptomatic therapies would imply a dramatic improvement in the quality of life of patient [26]. Unfortunately, our study could not prove the association between MTHFR 677CT gene polymorphism and dose of analgesics used in treatment of migraine or possible resistant to

therapy with different genotypes, a point which requires additional efforts to investigate.

#### *Conclusion:*

In conclusion, this study has demonstrated a significant association between MTHFR gene polymorphism (677CT) and migraine in Egyptian population. Patients with homozygous type (TT) were more susceptible to aura while patients with heterozygous type (CT) were more susceptible to attack of migraine associated with photophobia or phonophobia or both. A limiting factor in this study was the relatively small number of the sample due to cost constraints. Further studies at a larger scale are needed to build up on our findings with subsequent gene sequencing and concomitant measurement of homocysteine plasma level.

#### *Article highlights:*

- There is a significant association between MTHFR gene polymorphism (677CT) and migraine occurrence and severity in Egyptian patients.
- Measurements of homocysteine plasma level hand in hand with gene study might help clinicians putting in consideration that supplementation with folic acid, B6, and B12 can decrease homocysteine level and subsequently decrease the frequency and severity of migraines.

#### *Declaration:*

All authors declare that there is no financial or institutional conflict of interest.

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## المتغير الجيني 667CT لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي وزيادة القابلية للصداع النصفي في المصريين: دراسة حالة-مراقبة

خلفية: الصداع النصفي هو حالة إضطراب وعائى عصبى متكرر يقلل من جودة الحياة. والنساء فى منتصف العمر هن أكثر عرضه للصداع النصفي من الرجال. قد يكون الصداع النصفي مصحوباً بأعراض مرضية (MA) أو الصداع النصفي بدون أعراض مرضية مصاحبة (MO). حققت الدراسات الوراثية الجزيئية للصداع النصفي فى العديد من أشكال التغيرات الجينية التى ربما ترتبط بالصداع النصفي خاصة A1298C و rs4846049. ويعد المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي واحد من أهم التغيرات الجينية المقترحة التى تؤثر على الصداع النصفي.

الهدف: تهدف هذه الدراسة إلى تقييم دور المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي فى مرض الصداع النصفي بنوعيه فى المصريين وعلاقته بالأعراض التى تظهر معه وإيجاد صلة محتملة بينه وبين فعالية أو مقاومة المرض لبروتوكولات العلاج.

المواد وطرق البحث: أجريت هذه الدراسة على (١٨٠) شخصاً تم تقسيمهم إلى مجموعتين: مجموعة أولى (مجموعة المرضى) شملت (١٢٠) مريض بالغ مصاب بالصداع النصفي تم تشخيصهم بعبادة الصداع فى مستشفيات جامعة عين شمس وتم تشخيص إصابتهم بالصداع النصفي وفقاً لمعايير تشخيص ICHD، الإصدار التجريبي من الإصدار الثالث (٢٠١٣)، أما المجموعة الثانية (المجموعة الضابطة) والتى تضمنت (٦٠) من البالغين الأصحاء متطابقين فى العمر والجنس مع المجموعة الأولى. تم تقييم المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي باستخدام تقنية التفاعل البلمرى التسلسلى بطول الجزء المقيد (RFLP-PCR).

النتائج: أظهرت الدراسة وجود علاقة كبيرة بين المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي والصداع النصفي ( $p < 0.001$ )، نسبة الحدوث (OR=6.0، 95%CI: 1.84-19.59). كان كل من الإناث مختلفه الزيجوت (CT) والإناث متجانسة الزيجوت (TT) أكثر عرضه بشكل ملحوظ لتطور الصداع النصفي من كل من الذكور مختلفه الزيجوت (CT) والذكور متجانسة الزيجوت (TT) ( $X^2: 12.48$ ) ( $p < 0.05$ ). أظهر المرضى الذين يعانون من نوع متماثل الزيجوت (TT) خطراً إيجابياً كبيراً لحدوث الأعراض المصاحبة (50%) ( $p < 0.001$ ) ( $OR=45.0$ ، 95%CI: 5.26-385.19) بينما أظهر المرضى الذين يعانون من نوع مختلف الزيجوت (CT) إيجابية خطر كبير أن يكون لديهم نوبات من الصداع النصفي المرتبط بالخوف من الضوء (58.3%) ( $p < 0.001$ ) ( $OR=21.0$ ، 95%CI: 3.10-142.21) أو الخوف من الصوت (56.5%) ( $p < 0.001$ ) ( $OR=13.0$ ، 95%CI: 2.09-81.05) أو كلاهما. كما لوحظ وجود فرق إحصائى كبير فى المقارنة بين المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي وشدة نوبة الصداع النصفي ( $X^2: 8.57$ ،  $p < 0.05$ )، فى حين لم يلاحظ أى علاقة بين المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي وجرعة العلاج المسكن ( $X^2: 1.86$ ،  $p > 0.05$ ).

الخلاصة: توضح الدراسة وجود علاقة قوية بين المتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي وحدوث الصداع النصفي والأعراض التى تسبقه والمصاحبه له فى المصريين، وشدة نوبة الصداع وزيادة خطر حدوث المرض. لسوء الحظ، لم تجد الدراسة أى ارتباط إحصائى بين النمتغير الجيني 677C/T لجين الإختزال المثيلي لحمض الفوليك الرباعي المائي مع جرعة علاج مسكن.