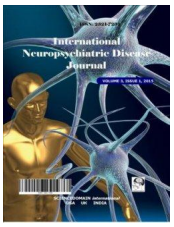

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C677T Methylenetetrahydrofolate Reductase Homozygosis and Vitamin Supplement in Migraineur Children

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Authors' contributions

This work was carried out in collaboration among all authors. Author ET designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SN performed the statistical analysis. Author AO managed the analyses of the study. Authors MM and GI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: Methylenetetrahydrofolate reductase (MTHFR) variant C677T has been associated with an increased genetic risk in migraine susceptibility. Individuals with the homozygous genotype show higher levels of plasma homocysteine (Hcy) [1,2,3].
Methods: Open-label study evaluating clinical trial was performed on 148 children migraineurs among 236 children suffering from headache, admitted to the Headache Regional Centre in l'Aquila (Italy)-Neuropsychiatric clinic, recruited and observed sequentially during the years 2015-2017. 31 patients (16,6% of all the sample) MTHFR homozygous were selected and underwent clinical evaluation of migraine characteristics (frequency, severity of pain and use of acute treatment) at baseline and after a 12 month-period of daily supplement of vitamins B9(2 mg), B6(25 mg),

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B12(400 mcg). In 20 patients with hyper Hcy (>95° percentile for age) the second collection of blood sample was obtained for Hcy assessment. Diagnosis of headache according to ICHD-III criteria. Statistical analysis is made by Wilcoxon test.

Results: 20 children suffer from Migraine without aura (MwoA) and 11 from Migraine with aura (MA). 86% of children aged >10 years had significantly higher Hcy values compared with controls ($p<0.0001$). 21 patients (70%), 14 F and 7 M (13 MwoA and 8 MA), have Hcy values > 95°percentile. In all the sample the vitamin supplementation was effective in producing significant reduction in Hcy levels ($p=0.0001$) and a significant improvement of migraine disability of frequency ($p<0.0001$), severity of pain ($p<0.0003$) and use of acute treatment ($p<0.0001$).

Conclusions: The study results are encouraging and emphasize the importance of a comprehensive therapy in paediatric migraines.

Keywords: Headache; children; genetic; homocysteine.

1. INTRODUCTION

Migraine is an extremely debilitating and highly prevalent neurovascular disorder presenting multiple symptoms. The methylenetetrahydrofolate reductase (MTHFR) gene variant C677T has been implicated as a genetic risk factor in migraine susceptibility, particularly in Migraine with Aura (MA) [4,5,6]. The MTHFR gene is located on chromosome 1p36.3 and encodes for a key enzyme for the metabolism of folate and homocysteine [7]. C677T polymorphism is characterized by a substitution of an alanine with a valine at position 222; this substitution leads to a reduction on enzyme activity [8,9]. Subjects with the C677T variant present reduced capacity to remethylate Homocystein to methionine, these conditions increase Homocystein plasma levels [9]. Individuals with the homozygous (TT) state for this mutation showed higher levels of plasma homocysteine [7]. Homocysteine acts as an excitatory amino acid and may influence the threshold for migraine headache. High levels of homocysteine are associated with vascular and endothelial damage and with cerebrovascular and cardiovascular disease; in particular, it has been assumed that hyperhomocysteinemia (HyperHcyA) is involved in reducing brain blood flow and producing the depolarization wave defined as CSD [10]. These facts associated with reduced oxygen transfer could act as trigger of migraine attack. The endothelial damage induced by high levels of homocysteine could decrease nitric oxide release and leads to the initiation and maintenance of migraine attacks [11]. High homocysteine levels are seen in individuals with migraine with aura [12,13]. A poor number of studies have found a significant association between the TT genotype and migraine [14,1]; especially MA [2] inconsistently [8,15]. The MTHFR C677T polymorphism has been reported to be associated with migraine and depression [16]; while other studies failed to show this association. Our results in previous study confirmed available data of literature [6]. In fact, the migraineurs had higher prevalence of MTHFR mutation, especially in homozygous with respect to tension type headache [15,13]. According to Lea [17] and other Authors [9,14], C677T MTHFR variant predisposes to a susceptibility to migraine with aura and not to that without aura, in particular, it is well- known for European Caucasian population. A meta-analysis of 15 case control studies showed a close correlation between TT variant and Caucasians suffering from MA, in non Caucasian population this genotype is associated with total migraine. The C677T allele (rs1801133), a common variant of the MTHFR gene has a frequency of approximately 23-41% in the Caucasian population [18,7,19]. The data of Italy population show mutation in homozygous in 8.8-10% of people [12]. Many authors have demonstred that the MA and MwoA have many diagnostic characteristics in common but in children aren't studies controlled of large study samples [9,3]. Individuals homozygous for this variant express approximately 30% of the mean activity of MTHFR enzyme levels, as compared with individuals without the substitution allele. Aim of this study is to verify if the homozygous genotype of allele CT of MTHFR determine the clinical variants of Migraine with and without aura (MwoA and MA) and if supplement of vitamin complex of VitB9, Vit B6 and Vit B12 effects the improvement of headache as intensity as frequency of attacks [9,20].

2. METHODS

The study population was enrolled in the Department of Neuropsychiatric Clinic affering to Regional Headache Center of Region Abruzzo,

University of L'Aquila. The patients were recruited according to temporal criteria by observations sequentially during the years 2015 and 2016 and 2017. The children are born in Italy from italian parents. The diagnosis of headache was made according to ICHD-III criteria [21]. The sample of study consists of 236 patients, 121 females and 115 males, aged between 8 to 13 years. Whole blood samples were collected using EDTA tubes and transferred in an upright position to local lab, where were stored at -20°C to freezer. During patient diagnostic evaluation we detected C677T variant of MTHFR based on the RealTime Polymerase Chain Reaction (RT-PCR), we considered the homozygous and heterozygous genotype for T allele. The polymorphism C677T was identified using SAMPLE PREP thrombo kit (Diatech Pharmacogenetics), using a TAQ man probe and a melting analysis to determinate genotypes [7]. The total homocysteine (tHcY) was determinate using a chemiluminescent immunoenzymatic assay (CMIA) homocysteine Architect [22]. 31 patients, aging from 6 to 17 years, 11 Males and 20 Females, with MTHFR homozygous genotype, were selected and underwent clinical evaluation of migraine characteristics. The following migraine parameters were considered: headache frequency expressed as number of days by month, pain intensity with the VAS scale (0-10) and use of drugs (number of administrations for month) at baseline and after a 12 month-period of daily supplement of vitamins B9(2 mg), B6(25 mg), B12(400 mcg) and homocysteine values. We compared homocysteine values to normal values found in a control sample of 30 children of same age and compared to literature data in a school age population [23]. Furthermore, homocysteine values were compared with those of heterozygous subjects. Clinical and laboratory data were divided according to the diagnosis of migraine. In 20 children suffer from MwoA and 11 from MA with hyperHcyA (>95° percentile for age) the second collection of blood sample was obtained for Hcy assessment. Diagnosis of headache was made according to ICHD-III criteria. Informed consent was obtained for participation in the study by the parents of the children. For the comparison of data referring to the characteristics of the headache (frequency, pain and use of the drugs) a repeated measures ANOVA on logtransformed data. Statistical analysis is made by Wilcoxon test (ANOVA).

3. RESULTS

96 patients are suffering from MwoA, 39 from MA, 13 from Chronic Migraine. The heterozygous genotype was present in 88% of sample, 50 (56%) females and 47 (60%) males, whereas the homozygous genotype in 31 patients (16,6%), 19 females and 12 males. Table 1 summarizes the clinical data of each of 31 patients homozygous type at time 0 and after 12 months of follow up. The diagnosis of headache, according to ICHD-III beta version 2013, is Chronic migraine in 11 pt, MwoA in 9 pt and MA in 11 patients. The vitamins supplement is efficacy to reduce values of homocysteine and to improve frequency, pain and disability of migraine. Fig.1 shows the results of statistical processing of frequency of headache. In fact the chronic type of migraine disappears and the intensity of pain is reduced by 80 percent of the cases. In Fig. 2 there are the results of pain intensity and in Fig. 3 the results of drug use of the whole sample, pre and post vitamins treatment.

There is a very significant difference in all parameters considered after 12 months of follow-up, while there is no difference in behaviour between the 2 types of migraine. The homocysteine values of all the sample is summarized in Fig. 4. Values are grouped according to the age of the children (> or < 10 years).It is noted that homozygous homocysteine values increase with age, unlike controls in which the increase with age is poor. 21 patients (70%), 14 females and 7 males have Hcy values >95° percentile. The values over 95° for age and the mean and SD compared to normal controls are 12,62 ± 6,86 (μM/l) vs 5,41 ±1,17 ($p<0,001$).

Among the cohort of children with hyperHcyA the diagnosis is: MwoA in 13 and MA in 8 patients. The values of Hcy after 12 months of vitamin supplement are reduced in the normal range for age. In all the sample the vitamin supplementation was effective in producing significant reduction in Hcy levels ($p=0.0001$) (Fig. 5) and a significant improvement of migraine disability in terms of frequency ($p<0.0001$) (Fig. 1), severity of pain ($p<0.0003$) (Fig. 2) and use of acute treatment ($p<0.0001$) (Fig. 3).

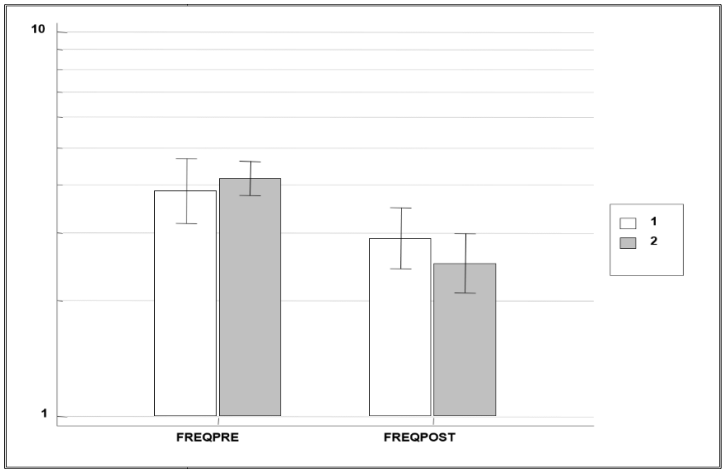

Fig. 1. Frequency of Migraine* attacks Pre-Post Vitamins treatment (p<0.0001)
**Migraine diagnosis. 1: MwoA; 2: MA*

Table 1. Clinical data of each patient at time 0 and after 12 months of follow up

Patients	Years	Sex	Migraine Diagnosis	Frequency*		Pain Severity**		Drugs	
				Pre	Post	Pre	Post	Pre	Post
1.	7	F	MwoA	3	2	2	2	2	1
2.	8,1	F	MA	17	7	3	2	10	4
3.	16,8	F	MwoA	15	10	2	1	9	3
4.	7,3	F	MwoA	6	4	2	2	4	2
5.	8,6	M	MwoA	20	11	3	1	11	5
6.	8,1	M	MwoA	18	5	3	2	3	0
7.	8,1	M	MwoA	11	6	1	1	2	0
8.	15,2	F	MwoA	13	5	2	2	3	0
9.	17,1	F	MA	4	2	2	2	4	1
10.	15,7	F	MA	5	2	3	1	3	0
11.	9,11	M	MwoA	3	1	4	3	3	0
12.	10,6	M	MwoA	14	5	3	2	7	0
13.	17,9	F	MwoA	7	4	3	3	5	3
14.	8,11	F	MA	20	8	2	2	12	6
15.	10	M	MwoA	5	4	2	2	4	1
16.	15,7	F	MwoA	11	4	2	2	5	3
17.	9,05	F	MwoA	16	4	3	2	9	0
18.	12,8	F	MwoA	7	2	2	2	5	2
19.	16,6	M	MA	8	5	2	1	7	0
20.	12,1	F	MwoA	2	1	2	2	2	1
21.	11,1	M	MwoA	5	2	2	2	3	0
22.	4,5	F	MA	4	2	2	1	3	0
23.	6,5	F	MwoA	3	1	2	2	2	0
24.	14,6	F	MwoA	17	2	3	2	7	0
25.	16,3	F	MA	2	1	3	2	1	0
26.	13,7	M	MwoA	21	11	3	1	9	2
27.	15,9	M	MA	12	7	2	1	7	4
28.	12,2	M	MA	19	8	2	3	13	4
29.	14	F	MwoA	16	7	3	3	12	3
30.	8	F	MA	16	7	2	3	9	2
31.	8,6	F	MA	8	4	4	4	0	0

**Frequency: headache days per month*
***Pain Severity. 1: low, 2: mild, 3: high, 4: very-high*

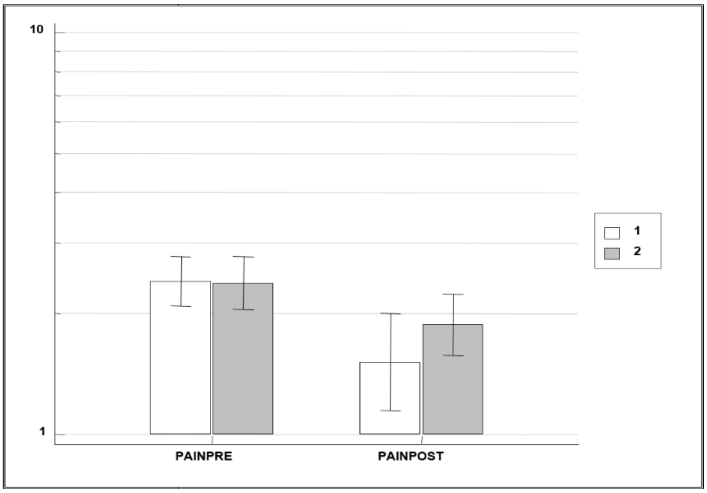

Fig. 2. Pain of Migraine*attacks Pre-Post Vitamins treatment (p<0.0003)
**Migraine diagnosis 1: MwoA; 2: MA*

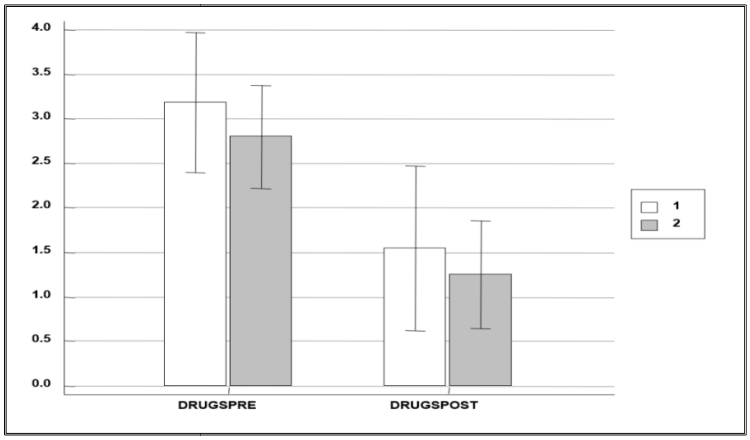

Fig. 3. Drugs of Migraine*attacks Pre-Post Vitamins treatment (p<0.0001).
**Migraine diagnosis. 1: MwoA; 2: MA*

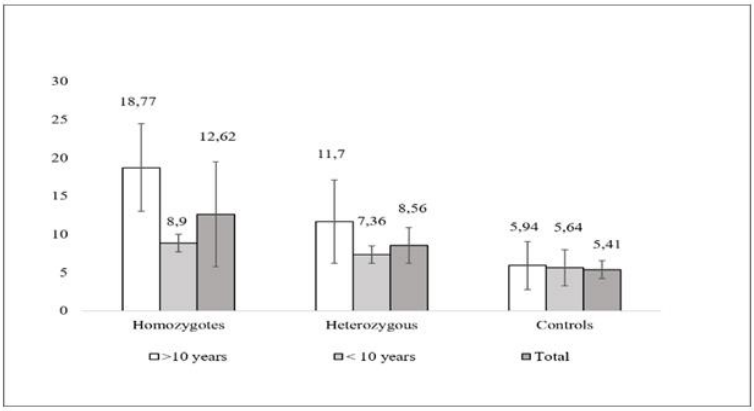

Fig. 4. Homocysteine values of all the sample on the basis of age

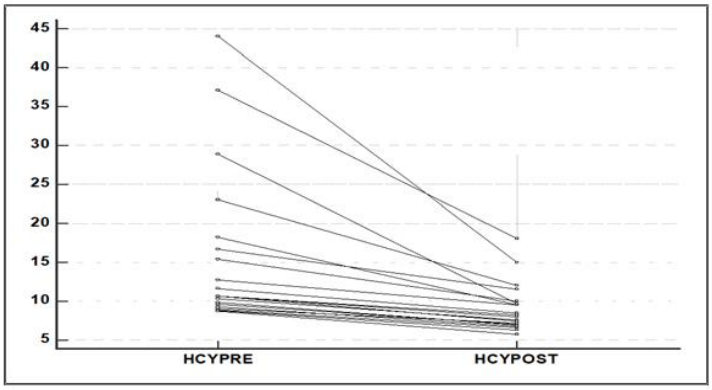

Fig. 5. Values of Hcy (μM/l) after 12 months of Vitamin supplement (p=0.0001)

4. DISCUSSION

Our data show that the heterozygous genotype was presented in 88% of migraineurs whereas the homozygous genotype in 16,6% of patients. Higher values than the general population, as already demonstrated in a previous study [6]. The data of Italy population show mutation in homozygous in 8,8-10% of people [12]. The observational literature suggests a consistent relationship between aspects of brain function and folate/B12 and/or homocysteine, a huge research effort predicated on the hypothesis that supplementation with these vitamins should decrease homocysteine levels and thereby either improve cognitive function or attenuate cognitive decline and the risk of dementia has generated largely equivocal results [24,19,10]. Indeed, reviews and meta-analyses published over more than a decade have provided scant evidence to support this hypothesis [25,9,26,27,28]. Reasons for hyperHcyA may include mutations in homocysteine metabolizing genes such as MTHFR, Methionine synthase reductase (MTRR), and cystathionin beta synthase (CBS), or possible nutritional deficiencies in cofactors involved in homocysteine metabolism. Folate is needed to drive the methionine synthesis pathway, as a lack of dietary folate and/or reduced MTHFR enzymatic activity can result in increased homocysteine levels in blood plasma hyperHcyA is caused by abnormal methionine biosynthesis due to deficiencies in folate, vitamin B12, and vitamin B6 [29]. High levels of homocysteine are associated with vascular and endothelial damage and with cerebrovascular and cardiovascular disease; in particular, it has been assumed that hyperHcyA is involved in reducing brain blood flow and producing the depolarization wave defined as cerebral spreading depression (CSD). These facts associated with reduced oxygen transfer could act as trigger of migraine attack. The endothelial damage induced by high levels of homocysteine could decrease nitric oxide release and leads to the initiation and maintenance of migraine attacks [30,31,9,32]. Naturally occurring folate found in food is present in the reduced, polyglutamated form with methyl or formyl as the one carbon substitution. FA found in fortified food and supplements are a synthetic, fully oxidized monoglutamate form of folate and is reduced to tetrahydrofolate prior to its participation in a metabolic reaction. Relative to naturally found folate, FA has a higher bioavailability, which is defined as the proportion of ingested folate that is absorbed and can be used for metabolic processes. Although there is a broad consensus that FA is more bioavailable than naturally found food folate, there are some intervention trials with food that have reported similar improvements in folate status compared with equimolar or small doses of FA from fortified foods or supplements [9,3,11,32]. Previous clinical trials by Lea and Menon [17,11] have provided evidence that homocysteine lowering by FA supplementation can reduce migraine disability, and that this effect may be modified by polymorphisms in genes coding for key enzymes that affect folate metabolism, such as the MTHFR genotype. T allele carriers of the MTHFR C677T genotype with a 3570% reduction in their enzymatic rate are genetically slower in homocysteine metabolism. The Author demonstred that an increase in dietary folate, especially the more bioavailable FA consumption levels, may effectively reduce migraine frequency in female migraineurs. If this is a causative relationship, then the T allele carriers of the MTHFR C677T variant may need higher levels of FA compared

with CC genotype carriers to experience a significant reduction in their migraine frequency. According to Lea data, we toke the dose of vitamins B9(2 mg), B6(25 mg), B12(400 mcg) and our results are good and encourage us to undertake this therapeutic procedure. The results demonstrate the effectiveness of the vitamin supplement on both clinical and laboratory parameters.

5. CONCLUSIONS

Our opinion is that the increase of homocysteine with age in homozygous type unlike heterozygous type and in controls in which this increase is very reduced, represents a very important observation. It occurs, that is, a sort of homocysteine accumulation with age and this affects both the brain and the progression of migraine. The data in paediatric literature are few and there are not longitudinal studies of migraineurs children treated compared to which one non treated. The migraine represent a disease with more comorbidities as cerebrovascular diseases, cognitive disabilities and attention deficit [24,30,10,3,28]. Setting up a therapeutic program must go beyond immediate response to a drug and immediate therapeutic success. Therefore, in the developmental age, the prevention of comorbidities is an important baggage to be dealt with correct.

CONSENT

Informed consent was obtained for participation in the study by the parents of the children.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Elisabetta Tozzi, Agnese Onofri, Giulia Iapadre, Martina Mazzilli, Stefano Necozione declare that they have no conflict of interests regarding the publication of this paper.

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