https://doi.org/10.48047/AFJBS.6.15.2024.14850-14861

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Genetic Polymorphism of Methylenetetrahydrofolate Reductase and Myocardial Infarction In Young Patients From Aurès Region In Algeria

Djaara Hayat*,[1,2], Boussif Abdelali [3,4]

[1] Laboratory of Biotechnology of Bioactive Molecules and Cellular Physiopathology (LBMBPC), Faculty of Natural and Life Sciences, University of Batna 2, Fesdis, Batna 05078, Algeria.
[2]Department of Biology of organisms, Faculty of Natural and Life Sciences, University of Batna 2, Fesdis, Batna 05078, Algeria.
[3]Department of Microbiology and Biochemistry, Faculty of Natural and Life Sciences, University of Batna 2, Fesdis, Batna 05078, Algeria.
[4]Laboratory of Applied Biochemistry, University Setif-1- Ferahat Abbas, Setif, Algeria.

***Corresponding Email : h.djaara@univ-batna2**

Volume 6, Issue 15, Oct 2024

Received: 15 Aug 2024

Accepted: 25 Sep 2024

Published: 21 Oct 2024

doi: 10.48047/AFJBS.6.15.2024.14850-14861

Abstract

The aim of this molecular study was to evaluate a possible association between hyperhomocysteinemia and the different genotypes of the C677T polymorphism of MTHFR gene, based on clinical and biochemical data, in pathological population, residing in Aurès region (Northeast Algeria). The present study included 14 young patients of rare age group, all patients were in the chronic phase of myocardial infarction (MI). Competitive immunoassay was used for the determination of vitamins (B9 and B12) and total homocysteine and the identification of MTHFR polymorphism was determined by Real-Time Polymerase Chain Reaction - Fluorescence Resonance Energy Transfer (Real-Time PCR -FRET). Results showed a non-significant association of moderate hyperhomocysteinemia (Hhcy) with the different genotypes of the C677T polymorphism (P = 0.117). However, a decrease in folate and B12 was recorded with moderate Hhcy for (TT) genotype but remains non-significant (P = 0.901, P = 0.304 respectively). No significant differences in the clinical and demographic patient characteristics according to the different genotypes of MTHFR C677T plymorphism were revealed. Same results were observed for the majority of biochemical variables. This is probably related in part to coronary patients who are in the chronic stage of the disease. Our study supports the hypothesis indicating that the polymorphism C677T MTHFR could be considered an important risk factor for MI, in the presence of moderate hyperhomocysteinemia. We suggest a strategy for the biological exploration of moderate or intermediate Hhcy, for informing the general public in our region (Aurès) and country face the risk of the evolution of this symptomatology.

Keywords: Myocardial infarction (MI), MTHFR C677T polymorphism, Hyperhomocysteinemia , RT-PCR-FRET®480II, Aurès, Algeria.

1. Introduction

Myocardial infarction (MI) is regarded as a polygenic disease with high mortality rate that results from the mutual action of environmental and genetic factors. There is growing interest in the importance of elevated plasma total homocysteine (tHcy) levels as a risk factor for coronary atherosclerotic disease (CAD).

Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine (Brustolin et al., 2010). Methionin is metabolised via re-methylation and transsulphuration pathway in which B-vitamins; folate, vitamin B12, vitamin B6 and vitamin B2 are required as cofactors (Hiraoka and Kagawa, 2017). Genetic defects of the enzymes or dietary deficiency of B vitamin cofactors involved in this metabolism result in elevated homocysteine levels.

The 5,10-methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes of Hcy metabolism (Frosst et al., 1995; Klerk et al., 2002), this enzyme catalyzes irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which acts as methyl donor for methionine synthesis from homocysteine (remethylation) (Goyette et al., 1998).The C-to-T transition at nucleotide 677, in exon 4 of MTHFR gene, is a point mutation that converts a cytosine (C) to a thymine (T) (Frosst et al., 1995), which converts an alanine to a valine codon in the N-terminal catalytic domain of the enzyme (Frosst et al., 1995; Klerk et al., 2002). This mutation renders the enzyme thermolabile and less active (Frosst et al., 1995) leading to increased plasma tHcy in association with low plasma folate (Frosst et al.,1995;Varga et al., 2005).

The MTHFR C677T polymorphism (rs1801133) is common (T-allele frequency 15 - 45%) in many populations and reduces enzyme efficiency (Clarke et al., 2012). In many populations without folic acid fortification, individuals with TT genotype had clearly higher plasma homocysteine levels (about 20%) than those with CC genotype (Jacques et al., 1996; Davey Smith and Ebrahim, 2003). However, conflicting results have been obtained with respect to the potential association of the MTHFR C677T gene variation with coronary artery disease and myocardial infarction.

Numerous studies have reported the relations ship between the MTHFR gene C677T polymorphism and risk of MI, it presents a heterogeneous worldwide distribution, and its frequency shows great ethnic and geographic variations. We previously studied the genotypic and allelic frequency of this polymorphism in healthy subjects of Aures region (Djaara et al., 2018). However, till present no study is carried out on young coronary patients who have suffered from a myocardial infarction in the *Aurès* region of Algeria, to determine the genotypic and allelic frequency of this polymorphism and their effect on the biochemical profile. This first study concerned sick subjects with coronary atherosclerosis and all the participants were among the inhabitants of the *Aurès* region (north-eastern Algeria).

The aim of our molecular study is to examine a possible association between hyperhomocysteinemia and the different genotypes of the polymorphism C677T of MTHFR gene, based on clinical and biochemical data, in young Algerian subjects with myocardial infarction in *Aurès* region, which remains unknown.

2. Materials and Methodes

Patients

A total of fourteen sick subjects, were volunteered to participate in this study (13 males and 01 female living in *Aurès* region), and were recruited, after obtaining their informed consent. The mean age of the

subjects studied was 33.35 ± 6.35 years, range [14-40 years, ≤40 years], a very rare cases of patients. Subjects of both sexes were from different localities of *Aurès* (Batna, Khenchela, Oum El Bouaghi...). All these patients had coronary atherosclerosis (chronic phase), and have been diagnosed by cardiologists in the cardiology unit during their hospitalizations. A clinical questionnaire was established for the collection of all necessary data for all subjects in the study population.

Blood collection and laboratory analysis

Whole blood was collected in dry or Ethylene Diamine Tetra-Acetic acid (EDTA)-containing tubes, centrifuged at 4000 rpm for 15 minutes the day of collection, then serum or plasma were collected. In order to carry out the extraction of the DNA for molecular study, venous blood samples (5ml) in EDTA-containing tubes are centrifuged immediately to isolate the cell pellets and plasma in separate tubes; the aliquots were stored at-20 °C until analyzed. This first step serves for DNA isolation from leukocytes.

Biochemical and clinical analyses of patients' serum and plasma were performed by the Nutrition-Genetics and Environmental Risk Exposure Laboratory (U1256 INSERM CHU of Nancy, France). The Competitive immunoassay was used for the determination of vitamins (B9 and B12), total homocysteine, CRP and BNP; while the enzymatic colorimetric method was used to explore lipid profile, Urea, ASAT and ALAT activities.

DNA extraction

Genetic analyzes was performed at the University Health Centre of Nancy (France), in the Laboratory of Nutrition-Genetics and Environmental Risk Exposure. Genomic DNA was obtained from blood leukocytes using the BACC3 NUCLEON® extraction Kit (Amersham Biosciences, Little Chalfont,UK). DNA extraction was performed according to the manufacturer's instructions.

Molecular genetic analysis

The MTHFR C677T genotypes were determined using the Real-Time Polymerase Chain Reaction-fluorescence resonance energy transfer (Real- Time PCR-FRET) in a Light Cycler®480 Instrument II (Roche Diagnostic, Meylan, France) (Ririe et al., 1997; Vossen et al., 2009).

The PCR primers and probes used in the assay are:
For the C677T polymorphism: Sequence of Forward primers (F): 5'TGGCAGGTTACCCCAAAGG 3', Reverse primer (R): 5'TGATGCCCATGTCGGTGC3'.
For probes: Probe Flu: TGAGGCTGACCTGAAGCACTTGAAGGAGAAGGTGTCTX.
Probe Red (LC Red): CGGGAGCCGATTTCATCAT p.

The amplification conditions were as follows: an initial denaturation cycle at 95°C for 10 minutes followed by 45 cycles of PCR each comprising; Denaturation at 95°C for 10 sec, hybridization at 55°C for 10 sec and extension at 72°C for 10 sec. The melting curve requires a step of 30 sec at 95°C, 2 min

at 40°C, 10 sec at 73°C and finally a cooling at 40°C for 30 sec ends the cycle, to allow the progressive fusion of the double strands of DNA and probes. Melting temperatures (Tm) for the three genotypes of MTHFR1 C677T are respectively: Wild genotype (CC): 61°C; Homozygote mutated (TT): 53°C; heterozygote (CT): 51°C + 63°C (Figure 1).

Statistical analyses

Data are expressed as percentages and frequencies for qualitative variables. The Pearson Chi-square test was used to compare these qualitative variables.

For the quantitative variables the data are expressed as mean ± standard deviation (SD), the multiple comparisons between the continuous variables were calculated by the Kruskal-Wallis test. All data were analyzed using the SPSS (Statistical Package for the Social Sciences) (version 23.0.0.0-2015 for Windows, SPSS Inc., Chicago, IL). $P<0.05$ was considered to be statistically significant.

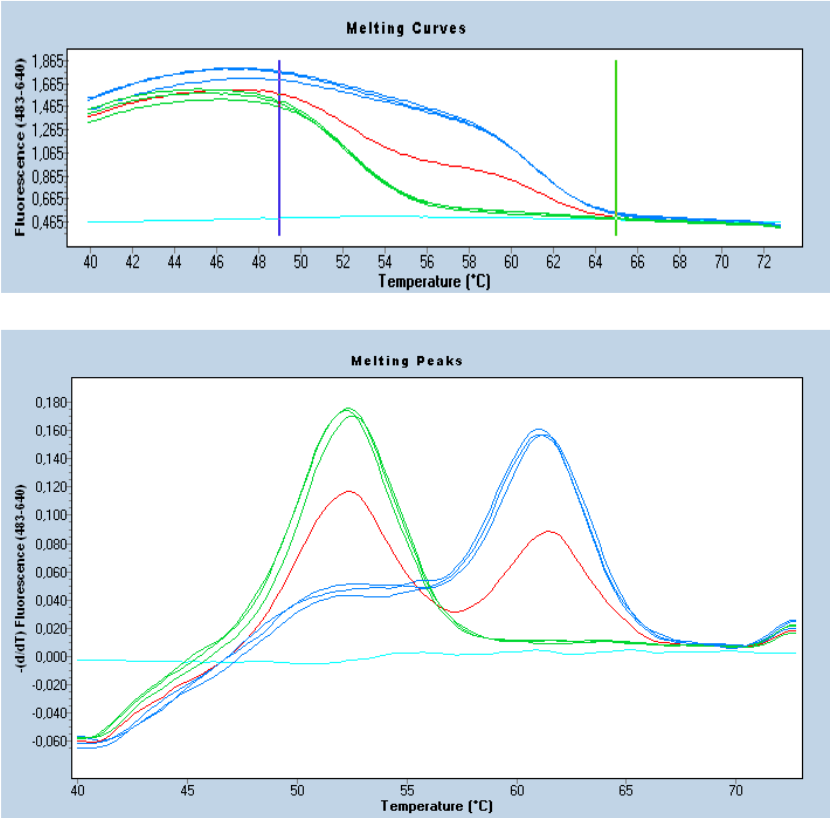

Figure1. Determination of the genetic polymorphism of MTHFR 1 with real-time PCR (fusion curve for the genotypes of the enzyme). In green ; mutated homozygot (TT), in blue; the wild homozygote (CC), in red; the heterozygotes (CT).

3. Results and discussion

The clinical and biochemical characteristics of patients according to the polymorphism of MTHFR C677T

Fourteen young patients with myocardial infarction (MI) were found during this survey conducted in different regions of the Aures. They are thus divided into 13 men (92.85%) and one woman (7.14%), with a larger proportion of men. Premature coronary artery disease is rare (6%) of acute coronary syndromes [ACS] (Van Der Vynckt et al., 2012).

As shown in Table 1, no significant differences in the clinical and demographic characteristics of patients according to the different genotypes of MTHFR C677T plymorphism were revealed: gender male (P = 0.688), body mass index (P = 0.197), smoking (P = 0.640), the age of smoking (P = 0.122) as well as the distribution of the number of patients with a family history of cardiovascular disease (P = 0.433). It is worth mentioning that we found only one diabetic woman during this research and she is CT genotype.

Regarding the distribution of the number of cases for moderate hyperhomocysteinemia (16-30 µmol / L), no significant difference was observed between the different genotypes of the MTHFR C677T polymorphism (P = 0.183). Therefore, no significant association between moderate hyperhomocysteinemia and this polymorphism was noted in our patients.

Concerning the concentrations of the various plasma lipid parameters, our results show no significant difference between the genotype subgroups (CC, CT, TT) for Trigly (P = 0.138) and HDL Chol (P = 0.821), Chol T (P = 0.088) and LDL Chol (P = 0.063) in our patients. The same results were observed for the variables: ASAT, ALAT, Urea, BNP, CRP (P = 0.189, P = 0.170, P = 0.453, P = 0.256, P = 0.293 respectively). Therfore, no significant associations with C677T polymorphism were revealed for these variables.

The average concentrations obtained are in the normal balance range, which is probably related in part to coronary patients who are in the chronic stage or because of the small size of our samples.

The clinical and biochemical characteristics of patients according to the C677T polymorphism of MTHFR are shown in Table 1.

Table 1. Clinical and biochemical characteristics of patients according to the C677T polymorphism of MTHFR.

Genotype	CC (n=05)	CT((n=08)	TT (n=01)	Total(14)	P value
Age (years)	31.40±9.83	33.75±3.24	40	33.35±6.35	0.263[a]
Gender, male, n	05	07	01	13	0.688[b]
Body Mass Index (BMI), Kg/m2	24.36±9.83	28.90±2.82	19.53	26.65±4.92	0.197[a]
Diabetes, n	00	01	00	01	-
Smoking, n	04	05	01	10	0.640[b]
Age of smoking, n	03	08	01	12	0.122[b]
Family history of cardiovascular disease, n	03	07	01	11	0.433[b]
Moderate hyperhomocysteinemia (16-30 µmol/L), n	03	02	01	6	0.183[b]
Chol t (g/L)	1.36±0.31	1.68±0.57	1.11	1.51±0.50	0.088[a]
Trigly (g/L)	1.02±0.37	1.67±0.95	0.95	1.37±0.87	0.138[a]
HDL Chol (g/L)	0.32±0.062	0.33±0.075	0.34	0.32±0.065	0.821[a]
LDL Chol (g/L)	0.83±0.25	1.08±0.45	0.67	0.96±0.37	0.063[a]
TGO (u/L)	23.40±6.10	36.62±15.49	28.00	31.28±13.52	0.189[a]
TGP (u/L)	9.60±4.33	24.62±15.56	23.00	19.14±13.81	0.170[a]
Urea (g/L)	0.26±0.021	0.30±0.077	0.32	0.29±0.062	0.453[a]
BNP (pg/mL)	10.40±6.06	11.00±6.18	36.00	12.57±8.80	0.256[a]
CRP (g/L)	3.46±3.76	10.78±16.05	6.10	7.83±12.49	0.293[a]

([a])Kruskal-Wallis test. ([b]) Khi-Square test. $p<0.05$ is considered significant.

The results obtained for age, male gender and smoking, agree with those found by Li et al.,(2017). However, Klerk et al.,(2002) reported in a meta-analysis evaluating the relationship between C677T polymorphism and the risk of coronary disease, a significant differences between the various genotypes with regard to risk factors; male sex and smoking.Similar results were observed by Inceetal (Ince et al., 2016) with respect to BMI, dyslipedimia, family history of cardiovascular disease and moderate hyperhomocysteinemia. A study by Joubranet al., (1998) concluded that hyperhomocysteinemia, which can result from mutations in the MTHFR gene, is independently associated with coronary heart disease in Arab men.

In contrast to our findings, Dharet al., (2010) found that diabetes and family history of coronary artery disease were significantly associated with genotypes (TT) and (CT). Moreover, Bennouar et al. (2007) found that the frequency distribution of traditional risk factors such as male sex, smoking, diabetes and others was higher in coronary patients, with genotypes of the C677T polymorphism, than controls, with an exception made for dyslipidemia and obesity in (TT) genotypes particularly. In patients, all frequencies of risk factors recorded were lower in genotypes (TT) than in the rest of the genotypes. These differences lead us to believe that conventional risk factors do not have the same effect on coronary artery disease depending on the different genotypes of the MTHFR C677T polymorphism. In fact, in subjects with wild genotype (CC), the increased risk of coronary artery disease was significantly associated with smoking habits, obesity and dyslipidemia (Bennouar et al., 2007).

Similarly to our results, Li et al., (2017) found no significant effect of C677T MTHFR polymorphism on CRP levels in subjects in the coronary group. However, Cho et al., (2006) observed that plasma concentrations of BNP were significantly higher in patients with MTHFR C677T mutation than in patients without mutation. Thus, Shenet al., (2015) found a significant increase in the concentration of

ASAT and ALAT in the sick group compared to the control group. In our patients and according to our results the renal physiological function was normal.

Association of C677T MTHFR genotype with total homocysteine, vitamin B12 and B9 in coronary subjects (with MI)

In our pathological population, only one patient was found with the homozygous genotype mutated TT (7.14%) (Table2). However, the frequencies of normal homozygous (CC) and heterozygous (CT) genotypes were 35.71% and 57.14%, respectively.

No significant differences were observed between the different genotypes of C677T MTHFR for total homocysteine levels, vitamin B12 and folate (P = 0.330, P = 0.733, P = 0.250, respectively). Therefore, the concentrations of these parameters were not significantly dependent on the MTHFR C677T polymorphism. Nevertheless, a high rate of tHcy (moderate hyperhomocysteinemia) is associated with the mutated TT genotype against the CC and CT genotype. As well as a low level of vitamin B12 and B9 was recorded in the mutated genotype (TT) compared to wild and heterozygous genotypes (Table 2), this shows the deleterious effect of the mutated genotype on the concentrations of tHcy, vitamin B12 and B9 in our patients. However, this effect remains not significant. (The mean concentrations of vitamins B12 and B9 are within the reference interval).

Table 02. Association of C677T MTHFR genotype with total Homocysteine, vitamin B12 And B9 in coronary subjects.

Genotype	CC	CT	TT	P value
N=14	05	08	01	-
Frequency (%)	35,71	57,14	07,14	-
Total homocysteine (µmol/l)	16.55±2.35	14.56±5.50	19.50	0.330[a]
Vitamin B12 (pM)	125.85±70.12	142.09±61.42	82.50	0.733[a]
Folate (nM)	12.07±3.14	14.42±4.90	05.25	0.250[a]

All parameters are expressed (mean ± sd). ([a]) Kruskal-Wallis test (p<0.05).

The Table 3 describes the association of C677T genotypes of MTHFR with homocysteinemia, vitamin B12 and folate in coronary patients. Our results (Table 3) revealed that no significant difference in homocysteine means was found between the different genotypes of the C677T MTHFR polymorphism, for both groups; normal homocysteinemia and moderate hyperhomocysteinemia (P = 0.182, P = 0.117, respectively). When concentrations of B12 and B9 vitamins of patients with moderate Hhcy were compared between the subgroups of the three previous genotypes, no significant difference was observed (P = 0.304, P = 0.901) respectively, the same result was noted for both groups of normal homocysteinemia (for B12 vit, P = 1.00 / for B9 vit, P = 0.402).

It is notable that folate and vitamin B12 concentrations in the moderate Hhcy groups are lower in the mutated genotypes (TT: 5.25 nM, CT: 12.44 ± 3.06 nM) for the B9 / vit (TT, 82.50 µM, CT: 115.13 ± 41.13 µM) for B12 compared to normal homocytinemia groups. Similarly, a high level of tHcy was observed particularly in carriers of the mutated genotypes: (CT) 22.45 ± 0.92 µmol/L and (TT) 19.50 µmol / L compared to those in the normal homocytinemia group.

Table 3. Association of C677T genotypes of MTHFR with homocysteinemia, vitamin B12 and folate in coronary patients

Génotype (N=14)	**CC**	**CT**	**TT**	**P value**
Group01. Normal Homocysteinemia (5-15 µmol/L)	14,45±1,06	11,94±3,03	-	0,182[a]
Group 02. Moderate Hyperhomocysteinemia (16-30 µmol/L)	17,96±1,78	22,45±0,92	19,50	0,117[a]
Vitamin B12 (*p*M)				
Group 01	115,75±41,75	151,08±26,51	-	1,00[a]
Group 02	132,58±51,39	115,13±41,13	82,50	0,304[a]
Folate (nM)				
Group 01	13,81±0,31	15,08±2,15	-	0,402[a]
Group 02	10,92±2,21	12,44±3,06	5,25	0,901[a]

All parameters are expressed as (mean ± sd). ([a]) Kruskal-Wallis test. $p<0.05$ is considered statistically significant.

These findings reveal a deleterious effect of the mutated genotype on the levels of vitamins B9, B12 and total homocysteine, in our patients. However, this action was not significant as mentioned above. Our results concerning the concentrations of total homocysteine, vitamin B12, and folate are probably explained by the small size of our samples to draw conclusions about the consequences for coronary patients. Contrarily to our results, Morita et al., (1997) reported that the frequency of the mutated genotype (TT) of the C677T MTHFR polymorphism was significantly higher in patients with coronary artery disease than in controls in a Japanese population. In addition, the frequency of this genotype was associated with the severity of coronary artery disease. Similar results were also found by Heidari et al., (2016) in an Iranian population.

In fact, our results are consistent with those of Eftychiouet al., (2012) suggesting that moderate hyperhomocysteinemia is not only associated with coronary artery disease, but also with MI and multi-vessel CAD. Recently, a study conducted by Liu et al., (2015) , including patients with coronary artery disease, of different categories, revealed that Hhcy was independently associated with the severity of coronary artery disease and correlated significantly with low levels of folic acid.

Actually, there is a debate about the causality, both of MTHFR genotype (TT) and hyperhomocytinemia, in their association with MI (Tanis et al., 2004). Therefore, in order to clearly establish this link, many other parameters need to be evaluated, for example, the timing necessary for the development of the disease during hyperhomocysteinemia must be evaluated more precisely (Kerkeni et al., 2006).

The results of Tanis and his collaborators, (2004) showed that genotype (TT) is associated with an increased risk of MI only when folic acid status is low. In young patients with low folate status and mutated homozygous genotype (TT), the risk of myocardial infarction was two times higher than in wild homozygous patients (CC) with an elevated folate statue. Al Mawiet al., (2004) demonstrated that the frequency of the T allele and the mutated genotype (TT) was associated with coronary artery disease and elevated levels of plasma homocysteine in a population of Bahreïn.

A study of 452 young adults, noted that genetic factors contributed to 9% of the variance of tHcy, compared with the 35% estimated for low folate and vitamin B12 levels (Kluijtmans et al., 2003) .In other study carried out on the Tunisian population suffering from coronary heart disease showed that the effect of the mutated genotype of MTHFR was more pronounced on homocysteinemia and coronary disease with low folate (<or = 6.1 ng / mL) (Belkahla et al., 2008) patients with (TT) genotype had a significantly higher tHcy level and significantly lower folate than those with genotype (CC) and (CT). Thus, genotype (TT) patients had more severe coronary lesions than those with genotypes (CC) and (CT) (Li et al.,(2017).

It should be noted that there is growing evidence of the role of aberrant DNA methylation in the development of the disease (Andreassi and Botto, 2003 ; Heijmans et al., 2003). Knowing that, furthermore, the major source of the methyl group used by S-adénosylmethionine (SAM) for the methylation reactions is the novo synthesis, dependent on the folate and monocarbonate units (Selhub et al., 1999). The results of Friso et al., (2002) show that the genomic methylation of DNA is directly correlated with the folate statue and inversely with the plasma homocysteine level. Genotypes (TT) had a low level of DNA methylation compared to the wild-type (CC). These searches have been confirmed in the study of Castro and collaborators, (2004). As a consequence, these DNA methylation abnormalities may promote both carcinogenesis, especially colorectal cancer (Shannon et al., 2002), and the occurrence of cardiovascular disease (Botto et al., 2003). In the C677T mutation of the MTHFR gene, the MTHFR enzyme becomes thermolabile and the MTHFR activity decreases by 65% and 30% in mutated homozygotes (TT) and heterozygous (CT), respectively (Frosst et al., 1995).

Some widely known results, like those presented by Gardemannet al., (1999) failed to find an association of C677T polymorphism with nonfatal myocardial infarction. Similarly, two Swedish case-control studies (on myocardial infarction and unstable angina) confirmed that high tHcy is a risk factor for coronary heart disease in both studies. In 'SHEEP' (Stockholm Heart Epidemiology Program), the association between tHcy and MI was observed in CC homozygotes and in heterozygotes but not in T-homozygotes, regardless of smoking, physical activity and obesity (Mehlig et al., 2013).

In Algeria, Houcher et al.,(2012) revealed that the mutation of the MTHFR C677T gene, in particular the genotypes (CT/TT), does not seem to be associated with an increase in the plasma tHcy level in cardiac patients (at a folate level <15.4 ng/ml and> 15.4 ng/ml), the authors suggested that this lack of correlation could be influenced by higher folate concentrations in this study.

A current meta-analysis includes forty-four studies, 9,693 MI survivors and 12,554 control subjects were included in this study based on the most recent information, showed no significant association between MTHFR C677T polymorphism and the risk of myocardial infarction. Nevertheless, this study did not investigate genetic-nutritional interactions (Li et al., 2016).

Finally, these discrepancies between the various results can be explained by differences in sample populations, such as ethnicity and dietary folate intake, due to fortified foods and / or vitamin supplementation Gueant-Rodriguez et al., (2005). We hope scientific community will quickly conduct further studies to provide more certainties and indications in this field.

4. Conclusion

Our study supports the hypothesis that the polymorphism C677T MTHFR can be a significant risk factor for MI in the presence of moderate Hhcy. We therefore suggest a strategy for the biological exploration of Hhcy to allow the assessment of a moderate or intermediate Hhcy as quickly as possible, and thus to the etiological diagnosis of intermediate to severe Hhcy that may be responsible for the onset and progression of several severe metabolic diseases such as coronary atherosclerosis. Furthermore, genetic investigation of various MI markers would be helpful to treat and predict the risks of this pathology.This first study must be followed by further studies with a larger sample size are needed, to better clarify the relationship between genotypes of the MTHFR gene and coronary atherosclerosis in young patients with Myocardial Infarction, in our region of *'Aurès* ' (Algeria).

Acknowledgments

We would like to extend our gratitude and appreciation to Professor Rosa-Maria Guéant-Rodriguez, Professor Jean-Louis Guéant, all the personnel of the INSERM Unity 1256 Nutrition-Genetics-Environmental Risk Exposure Laboratory, Nancy (France), for their technical assistance to carry out this interesting work.

Disclosure of conflict of interest

The Authors declare no conflict of interest.

References

Brustolin S, Giugliani R and Felix TM: Genetics of homocysteine metabolism and associated disorders. Braz J Med Biol Res 43: 1–7,(2010).

Hiraoka M and Kagawa Y: Genetic polymorphisms and folate status. CongenitAnom**57**: 142-149,(2017).

Frosst P, Blom HJ, Milos R, et al:A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolatereductase. Nat Genet **10**:111–3, (1995).

Klerk M, Verhoef P, Clarke R, et al: MTHFR 677CRT polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 288: 2023–31,(2002).

Goyette P, Pai A, Milos R, et al: Gene structure of human and mouse methylenetetrahydrofolatereductase (MTHFR). Mamm Genome **9**: 652-656, (1998).

Varga EA, Sturm AC, Misita CP,et al: Cardiology patient pages. Homocysteine and MTHFR mutations: relation to thrombosis and coronary artery disease. Circulation 111: e289–93,(2005).

Clarke R, Bennett DA, Parish S, et al: Homocysteine and coronary heart disease: metaanalysis of MTHFR case-control studies, avoiding publication bias. PLoS Med 9: e1001177, (2012).

Jacques PF, Bostom AG, Williams RR, et al: Relation between folate status, a common mutation in methylenetetrahydrofolatereductase, and plasma homocysteine concentrations. Circulation 93: 7–9, (1996).

Davey Smith G and EbrahimS :'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?. Int J Epidemiol32: 1–22, (2003).

Djaara H, Yahia M and Boussif A :Frequency and coexistence of the C677T and A1298C polymorphisms of the MTHFR gene in Aures region of Algeria. Biodiversitas**19 (3)**: 1169-1175,(2018).

Ririe KM, Rasmussen RP and Wittwer CT: Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal Biochem245: 154-160, (1997).

Vossen R,Aten E, Roos A and Dunnen J T: High-Resolution Melting Analysis (HRMA)-More than just a sequence variant screening. Human Mutation 30: 860-866, (2009).

Van Der Vynckt C, Kerneis M, Abtan J, et al: Maladie coronaire prématurée. Sang ThromboseVaisseaux4: 173-82, (2012).

Li MN, Wang HJ, Zhang NR, et al: MTHFR C677T gene polymorphism and the severity of coronary lesions in acute coronary syndrome. Medicine 96: 49, (2017).

Ince FD, Atay A, Koseoglu MH, et al: The severity of Coronary Artery Disease and MethylenetetrahydrofolateReductase (MTHFR) Enzyme Gene Polymorphism. IntCardiovasc Res J 10(4): 172-178.icrj.35166, (2016).

Joubran R, Asmi M, Busjahn A, et al: Homocysteine levels and coronary artery disease in Syria. J CardiovascRisk **5**: 257-261,(1998).

Dhar S, Chatterjee S, Ray S, et al: Polymorphisms of methylenetetrahydrofolatereductase gene as the genetic predispositions of coronary artery diseases in eastern India. J Cardiovas Dis Res **1(3)**: 152-157, (2010).

Bennouar N, Allami A, Azeddoug H, et al: ThermolabileMethylenetetrahydrofolateReductase C677T Polymorphism and Homocysteine Are Risk Factors for Coronary Artery Disease inMoroccan Population. J Biomed Biotechnol80687: 9 pages,(2007).

Cho SE, Hong KS, Shin GJ ,et al: The methylenetetrahydrofolatereductase C677T gene mutation is associated with hyperhomocysteinemia, cardiovascular disease and plasma B-type natriuretic peptide levels in Korea. ClinChem Lab Med 44(9): 1070-1075, (2006).

Shen J, Zhang J, Wen J, et al: Correlation of serum alanine aminotransferase and aspartate aminotransferase with coronary heart disease.Int J ClinExp Med 8(3): 4399-4404(2015).

Morita H, Taguchi J, Kurihara H, et al: Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. Circulation 95(8): 2032-6, (1997).

Heidari MM, Khatami M, Hadadzadeh M, et al: Polymorphisms in NOS3, MTHFR, APOB and TNF-alpha Genes and Risk of Coronary Atherosclerotic Lesions in Iranian Patients. Res CardiovascMed 5(1): e29134, (2016).

Eftychiou C, Antoniades L, Makri L, et al:Homocysteine Levels and MTHFR Polymorphisms in Young Patients with Acute Myocardial Infarction: A Case Control Study. Hellenic J Cardiol**53**: 189-194. (2012).

Liu C, Yang Y, Peng D, et al: Hyperhomocysteinemia as a metabolic disorder parameter is independently associated with the severity of coronary heart disease. Saudi Med J **36**: 839-46, (2015).

Tanis BC, Blom HJ, Bloemenkamp DG, et al: Folate, homocysteine levels methylenetetrahydrofolatereductase (MTHFR) C677T variant, and the risk of myocardial infarction in young women: effect of female hormones of homocysteine levels. J. Thromb. Haemost**2**: 35-40,(2004).

Kerkeni M, Addad F, Chauffert M, et al: Hyperhomocysteinaemia, methylenetetrahydrofolatereductase polymorphism and risk of coronary artery disease. Ann ClinBiochem43: 200-206,(2006).

Al Mawi WY, Ameen G, Tamim H, et al: Factor V G1691A, prothrombin G20210A, and methylenetetrahydrofolatereductase [MTHFR] C677T gene polymorphism in angiographically documented coronary artery disease. J ThrombThrombolysis 17(3): 199-205,(2004).

KluijtmansLA,Young IS, Boreham CA, et al: Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. Blood 101: 2483-2488, (2003).

Belkahla R, Omezzine A, Kchok K, et al: Effet des polymorphismes des enzymes clés du métabolisme de lřhomocystéine sur lřhomocystéinémie et le risque coronarien chez une population tunisienne. Ann CardiolAngeiol57: 219-224:(2008).

Andreassi MG and BottoNDNA : damage as a new emerging risk factor in atherosclerosis. Trends Cardiovasc Med13: 270-5, (2003).

Heijmans BT, Boer JM, Suchiman HE, et al: A common variant of the methylenetetrahydrofolatereductase gene (1p36) is associated with an increased risk of cancer. Cancer Res 63:1249-53,(2003).

Selhub J, Jacques PF, Rosenberg IH, et al: Serum Total Homocysteine Concentrations in the Third National Health and Nutrition Examination Survey (1991-1994): Population Reference Ranges and Contribution of Vitamin Status to High Serum Concentrations. Ann Intern Med 131 (5): 321-330, (1999).

Friso S, Choi SW, Girelli D, et al: A common mutation in the5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. ProcNatlAcadSci USA 99: 5606-11,(2002).

Castro R, Rivera I, Ravasco P, et al: 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C mutations are associated with DNA hypomethylation. Med Genet 41: 454-458, (2004).

Shannon B, Gnanasampanthan S, Beilby J,et al: A polymorphism in the methylenetetrahydrofolatereductase gene predisposes to colorectal cancers with microsatellite instability. Gut 50: 520-524,(2002).

Botto N, Andreassi MG, Manfredi S, et al: Genetic polymorphisms in folate and homocysteine metabolism as risk factors for DNA damage. Eur J Hum Genet 11: 671-678,(2003).

Gardemann A, Weidemann H, Philipp M, et al: The TT genotype of the methylenetetrahydrofolatereductase C677T gene polymorphism is associated with the extent of coronary atherosclerosis in patients at high risk for coronary artery disease. Eur Heart J 20: 584-592,(1999).

Mehlig K, Leander K, De Faire U, et al: The association between plasma homocysteine and coronary heart disease is modified by the MTHFR677C>T polymorphism. Heart 99 : 1761-1765, (2013).

Houcher Z, Houcher B, Touabti A, et al: Nutritional factors, homocysteine and C677T polymorphism of the methylenetetrahydrofolatereductase gene in Algerian subjects with Cardiovascular Disease:23(1); (2012).

Li WX, Li W, Cao JQ, et al: Folate Deficiency Was Associated with Increased Alanine Aminotransferase and GlutamylTranspeptidase Concentrations in a Chinese Hypertensive Population: A Cross-Sectional Study. J NutrSciVitaminol (Tokyo) 62(4): 265-271, (2016).

Guéant-Rodriguez RM, JuillièreY, Candito M, et al: Association of MTRR A66G polymorphism (but not of MTHFR C677T and A1298C, MTR A2756G, TCN C776G) with homocysteine and coronary artery disease in the French population. ThrombHaemost94: 510-5, (2005).