Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol. XV/B Anul15, 2016

MTHFR C677T POLYMORPHISM IN PATIENTS WITH CARDIOVASCULAR DISEASES IN BIHOR COUNTY

Maghiar Adriana Laura*, Bembea Marius, Jurca Claudia*, Kozma Kinga***

*University of Oradea, Faculty of Medicine and Pharmacy, Pta 1 Decembrie, no. 10, Oradea, Romania, e-mail: maghiar_adi@yahoo.com, claudiajurca70@yahoo.com, kozmaki@gmail.com
** Municipal Clinical Hospital "Dr.G. Curteanu" Oradea, Department of Medical Genetics, str. C. Coposu, no. 12, Oradea, zip code 410 469, email: bembea13@yahoo.com

Abstract

The Polymorphism of MTHFR gene is associated in many studies with risk of cardiovascular disease.

The purpose of the study was to determine the genotype of MTHFR 677 and establishing the correlation with cardiovascular disease in a group of patients in Bihor County.

We conducted a case-control study on two equal groups of patients, a study group (SG) consisting of patients with acute myocardial infarction and thromboembolism and a control group (CG) consisting of a number of 51 healthy individuals coming from Bihor County.

To the participants in both groups was performed the genotyping on gene MTHFR 677 through real-time PCR method.

The proportion and significance of the three genotypes CC, CT and TT of the MTHFR 677 gene were analyzed by comparison in the two groups. The proportions found were: men of SG vs CG, MTHFR genotype C677C (wild type) was 22.86% vs. 40.67%; MTHFR C677T (heterozygous mutant) 40.0% vs. 45% and MTHFR T677T (homozygous mutant) 37.14% vs. 12.50%.
Women of SG vs CG, MTHFR genotype C677C (wild type) was 31,25% vs. 40,74%; MTHFR C677T (heterozygous mutant) 43,75% vs. 51,85% and MTHFR T677T (homozygous mutant) 22,0% vs. 7,41%.

T677T homozygous mutant genotype of MTHFR gene is present in a higher percentage of both women and men in the study group compared with the control group, suggesting its predisposing role to cardiovascular disease. In the presence of the mutant allele T the risk of disease increases on the carriers, both women and men.

Key words: polymorphism of MTHFR, early-onset myocardial infarction

INTRODUCTION

Worldwide, cardiovascular disease (CVD) is the leading cause of death. In Europe cardiovascular diseases are responsible for over half of all deaths, more than deaths caused by all cancers combined, with a higher percentage in women (43% of all deaths) than in men (36% of all deaths). Over three-quarters of deaths from cardiovascular disease occur in low- and middle-income countries. (Nichols M. et al, 2012).

According to the latest report of the World Health Organization (WHO) on noncommunicable diseases in 2014, cardiovascular diseases caused in 2012 a total of 17.5 million deaths, ie 46% of all deaths from noncommunicable diseases and 31% of all deaths in the world. Of these 7.5 million are due to myocardial infarction (heart disease) and 6.7 million due

to stroke. More than three quarters of these deaths are recorded in countries with low or medium income. It is estimated that the number of deaths from cardiovascular disease could reach 23.3 million by 2030. (WHO, 2016)

Disease burden in the population of Romania is great because although life expectancy at birth increased, healthy life expectancy decreased in Romania, according to the survey SILC (Statistics of Income and Living Conditions), contrary to the situation in the European Union. Healthy life expectancy, HLY (Healthy Life Years) in Romania in 2013 for women age 65 years was 3.4 years and 2.7 years for men, below the European average (8.6 years for women and 8.5 years for men). However, in comparison to men women live in a higher proportion of their lives with serious health problems. (EHLEIS, 2015; Eurostat)

Even if life expectancy in Romania increased in recent years, from 70 years in 1999 to 74 years in 2012 for both sexes, mortality from cardiovascular diseases remains high in 2000 being 467.5 ‰ 00 deaths and 364 ‰ 00 deaths in 2012, according to data published by WHO. Although there is a decrease in mortality from this cause, Romania remains in second place compared to other EU countries, Bulgaria is ranked first in both periods presented (WHO, 2014).

Most cardiovascular diseases are multifactorial disorders in whose etiology enter many risk factors. The risk of developing cardiovascular disease is so under the influence of environmental factors and genetic factors. Genetic polymorphism is defined by the existence of multiple alleles (different forms of the same gene) that exceed 1% in the general population. About 90% of genetic polymorphisms are represented by single nucleotide polymorphisms - SNPs. Heredity was suspected in cardiovascular disease especially when the disease was installed at a young age or if that family history includes cardiovascular disease in first degree relatives, particularly if the death occurred before the age of 55 years for men and 65 years women. (Covic M. et al, 2011)

A large number of genes have been studied in order to determine their relationship to the risk of developing cardiovascular disease. There were studied different variants of genes involved in lipid metabolism, such as apolipoprotein E (ApoE), apolipoprotein B (ApoB), lipoprotein lipase (LPL) transfer protein cholesterol esters (CETP = cholesterol ester transfer protein) , etc. Other genes involved in coagulation inhibitor-1 plasminogen activator (PAI-1 = plasminogen activator inhibitor 1), factor V (FV) and in various aspects of endothelial function as synthetase endothelial nitric oxide synthase (eNOS = endothelial nitric oxide synthase), or folate metabolism such as methylene-tetrahydrofolate reductase (MTHFR). (Covic M. et al, 2011).

MTHFR (OMIM*607093) is the symbol for the gene encoding the enzyme methylenetetrahydrofolate reductase and is located on chromosome 1p36.3. (Goyette P. et al, 1993). There are over 41 polymorphisms of this gene, but the best studied are C677T and A1298C. Both are produced by the substitution of one amino acid, which will result in the synthesis of thermos-sensitive proteins with reduced enzyme activity. (Leclerc D. et al, 2004).

The most common and most extensively studied polymorphism is C677T, identified by Frosst et al, 1995. It is located in exon 5 and is caused by a missense mutation, the cytosine (C) is replaced with thymine (T) at position 677 of the MTHFR gene. The two alleles are C allele or non-mutant allele and the T allele or mutant allele. Modified codon (A222V) will encode instead of alanine (Ala) valine (Val), yielding a thermolabile protein with reduced enzyme activity. Thus, C677T mutation may represent an important genetic risk factor in cardiovascular diseases, stroke, thromboembolic disorders, recurrent miscarriage, but has been associated in some studies with neurological disorders, etc. (Cao Y. et al, 2013; Xuan C. et al, 2011; U. Yadav et al, 2016; Liew S. et al, 2015; Casas J.P. et al, 2004).

MTHFR C677T polymorphism role in the development of coronary artery disease and myocardial infarction was confirmed in several studies. Two recent meta-analyzes that included a large number of patients with myocardial infarction or coronary artery disease showed that MTHFR C677T polymorphism was associated with risk of myocardial infarction in young middle age OR 1.275 (1077-1509; 95% CI) or coronary artery disease TT vs CC, OR = 1.88 (1.54-2.30; CI 95%). (Xuan C. et al, 2011; Shan J. G. et al., 2016).

MATERIAL AND METHOD

The purpose of the study was to determine the gene's MTHFR C677T polymorphism and establishing the correlation with major cardiovascular incidents, on a group of patients in Bihor County, compared with a control group of healthy individuals without cardiovascular medical history.

The study group (SG) consists of a number of 51 patients with acute myocardial infarction or pulmonary embolism.

The inclusion criteria were:

- Patients who have had a myocardial infarction or pulmonary embolism under the age of 50 years;

- Myocardial infarction was confirmed by chest pain, specific ischemia changes on the electrocardiogram , elevated serum levels of troponin T or I and CKMB increased activity;
- Patients had not symptoms of coronary heart disease before the first infarction.

Exclusion criteria from the study group:

- people over 50 years at the time of infarction or thromboembolism;
- people with diabetes or hypertension.

The control group (CG) consists of 51 people asymptomatic, apparently healthy, and randomly selected.

Criteria for inclusion in the control group were:

- Age up to 50 years;
- Without cardiovascular disease;
- The geographical area of origin correspond to the region from which the patients in the study group come from.

Exclusion criteria:

- People with cardiovascular diseses and diabetes were not included in the control group;

All persons have given their informed consent, identical for both groups studied.

Working method:

Both groups of patients were collected 2 ml sample of venous blood from the collection tube, containing EDTA as an anticoagulant. If DNA extraction cannot be done after harvesting, the sample blood can be stored at 2-8° C within 7 days or in a freezer at ≤ - 20° C for maximum two months. After extraction, the DNA can be preserved in a freezer in sterile tubes at - 80° C with unlimited validity.

Genomic DNA extraction was performed using the kit Quick gDNA miniprep ™, produced by Zymo Research USA. This technique allows for extraction of genomic DNA of high purity from venous blood collected in EDTA anticoagulant. The aim is to obtain the DNA from the nucleus of white blood cells which will then move in the suspension and then be recovered using a buffer solution. This technique avoids mechanical destruction or enzymatic nucleic acids and RNA contamination.

Analysis method: genotyping was performed by polymerase chain reaction of DNA in real time (real-time PCR). For detection of the polymorphism of the gene methylene-tetrahydrofolate reductase MTHFR C677T, I used the kit from GeneProof^{R} Czech Republic. Real-time PCR (RT-PCR) called quantitative PCR (qPCR), uses oligonucleotides labeled with the fluorescent dye, which will be detected as it occurs amplification and can be monitored in real time, which means that the PCR product does

not require further processing. In-vitro amplification of DNA fragments was performed using a thermocycler device Eco Illumina.

RESULTS AND DISSCUSIONS

From the study group (SG) were part 51 patients, 35 men and 16 women aged 25-50 years.

- 31.37% were women
- 68.63% were male

The control group (CG) was made out of 51 people, 24 men and 27 women aged 25-50 years.

- 47.06% were male
- 52.94% were women

Notice in Table 1 and Figure 1 that in the study group in patients with myocardial infarction, homozygous MTHFR mutation T677T occurs in 33.33% of cases compared to 9.8% in the group of healthy individuals. Also the proportion of people without the mutation on the analyzed gene is higher among people in the control group.

Tabel 1

MTHFR polymorphisms distribution of all patients from the groups

MTHFR genotype distributions of all patients	**SG (51 cases)**	**CG (51 cases)**	**Total cases 102**
Normal heterozygous genotype MTHFR C677C (wild type)	**13** *(25,49%)*	**21** *(41,18%)*	**34** *(33,33%)*
Mutant heterozygous genotype MTHFR C677T	**21** *(41,18%)*	**25** *(49,02%)*	**46** *(45,10%)*
Mutant homozygous genotype MTHFR T677T	**17** *(33,33%)*	**5** *(9,80%)*	**22** *(21,57%)*

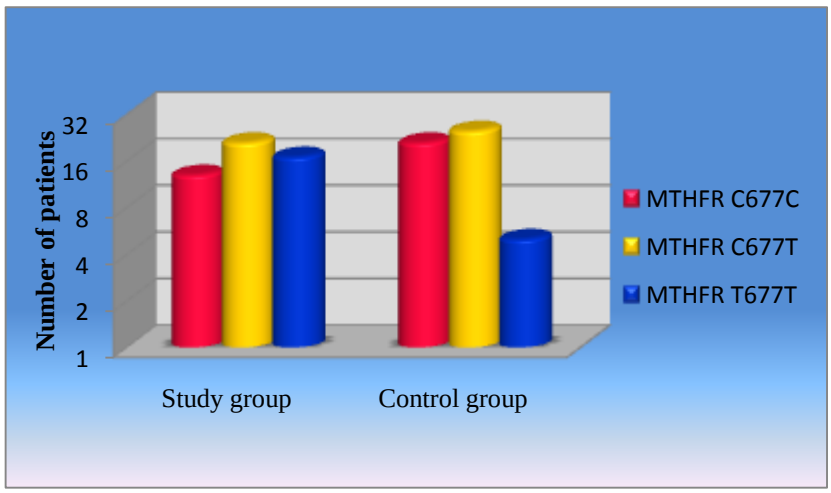

Fig. 1 Distribution of CC, CT and TT genotypes in the groups

In men, the situation is shown in Table 2 and Figure 2. In patients with cardiovascular disease, homozygous mutation MTHFR T677T is present in 37.14% of cases, compared to 12.5% in the group of men without cardiovascular disease. Also the proportion of people without the mutation on the analyzed gene is higher among people in the control group.

Tabel 2

MTHFR polymorphisms distribution for men's tested groups

MTHFR genotype distributions for men	SG 35 cases	CG 24 cases
Normal heterozygous genotype MTHFR C677C (wild type)	**8** *(22,86%)*	**10** *(41,67%)*
Mutant heterozygous genotype MTHFR C677T	**14** *(40,00%)*	**11** *(45,83%)*
Mutant homozygous genotype MTHFR T677T	**13** *(37,14%)*	**3** *(12,50%)*

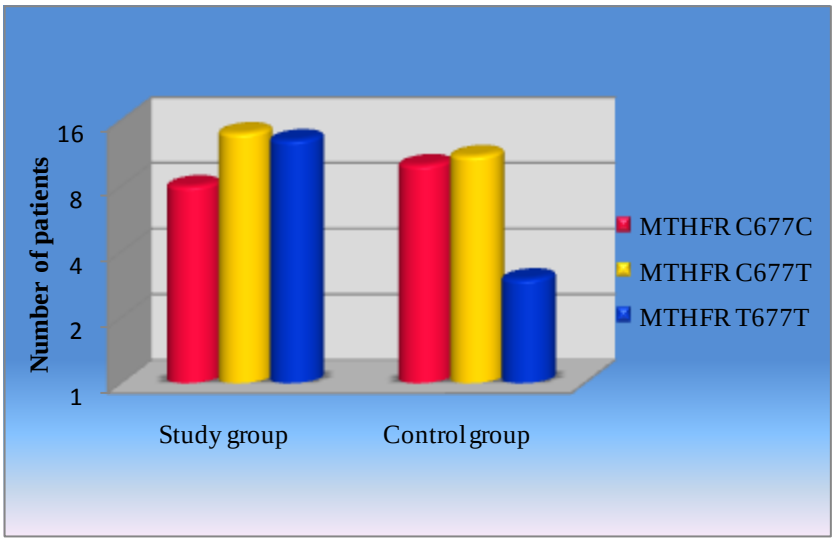

Fig. 2 Distribution of CC, CT and TT genotypes in men's groups

In the case of women, the situation is shown in Table 3 and Figure 3. In patients with cardiovascular disease, homozygous MTHFR mutation T677T is present in 25% of cases, compared to 7.41% in the group of women without cardiovascular disease. Also the proportion of people without the mutation on the analyzed gene is significantly higher among people in the control group.

Tabel 3

MTHFR polymorphisms distribution for woman's tested groups

MTHFR genotype distributions for woman	**SG** **16 cases**	**CG** **27 cases**
Normal heterozygous genotype MTHFR C677C (wild type)	**5** *(13,25%)*	**11** *(40,74%)*
Mutant heterozygous genotype MTHFR C677T	**7** *(43,75%)*	**14** *(51,85%)*
Mutant homozygous genotype MTHFR T677T	**4** *(25,00%)*	**2** *(7,41%)*

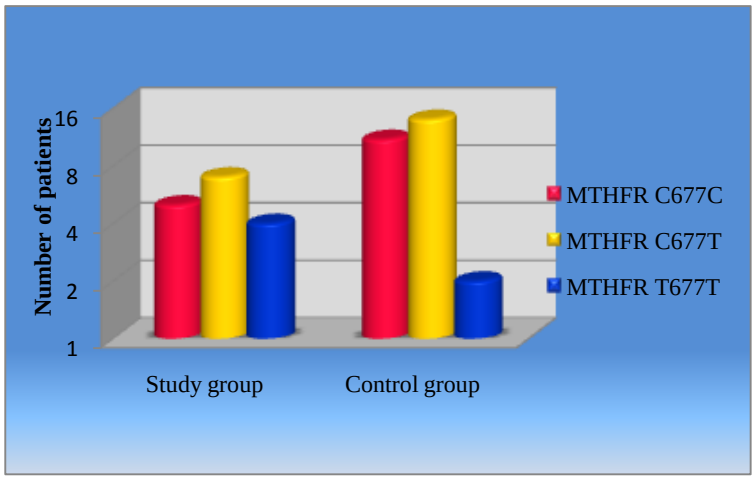

Fig. 3 Distribution of CC, CT and TT genotypes in woman's groups

CONCLUSIONS

1. Homozygous mutation T677T of the MTHFR gene is present in a higher percentage in both women and men from the study group compared to the control group, suggesting its predisposing role to cardiovascular disease. In the presence of the mutant allele T the risk of the disease increases in the carriers, both women and men.
2. The number of women with cardiovascular disease in the study group was significantly lower than that of men, a possible explanation could be the protective influence of estrogen hormones before menopause.

I thank by this means the cardiologists and geneticists and staff from Medical Genetics who made this study possible and patients and persons who have consented to participate in the two groups.

REFERENCES

1. Cao Y., Xu J., Zhang Z., Huang X., Zhang A., Wang J., Zheng Q., Fu L., Du J., 2013, Association study between methylenetetrahydrofolate reductase polymorphisms, Gene, Feb 10;514(2):105-11.
2. Casas J. P., Hingorani A. D., Bautista L. E., et al., 2004, Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18,000 cases and 58,000. Arch Neurol., Nov; 61(11):1652-61.
3. Clarke R., Collins R., Lewington S., et al., 2002, Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis, JAMA, 288:2015- 2022.
4. EHLEIS, European Health and Life Expectancy Information System, Country Reports Issue 9- October 2015. Available from: http://www.eurohex.eu.
5. Eurostat Mortality and life expectancy statistics. Available from: http://ec.europa.eu/eurostat/statistics-explained/index.php/Mortality_and_life_expectancy_statistics.
6. Frosst P., Blom H. J., Milos, R., Goyette P., Sheppard C. A., Matthews R. G., Boers G. J. H., den Heijer M., Kluijtmans L. A. J., van den Heuvel L. P., Rozen R. A., 1995, Candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase, Nature Genet, 10: 111-113.
7. Goyette P., Sumner J. S., Milos R., Duncan A., Rosenblatt D., Matthews R., Rozen R., 1993, Isolation of a cDNA for human methylenetetrahydrofolate reductase (MTHFR) and identification of mutations in MTHFR-deficient patient, 1993, Am. J. Hum. Genet, 53 (suppl.): A153.
8. Leclerc D., Rozen R., 2007, Molecular genetics of MTHFR: Polymorphisms are not all benign Medecine sciences, 23(3):297-302.
9. Liew S., Gupta E. D., 2015, Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases, Eur J Med Genet., 58(1): 1-10.
10. Mircea Covic, Dragoș Ștefănescu, Ionel Sandovici, 2011, Genetică medicală. Ediția a II-a, Editura Polirom, Iași.
11. Nichols M., Townsend N., Luengo-Fernandez R., Leal J., Gray A., Scarborough P., Rayner M., 2012, European Cardiovascular Disease Statistics 2012. European Heart Network, Brussels, European Society of Cardiology, Sophia Antipolis.
12. OMIM,Online Mendelian Inheritance in Man,http://www.ncbi.nlm.nih.gov/omim/
13. Shan J. G., Xue S., 2016, MTHFR C677T polymorphism and coronary artery disease risk in the Chinese population: a meta-analysis based on 33 studies, Int J Clin Exp Med, 9(2):2822-2830.
14. Ueland P. M., Vollset S. E., 2004, Homocysteine and folate in pregnancy, Clin Chem, 50:1293-1295.
15. World Health Organisation, 2016, Cardiovascular diseases. Fact sheet N°317. Available from: http//:www.who.int/mediacentre/factsheets/fs317/en/index/html.
16. World Health Organization. Rapport sur la situation mondiale des maladies non transmissibles, 2014, «Atteindre les neuf cibles mondiales: une responsabilité partagée», Ed. OMS.
17. Xuan C., Bai X. Y., Gao G., Yang Q., He G. W., 2011, Association between polymorphism of methylenetetrahydrofolate reductase (MTHFR) C677T and risk of myocardial infarction: a metaanalysis for 8,140 cases and 10,522 controls, Arch Med Re, 42(8):677–685.
18. Yadav U., Kumar P., Gupta S., Rai V., 2016, Role of MTHFR C677T gene polymorphism in the susceptibility of schizophrenia: An updated meta-analysis, Asian J Psychiatr, 20:41-51.