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**SHORT COMMUNICATION**

# Effect of C677T Methylenetetrahydrofolate Reductase Gene Polymorphism on Plasma Homocysteine Levels in Ethnic Groups

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## Summary

The objective of this study was to examine plasma homocysteine levels and C677T methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in two ethnic groups from Slovakia. The samples consisted of general Slovak-Romany population (68 men and 81 women) from Southwestern Slovakia and the Slovak-Caucasians (174 men and 177 women) who participated in the CINDI project. The homocysteine levels were examined by HPLC, the analysis of MTHFR genotypes was done by PCR. The Slovak-Romany men (12.0±5.6 (S.D.) μmol/l) and women (9.2±2.6 μmol/l) have significantly lower plasma homocysteine levels ($p<0.024$ and $p<0.00001$) when compared to Caucasians (13.3±5.1 μmol/l in men and 11.3±4.3 μmol/l in women). The genetic equilibrium is assumed for the gene frequencies of the MTHFR polymorphism in both samples. The distribution of MTHFR genotypes did not differ between the two populations (TT 13 vs. 10.6 %; CT 46.6 vs. 41.7 %; CC 40.4 vs. 47.7%, $\chi^2 = 2.315$, df=2, ns). The effect of MTHFR genotypes on homocysteine levels was not confirmed in the Slovak-Romanies and TT homozygosity significantly increased plasma homocysteine levels only in Slovak-Caucasians (11.5±4.4 μmol/l, ns; vs. 14.8±4.8 μmol/l, $p<0.002$, respectively). To our knowledge, this is the first epidemiological study in the Romany population examining distribution of the MTHFR genotypes and their effect on homocysteine levels. Further studies are needed to establish the variety of cardiovascular risk factors among Romanies in order to evaluate the significance of particular factors.

**Key words**

C677T methylenetetrahydrofolate reductase polymorphism • Homocysteine • Slovak-Romanies • Slovak-Caucasians • Ethnic groups

Plasma homocysteine levels are influenced by many dietary and environmental factors as well as genetically based alterations of homocysteine transsulphurylation or remethylation (Lynch *et al.* 1995, Toborek and Henneig 1996). The decreased activity of the enzyme 5,10-methylenetetrahydrofolate reductase



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(MTHFR) is one of the most frequent genetic causes of moderately increased plasma homocysteine (Ueland *et al*. 1993). A polymorphism (nucleotide 677 C⟶T) appears to be responsible for this functional defect. Consequently, higher levels of homocysteine have been considered as an independent risk factor for vascular diseases (Frosst *et al.* 1995). The frequencies of this polymorphism and its impact on plasma homocysteine concentrations vary among different populations (Gudnason *et al*. 1998, Schneider *et al*. 1998, Belkovets *et al*. 2001, Cappuccio *et al.*2002). Considering these data, the objective of this study was to examine the relationship between the C677T MTHFR genotype and homocysteine concentrations in two population samples, Slovak-Romanies and Slovak-Caucasians.

The Romany subjects (Slovak-Romanies, 68 men and 81 women) were inhabitants of a village situated in Southwestern Slovakia, which is characterized by a large Romany population. At the time of data collection there was a total of 3322 inhabitants and nearly half of them were Romanies. The sample is representative of the general Romany population. The medical examination was done in cooperation with their general practitioner. Only adult probands were invited to participate in this study. The Slovak-Caucasians (174 men and 177 women) were participants of the CINDI (Countrywide Integrated Noncommunicable Diseases Intervention) survey from Banská Bystrica in Central Slovakia (Avdičová *et al*. 2000).

Two indices were calculated to characterize the examined subjects. Body mass index (BMI) and the waist to hip ratio (WHR). Personal and family history, life style habits and socioeconomic status were examined using a questionnaire that was completed by the subjects and reviewed by the investigating personnel. The homocysteine concentrations were analyzed by HPLC using a standard kit (CHROMSYSTEMS Instruments & Chemicals GmbH, Munich) after collection of blood into EDTA tubes and centrifugation for 15 min within one hour at 4 °C.

The C677T MTHFR genotypes were analyzed by PCR using a forward primer 5`-TGAAGGAGAAGGTGTCTGCGGGA and a reverse primer 5`- AGGACGGTGCGGTGAGGAGGTG. The amplified DNA was digested by *Hinf* I at 37 °C for one hour (Frosst *et al*. 1995).

The normal distribution of the data was analyzed using the Kolmogorov-Smirnov test. Allele frequencies were tested for the Hardy-Weinberg equilibrium. The differences between the quantitative parameters were examined using Student´s t-test and Wilcoxon´s test. Correlation analysis was done by analysis of variance (ANOVA) and partial Pearson correlation tests.

Statistical analyses were performed using the software SPSS 6.0.1 for Windows. The results are expressed as means ± S.D. The Ethics Committee of the Institute of Preventive and Clinical Medicine (Bratislava) approved the study.

Table 1 shows some of the characteristics of the population samples separated according to gender. The variables characterizing the degree of obesity demonstrate a very high average value of BMI in both groups. The Slovak-Caucasians, both men and women, were characterized as overweight, and the Slovak-Romanies were characterized as obese. These differences were statistically significant. Both, the Romany men and women, attained limited values of central obesity (WHR index) and they differed significantly in comparison with Slovak-Caucasians. The Romanies also had much higher BMI and WHR values compared to Slovak-Caucasians patients who survived premature myocardial infarction and who showed many typical signs of the metabolic syndrome (Rašlová *et al*. 2001). On the other hand, homocysteine concentrations were significantly lower in Slovak-Romanies than in Slovak-Caucasians (Tables 1 and 2). The distribution of genotypes and allele frequencies of MTHFR polymorphisms and corresponding homocysteine concentrations in the studied samples are shown in Table 2. The observed and expected genotype frequencies were in agreement and a genetic equilibrium can therefore be assumed for this polymorphic system in both samples (Slovak-Romanies $\chi^2$=0.00797, df=1, p=0.05; Slovak-Caucasians $\chi^2$=0.363, df=1, p=0.05). In particular, there were no indications of increased frequencies of homozygous genotypes, which would be expected in a genetically isolated population, such as the Slovak-Romanies. The effect of MTHFR genotypes on homocysteine levels has not been confirmed in the Slovak-Romanies. However, TT homozygosity significantly increased plasma homocysteine levels in the Slovak-Caucasians (Table 2), in both men ($TT_{n=21}$: 15.6±4.9, $CT_{n=67}$: 13.4±5.7, $CC_{n=85}$: 12.6±4.4 μmol/l, p<0.05) and women ($TT_{n=16}$: 13.9±4.6, $CT_{n=79}$: 10.9±4.3, $CC_{n=82}$: 11.2±4.1 μmol/l, p<0.04), in comparison with the Slovak-Romany men ($TT_{n=5}$: 14.7±5.0, $CT_{n=31}$: 11.5±4.1, $CC_{n=31}$: 12.2±6.9 μmol/l, ns) and women ($TT_{n=14}$: 10.3±3.6, $CT_{n=37}$: 8.8±1.9, $CC_{n=28}$: 8.9±2.71 μmol/l, ns ).

**Table 1**. Characteristics of subjects and homocysteine (Hcy) concentrations in the studied samples

| | Men | | Women | |
|---|---|---|---|---|
| | **Slovak-Romanies n=68** | **Slovak-Caucasians n=174** | **Slovak-Romanies n=81** | **Slovak-Caucasians n=177** |
| *Age* | 42.1±13.9** | 47.7±8.8 | 40.9±13.7** | 46.2±8.1 |
| | 18-79 | 30-60 | 18-71 | 30-60 |
| *Weight (kg)* | 89.1±17.7* | 83.4±12.3 | 75.3±19.8 | 70.6±13.7 |
| *Height (cm)* | 171.9±6.0 ** | 175.1±6.7 | 159.5± 5.7** | 163.9±5.7 |
| *BMI (kg/m$^2$)* | 30.1±5.7*** | 27.2±3.8 | 29.6± 7.6** | 26.2±4.9 |
| *WHR* | 0.96±0.07 *** | 0.91±0.06 | 0.86±0.08*** | 0.80±0.06 |
| *Hcy (μ mol/l)* | 12.0±5.6* | 13.3±5.1 | 9.2±2.6*** | 11.3± 0.3 |

Data are means ± S.D. Significantly different from Slovak-Caucasians of the same gender: * p<0.05, ** p<0.01, *** p<0.001.

**Table 2.** C→T MTHFR allele and genotype frequencies and corresponding homocysteine levels

| | Slovak-Romanies | | Slovak–Caucasians | |
|---|---|---|---|---|
| | Homocysteine | | Homocysteine | |
| **Genotypes** | **% (n)** | **(μmol/l)** | **% (n)** | **(μmol/l)** |
| CC | 40.4 (59) | 10.7±5.5 | 47.7 (167) | 11.9±4.3 |
| CT | 46.6 (68) | 10.0±3.4 | 41.7 (146) | 12.0±5.1 |
| TT | 13.0 (19) | 11.5±4.4 | 10.6 (37) | 14.8±4.8** |
| *Alleles* C | 63.7 | | 68.6 | |
| T | 36.3 | | 31.4 | |

Data are means ± S.D. **p<0.01: significant effect of TT genotype on homocysteine levels in Slovak-Caucasians; % – frequency of genotypes and alleles, n – number of subjects with the respective genotype.

There were no significant differences in the distribution of MTHFR genotypes between men and women in both ethnic groups. Cappuccio *et al.* (2002) have recently published a study in which homocysteine concentrations and MTHFR genotypes were examined in Caucasian, African and South Asian – Indian (Hindu and Muslim) populations. The frequencies of TT MTHFR genotypes in the Indian population were significantly lower than in the Caucasians (2 % vs 10 %). They were also much lower than those found in our Romany population despite the generally accepted view that European Romanies came from India (Mastana and Papiha 1992). However, conclusive heterogeneity exists among Romany populations which may explain these differences. The factors responsible for moderate diversification of East European Romany groups may be due to a high rate of migration, isolation and random drift, while among Western groups admixture seems to be an important factor. The frequencies of MTHFR genotypes of Slovak-Romanies and Slovak-Caucasians were in accordance with many other studied European groups (Gudnason *et al.*1998). Homocysteine levels also depend on the nutrient content, especially folate and vitamins $B_6$ and $B_{12}$, so that their insufficient dietary intake could be responsible for higher homocysteine levels. Rowley *et al.* (2001) have shown that a low socio-economic status affects homocysteine levels, especially as the result of unhealthy dietary habits. One marker of low socioeconomic conditions of Slovak-Romanies could be the level of education. In our study, 72 % and 28 % of Slovak-Romanies had only elementary or apprentice education, respectively, which was significantly lower when compared with Slovak-Caucasians in whom 8 % of population had elementary, 17 % apprentice and 75 % higher education. Despite this, more favorable homocysteine concentrations were found in Slovak-Romanies than in Slovak-Caucasians. The body mass index (BMI) is often used as a proxy measure for body fatness because it correlated with body mass and percentage of body fat in the general population (Bouchard *et al.* 1988). Since the two studied populations strongly differed in the body fatness, we have examined the relationship between BMI, WHR and plasma homocysteine concentrations. Neither univariate or

multivariate analysis showed any effect of BMI and WHR on homocysteine levels in the studied ethnic groups.

To our knowledge, this is the first epidemiological study in the Romany population examining MTHFR genotypes and homocysteine levels. Further studies are needed to establish the variety of cardiovascular risk factors among Romanies in order to specify their significance.

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