Contribution of Haptoglobin and MTHFR polymorphisms to hyperhomocysteinemia and hypercysteinemia in type 2 diabetic patients

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Introduction

There is a lack of epidemiological data on the distribution of haptoglobin and C677T of MTHFR polymorphisms in Caucasians type 2 diabetic patients [1]. The contribution of these two genetic factors to hyperhomocysteinemia and hypercysteinemia can be useful to prevent cardiovascular events and to reduce public health costs.

Aim

To evaluate the contribution of haptoglobin and C677T polymorphisms for hyperhomocysteinemia and hypercysteinemia in Portuguese type 2 diabetic patients with and without angiopathy.

Methods

An observational analytical case-control study in 150 Portuguese type 2 diabetic patients (40-75 years) was performed. The study population was divided into two groups: group I - 75 patients with angiopathy, group II - 75 patients without angiopathy. Inclusion criteria for both groups were: caucasians and diagnosis of type 2 diabetes for at least 1 year. Additional criterion for group I was the presence of at least one of the following complications: cerebrovascular disease, ischemic stroke, peripheral vascular disease or retinopathy. Homocysteine and cysteine plasma levels were obtained by validated HPLC methods [2]. The haptoglobin polymorphism was identified by polyacrylamide gel electrophoresis and peroxidase staining, and C677T MTHFR polymorphism by PCR and RFLP. Statistical analysis was performed by odds ratio calculation.

Results

The baseline characteristics of diabetic patients studied are presented in Table 1. The Hp 2-1 was the most prevalent phenotype (Figure 1). The CT + TT genotypes were more frequent in group I (46.4%; 7.2%) than in group II (41.5%; 6.2%) (Figure 2). Homocysteine and cysteine mean concentrations according with Hp and MTHFR genotypes are presented in Figures 3, 4, 5 and 6, respectively. The diabetic patients of group I with the genotypes Hp 2-1 + Hp 2-2 had a higher probability to have hyperhomocysteinemia (OR: 4.33; p = 0.014) when compared to diabetic patients without angiopathy (Table 2). The presence of C677T + 677TT genotypes in group I increased five times (OR: 5.37; p = 0.040) the probability to have hyperhomocysteinemia compared to group II (Table 3). The association between Hp 2-1 and C677T polymorphisms increased the probability (OR: 4.40 p = 0.006) to have hypercysteinemia in group I (Table 4).
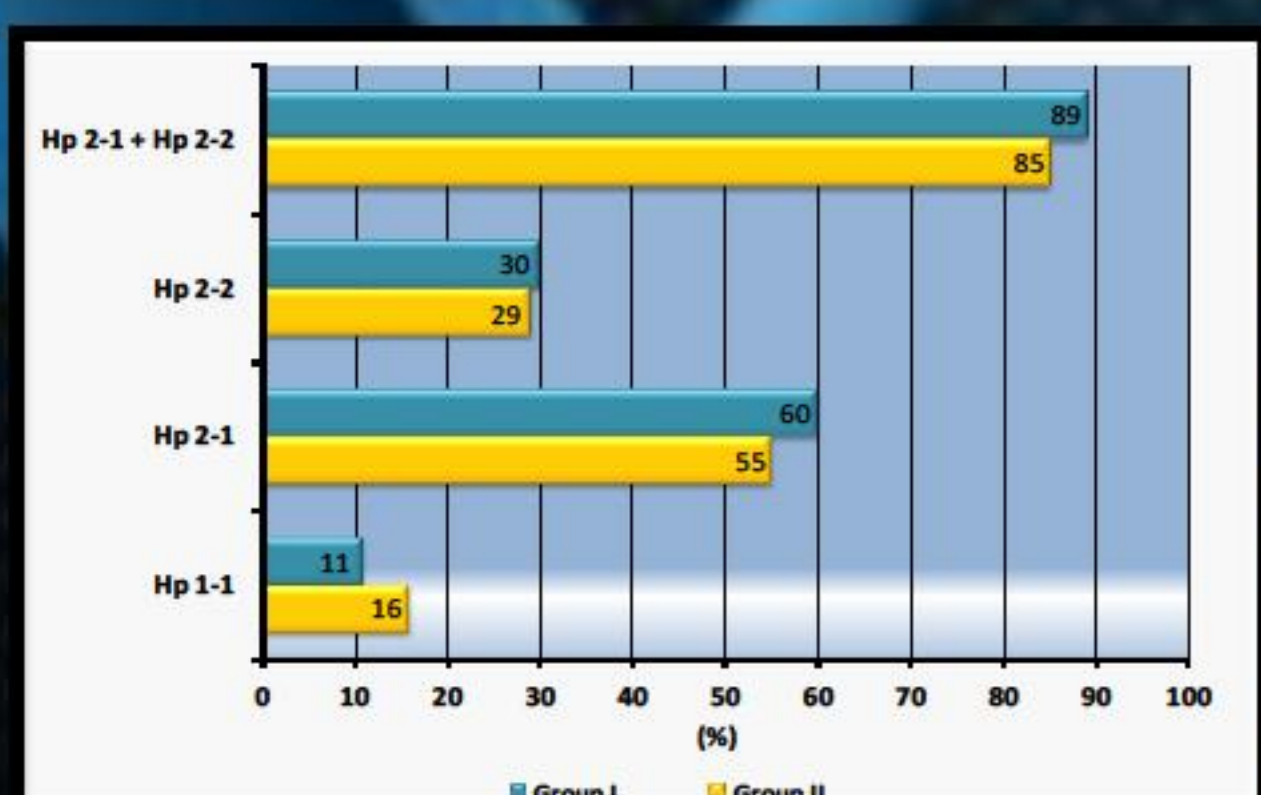

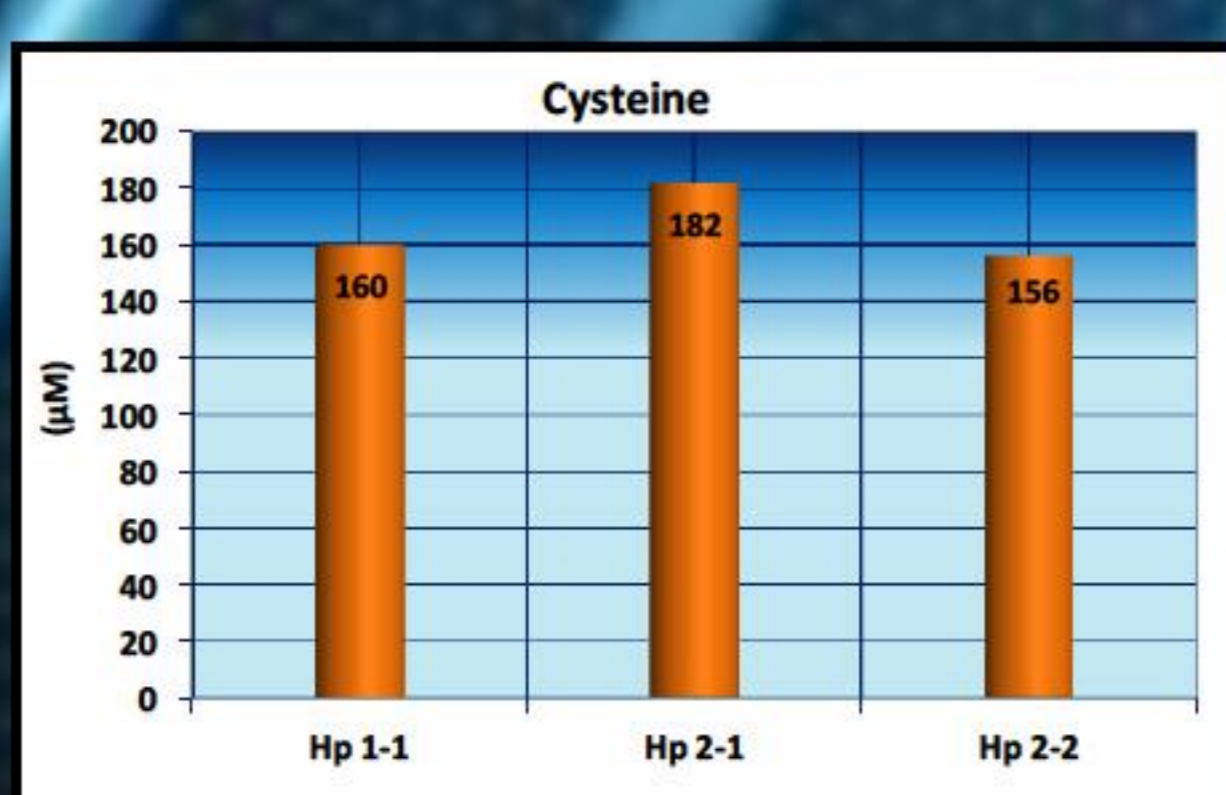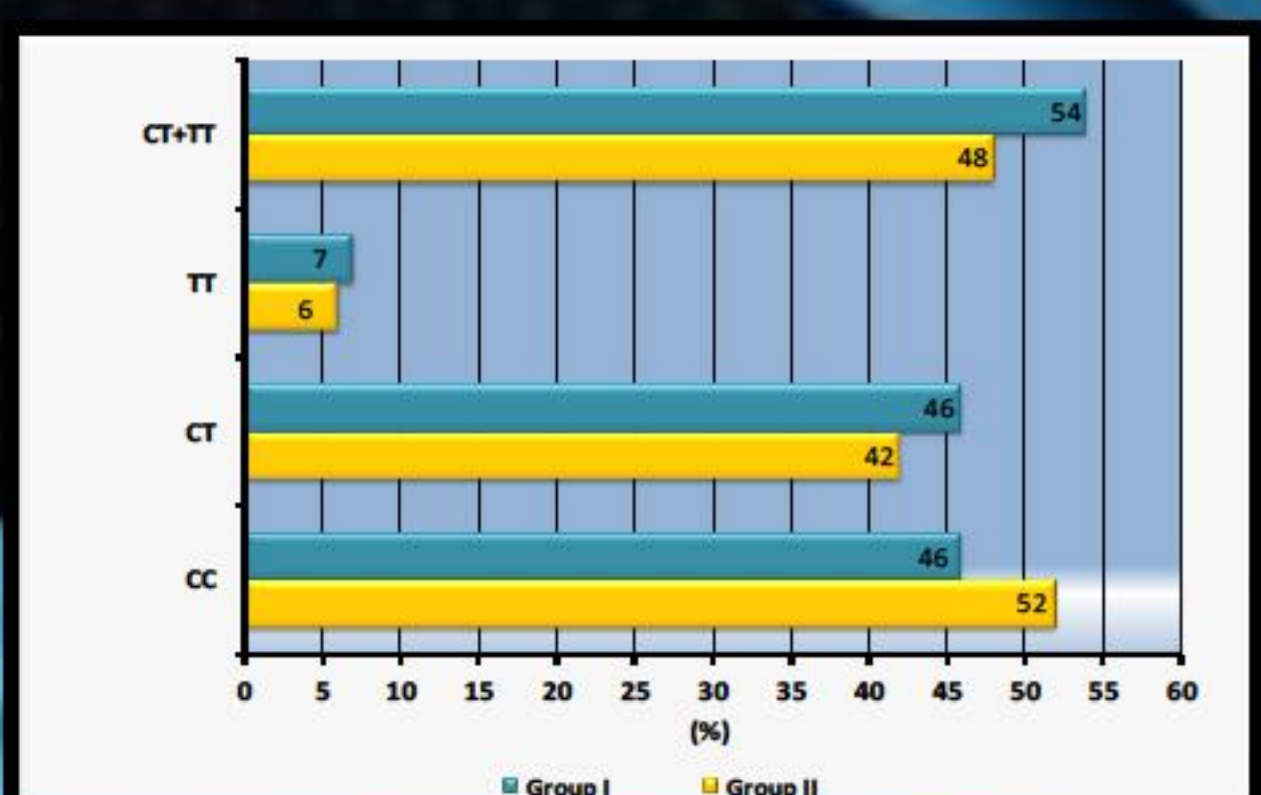Fig. 1 - Haptoglobin phenotype frequency in study population.

Fig. 2 - Frequency of C677T MTHFR genotype in study population.

Fig. 3 - Mean plasma levels of homocysteine according to haptoglobin phenotype.

Fig. 4 - Mean plasma levels of cysteine according to haptoglobin phenotype.
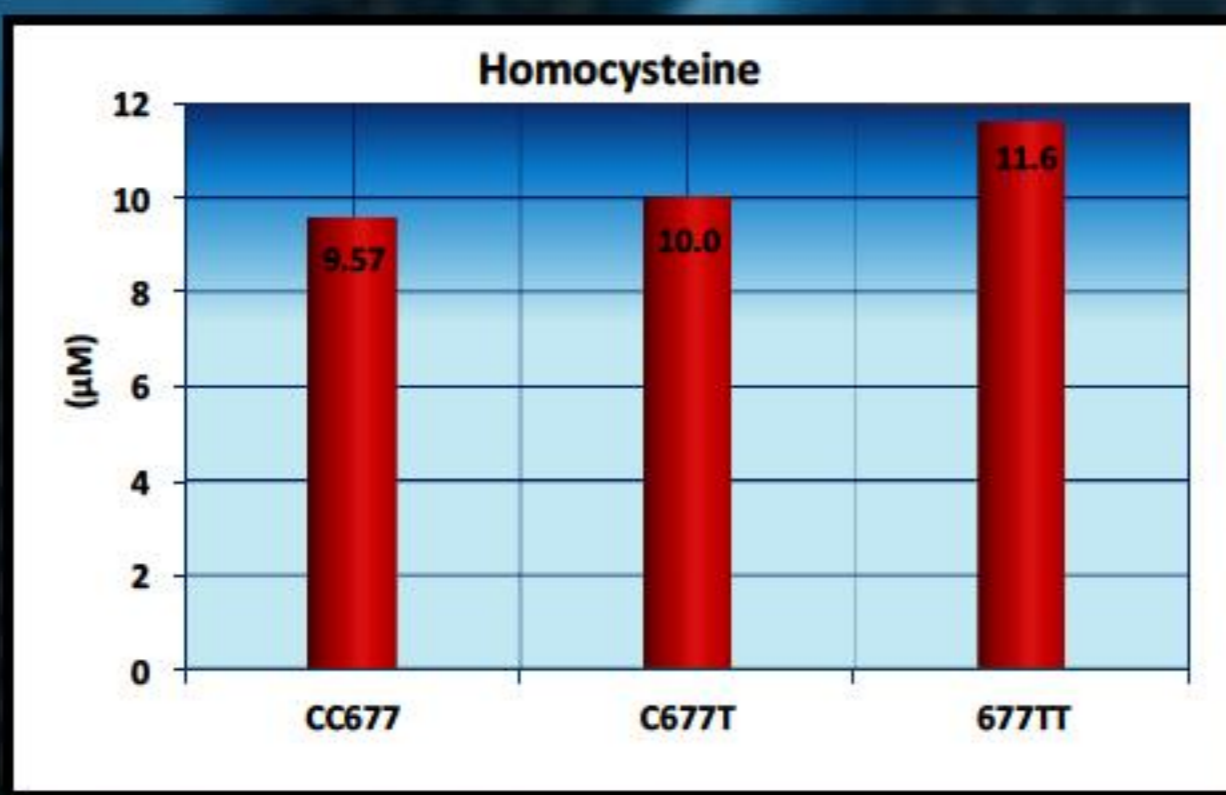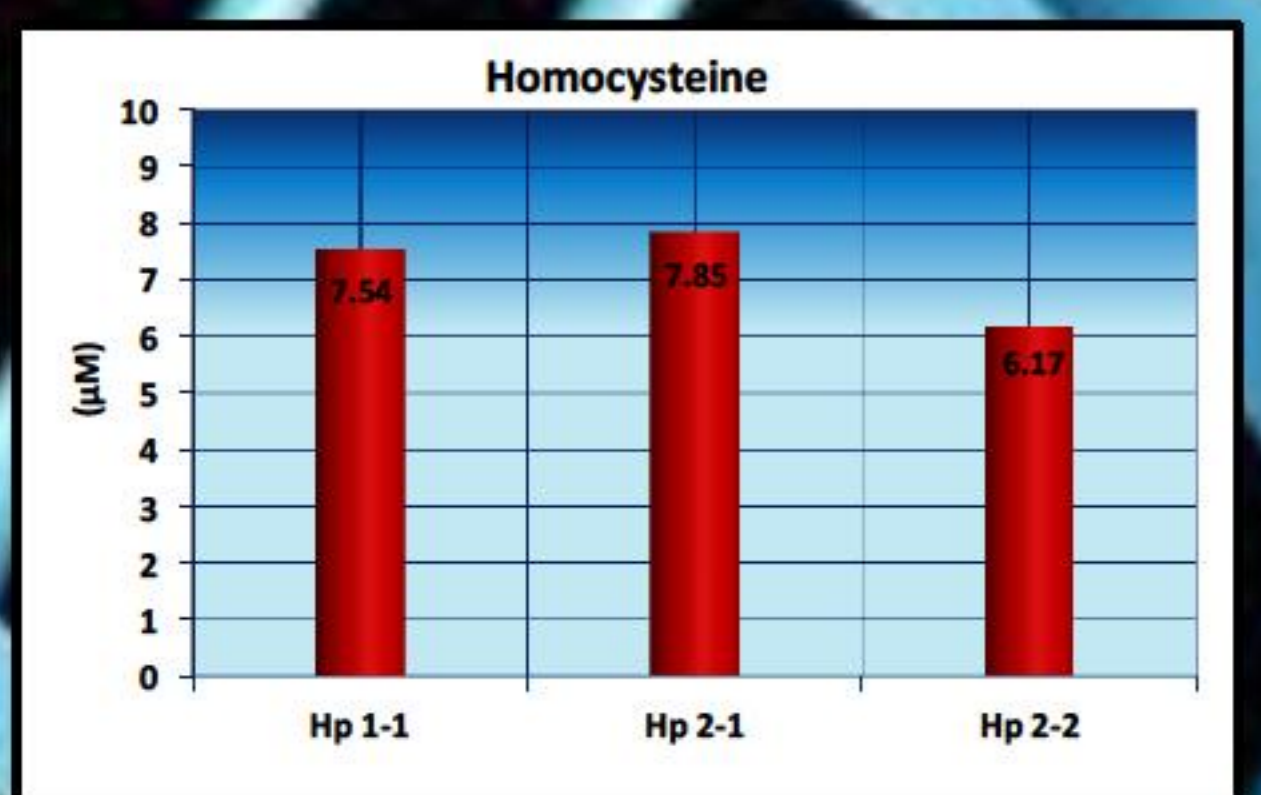

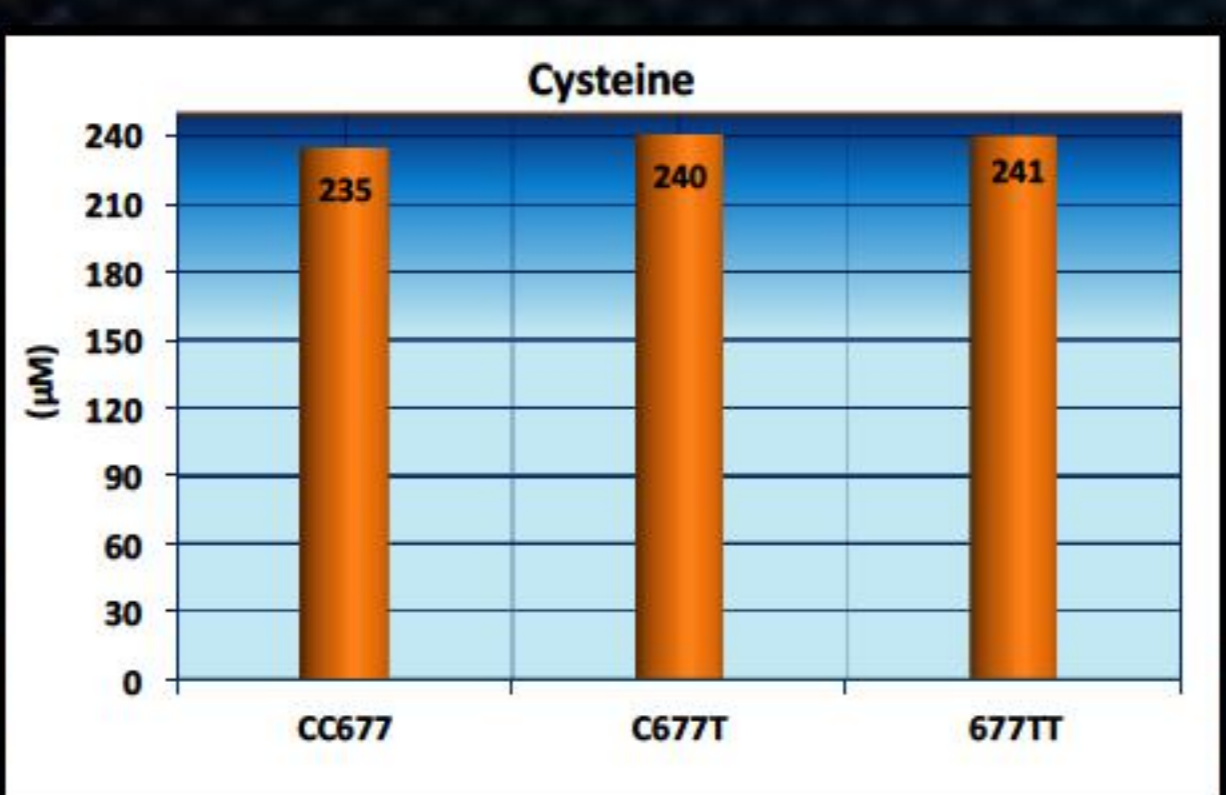Fig. 5 - Mean plasma levels of homocysteine according to C677T polymorphism of MTHFR.

Fig. 6 - Mean plasma levels of cysteine according to C677T polymorphism of MTHFR.

Table 1 - Baseline clinical and laboratory characteristics of the study population.

Characteristics	Group I (n = 75) Mean	SD	Group II (n = 75) Mean	SD
Age (years)	62.9[a]	7.17	62.8[a]	7.01
Diabetes duration (years)	19.4[a]	9.33	13.5[b]	7.95
Hb (g/dl)	13.4[a]	1.72	13.7[a]	1.42
HbA1c (%)	8.70[b]	1.42	8.22[a]	1.39
Hematocrit (%)	39.3[a]	4.74	40.3[a]	4.03
Erythrocytes (millions/μl)	4.58[b]	0.59	4.64[a]	0.44
Total cholesterol (mmol/l)	5.24[a]	1.22	5.18[a]	0.97
HDL-Cholesterol (mmol/l)	1.27[b]	0.38	1.34[a]	0.35
LDL-Cholesterol (mmol/l)	3.34[a]	0.96	3.22[a]	0.85
Triglycerides (mmol/l)	2.19[a]	1.07	1.94[a]	1.21
Systolic blood pressure (mmHg)	154[a]	20.3	148[b]	25.9
Diastolic blood pressure (mmHg)	79.2[a]	13.1	82.0[a]	12.7
Resting heart rate (beats/min)	73.2[a]	12.0	77.6[a]	12.9

NA, not applicable; Hb, haemoglobin; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Means in a row with different superscript letters are significantly different by post hoc Bonferroni test (P<0.05)

Table 2 - Effect of Hp 2-1 + 2-2 phenotypes on the variation of homocysteine and cysteine plasma levels.

Parameters	Group I Hp 2-1 + Hp 2-2 (n = 67) exposed / non-exposed	Group II Hp 2-1 + Hp 2-2 (n = 64) exposed / non-exposed	OR	CI (95%)	p
Hyperhomocysteinemia[a]	15/52	4/60	4.33	1.35-13.9	0.014*
Hypercysteinemia[b]	13/54	5/59	2.84	0.95-8.50	0.062

CI, confidence interval; OR, Odds ratio. *statistically significant (p<0.05).
[a]homocysteine ≥15 μM; [b]cysteine ≥300 μM

Table 3 - Effect of C677T and T677T genotypes of MTHFR on the variation of homocysteine and cysteine plasma levels.

Parameters	Group I C677T + 677TT (n = 37) exposed / non-exposed	Group II C677T + 677TT (n = 31) exposed / non-exposed	OR	CI (95%)	p
Hyperhomocysteinemia[a]	10/27	2/29	5.37	1.08-26.8	0.040*
Hypercysteinemia[b]	8/29	2/29	4.00	0.78-20.5	0.096

CI, confidence interval; OR, Odds ratio. *statistically significant (p<0.05).
[a]homocysteine ≥15 μM; [b]cysteine ≥300 μM

Table 4 - Combined effect of Hp 2-1 phenotype and C677T genotype of MTHFR on the variation of homocysteine and cysteine plasma levels.

Parameters	Group I Hp 2-1 + C677T (n = 76) exposed / non-exposed	Group II Hp 2-1 + C677T (n = 71) exposed / non-exposed	OR	CI (95%)	p
Hyperhomocysteinemia[a]	21/55	6/65	4.14	1.59-11.0	0.004*
Hypercysteinemia[b]	19/57	5/66	4.40	1.54-12.5	0.006*

CI, confidence interval; OR, Odds ratio. *statistically significant (p<0.05).
[a]homocysteine ≥15 μM; [b]cysteine ≥300 μM

Conclusion

The haptoglobin 2-1 and C677T of MTHFR polymorphisms are associated with a predisposition to have hyperhomocysteinemia and their combined effect increased the probability to have hypercysteinemia. These polymorphisms should be considered risk factors for angiopathy development of Caucasians type 2 diabetic patients.

REFERENCES
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[2] Valente A, Bronze MR, Bicho M, Duarte R, Costa HS. Validation and clinical application of an UHPLC method for simultaneous analysis of total homocysteine and cysteine in human plasma. J Sep Sci 2012; 35(24):3427-3433.