Environmental influence on the world-wide prevalence of a 776C>G variant in the transcobalamin gene (TCN2)

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Environmental influence on the world-wide prevalence of a 776C>G variant in the transcobalamin gene (*TCN2*)

Running title: **Environmental influence on *TCN2* 776C>G**

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ABSTRACT

Background. A 776C>G variant (dbSNP ID: rs1801198) in the transcobalamin gene (*TCN2*; MIM# 275350) decreases the cellular and plasma concentration of transcobalamin and thereby influences the cellular availability of vitamin B12.
Objective. To evaluate the world-wide prevalence of this variant and its association with homocysteine plasma level.
Methods. The study was performed in 1433 apparently healthy subjects, including Afro-Americans and Afro-Africans and in 251 Afro-Africans subjects with severe malaria.
Results. The frequencies of 776G allele were the highest in China (0.607 [95% confidence interval:0.554-0.659]), low in West Africa (Benin and Togo, 0.178 [0.154-0.206]) and intermediate in France (0.445 [0.408-0.481]), Italy (0.352 [0.299-0.409]), Marocco (0.370 [0.300-0.447]) and Mexico (0.374 [0.392-0.41.9]). The 776GG genotype was more frequent in Afro-Americans from New York (16.7 [8.4-30.7]) and in Afro-African patients with severe malaria (6.0% [95%CI: 3.7-9.6]) than in healthy Afro-African volunteers (p=0.0004 and p=0.0329, respectively), while no difference was observed for *MTHFR* 677TT and 677T allele. A disequilibrium of *TCN2* genotype distribution was recorded in the patients with severe malaria, with a 2-fold higher *GG* genotypes than expected (p=0.010). An association between the *TCN2* polymorphism and homocysteine was observed only in Mexico and France, the two countries with the highest rate of low plasma concentration of vitamin B12 (<100 pmol/L).
Conclusion. Our results suggest therefore that, given the dramatic heterogeneity of the *776G* allele frequency worldwide, this polymorphism may be prone to a selective pressure or confers an evolutionary advantage in confronting environmental factors, one of which being malaria.

Key words: Transcobalamin; homocysteine; nutrigenetics; malaria

INTRODUCTION

Accumulating evidence suggests that the metabolisms of homocysteine and of vitamin B12 play a role in developmental disorders [1]. Homocysteine is a cross point substrate of the so called one-carbon metabolism. This metabolism is crucial for DNA synthesis and repair and for a wide range of trans-methylation reactions, including those of DNA and histones that are implicated in the regulation of gene expression [2]. The concentration of homocysteine in humans is influenced by the dietary intake of folate and vitamin B_{12} and by the polymorphisms in genes that encode enzymes or proteins involved in the specific transport of folate and vitamin B_{12} [3]. One of these enzymes, methylenetetrahydrofolate reductase (MTHFR), catalyzes the synthesis of 5-methyltetrahydrofolate, the methyl donor for vitamin B12-dependent re-methylation of homocysteine to methionine. A 677 C >T variant (dbSNP ID rs1801133) of *MTHFR* (MIM# 607093) decreases by 4-fold the catalytic rate of the enzyme and consequently, increases t-Hcys especially in low folate status [4;5]. This variant is also a genetic determinant of folate imbalance between DNA synthesis and/or methylation [6]. Its world wide distribution is very heterogeneous, the highest and the lowest *T* allele frequency being reported in Mexico and sub-Saharian Africa, respectively [7,8,9]. It has been hypothesized that dietary folate is one of the factors that has influenced the prevalence of 677 T allele world wide [10,11].

Another common genetic polymorphism that influences homocysteine and vitamin B12 metabolisms is a 776C>G variant in the transcobalamin gene (*TCN2*, MIM# 275350) that results in the substitution of proline for arginine in codon 259 (dbSNP ID: rs1801198 [12,13]. *TCN2* 776G allele decreases the transcription and the cellular and plasma concentration of transcobalamin, the carrier protein which delivers vitamin B12 to cells [13]. The influence of *TCN2* 776 G allele on the cellular availability of vitamin B12 has been documented by its association with plasma apotranscobalamin, holotranscobalamin, homocysteine and methylmalonic acid, in Caucasians [13,14,15]. This polymorphism seems to be associated with an increased risk of spontaneous abortion [16]. The possibility of a gene-gene interaction between the *MTHFR* and *TCN2* polymorphisms has been also suggested in this context [17]. More recently, *TCN2* polymorphism was found to be associated with non-syndromic cleft lip with or without cleft palate [18]. Taken together, these data suggest an association between *TCN2* 776 G allele and the pathological and developmental events of the very early steps of life. Whether this association is influenced by environmental factors is not known and if it were the case, it could reasonable to predict its heterogeneous distribution in populations that are exposed to a contrasted environment. To address this issue, we studied therefore the worldwide distribution of *TCN2* 776 G allele in populations from Europe, Mediterranean area, West Africa, China and Mexico and we compared Afro-Africans from West Africa with Afro-Americans. We also evaluated the influence of this variant on plasma homocysteine concentration in those populations that were accessible to plasma collection.

METHODS

The study was carried out on a population of 1433 apparently healthy subjects, with an age range of 20-60 years. Ethics approval for study of DNA variants in the untraceable DNA samples was obtained through the Research Ethics Board of Troina (Sicily, Italy). Similarly, local approval was obtained for DNA samples from New York and China. All the other subjects were medically examined to rule out cancer, cardiovascular, renal, hepatic and genetic disease and recurrent vitamin supplementation prior to inclusion in the study. Institutional review board approval was obtained from the ethical committees of the medical center of Troina (IRCCS Associazione Oasi Maria S.S., Troina, Sicily, Italy), University Hospital Center of Nancy (Nancy, France), University of Bénin (Lomé, Togo), University of Cotonou (Cotonou, Bénin), Instituto Nacional de Ciencias Medicas y Nutricion (Mexico DF, US of Mexico) and University Hospital of Casablanca (Marocco). Informed consent was obtained from the subjects. The volunteers from Sicily were from a rural area, in the North-East of Sicily. Volunteers of Togo were recruited in the coast (Lomé) and the savanna. They have been already described in a recent publication [8]. The volunteers from Benin were recruited in the coastal urban area of Cotonou. The volunteers from Mexico were blood donors at the Instituto Nacional de Ciencias Medicas y Nutricion and originated from the periphery of Mexico City and those from China and New York were blood donors from the region of Wuhan and New York City, respectively. A group of 251 patients with severe malaria was also recruited in the university hospital center of Cotonou (Benin). All blood specimens were tested in a single laboratory (Nancy, France). Fasting venous blood was collected in EDTA-containing tubes and after immediate centrifugation, aliquots were stored at -30° C until analysis. Plasma homocysteine concentrations (protein-bound and free homocysteine) were determined by the Fluorescence Polarization Immunoassay (FPIA) IMx-Homocysteine method developed by Abbott (ABBOTT Laboratories Diagnostics Division, Abbott Park–IL, USA). Buffy coat was prepared from the previously collected blood and genomic DNA was isolated with the Qiagen kit (Qiagen-France, Courtaboeuf cedex France). Plasma vitamin B12 and Folate concentrations were determined by the immunoassay kit “VB12 and Folates” on an ACS 180 automated chemiluminescent system (Chiron Diagnostics Corporation East Walpole MA, USA) with threshold values established respectively at 100 pmol/L and 7 nmol/L, respectively [11]. Genotyping of the *TCN2* 776 C > G polymorphism was performed by the amplification-refractory mutation system, as described recently by us [12]. PCR-based RFLP method was used to determine the genotypes of *MTHFR* [8]. Allele and genotype frequencies were reported as percentages and 95% exact confidence interval and a Fisher test was used to assess differences between the groups. Continuous variables were reported as mean and standard deviations. Comparisons of continuous variables were performed by Mann-Whitney test. P-values lower than 0.05 were considered significant. Data were prospectively collected and analysed using the SAS software for Windows (SAS Institute, Berkeley, California, USA).

RESULTS

The distribution of the genotypes of *TCN2* 776 C>G polymorphism among the 7 populations is presented in table 1.

Table 1: Distribution of *TCN2* 776C>G genotypes worldwide

Geographical area	**Ethnicity**	**Number of subjects**	**Genotype counts Number, % [95%confidence interval]**		
			776CC	*776CG*	*776GG*
Togo	Afro-Africans	195	134, 68.7 [62.0-74.9]	52, 26.7 [20.8-33.1]	9, 4.6 [2.2-8.2]
Benin	Afro-Africans	214	142, 66.3 [59.9-72.4]	68, 31.8 [25.8-38.2]	4, 1.9 [0.6-4.3]
West Africa (Togo + Benin)	Afro-Africans	409	276, 67.5 [62.8-71.8]	120, 29.3 [25.1-33.9]	13, 3.2 [1.9-5.4]
New York	Afro-Americans	42	21, 50.0 [35.4-64.5]	14, 30.3 [21.0-48.5]	7, 16.7 [8.4-30.7]
North East United States*	Afro-Americans	51	19, 37.2 [25.9-50.7]	27, 52.9 [39.5-19.9]	5, 9.8 [3.6-19.9]
New York + North-East United States	Afro-Americans	93	40, 43.0 [33.4-53.2]	41, 44.1 [34.4-54.2]	12, 12.9 [7.6-21.2]
Marocco	Arabs-Caucasians	81	35, 43.2 [33.0-54.1]	32, 39.5 [29.6-50.4]	14, 17.3 [10.6-29.0]
France	Caucasians	353	109, 30.9 [26.3-35.9]	174, 49.3 [44.1-54.5]	70, 19.8 [16.0-24.3]
Italy (Sicily)	Caucasians	142	58, 40.8 [33.1-49.1]	68, 47.9 [39.8-56.1]	16, 11.3 [7.1-17.5]
Mexico	Hispanics	239	102, 42.7 [36.6-49.0]	95, 39.7 [33.8-46.1]	42, 17.6 [13.3-22.9]
Central China	Hans	167	34, 20.4 [15.0-27.1]	63, 37.7 [30.7-45.3]	70, 41.9 [34.7-49.5]

* Data from reference 19

The highest frequency was reported in China, this genotype being even more frequent that the 776CC genotype in this population. A low frequency of GG genotype was observed in the populations from West Africa (table 2).

Table 2: *TCN2* 776G and *MTHFR* 677T allele frequencies worldwide

Geographical area	Ethnicity	Number of alleles	Allele frequency [95%confidence interval]	
			TCN 776G	*MTHFR* 677T
Togo	Afro-Africans	390	0.179 [0.144-0.219]	7.4 [5.1-10.3]
Benin	Afro-Africans	428	0.177 [0.143-0.216]	10.7 [5.1-10.3]
West Africa	Afro-Africans	818	0.178 [0.154-0.206]	0.093 [0.075-0.115]
New York	Afro-Americans	84	0.333 [0.242-0.440]	0.107 [0.057-0.191]
North East United States*	Afro-Americans	102	0.363 [0.274-0.458]	
New York + North East United States	Afro-Americans	186	0.349 [0.284-0.349]	
Marocco	Arabs-Caucasians	162	0.370 [0.300-0.447]	0.289 [0.218-0.373]
France	Caucasians	706	0.445 [0.408-0.481]	0.354 [0.318-0.392]
Italy (Sicily)	Caucasians	284	0.352 [0.299-0.409]	0.438 [0.382-0.496]
Mexico	Hispanics	478	0.374 [0.392-0.41.9]	0.581 [0.538-0.622]
Central China	Hans	334	0.607 [0.554-0.659]	0.392 [0.344-0.442]

* Data from reference 19

In addition, no difference was observed between the subjects from Togo and those from Benin, nor between those from the coast of Togo and those from the Savannah. In contrast, a two fold higher frequency of *TCN2* 776GG genotype was reported in the patients from Benin with severe malaria (6.0% [95%CI: 3.7-9.6], Figure 1). Surprisingly, the frequency of 776GG genotype in Afro-Americans was not significantly different from that reported in Caucasian populations (p=0.8387), but it was dramatically higher than that observed in the Afro-African subjects (table 1, p= 0.0004). It was in the same order of magnitude as that reported in another group of Afro-Americans from North eastern United States [19]. By comparison, the frequency of *MTHFR* 677TT genotype was similar in both African populations from West Africa and New York [8], indicating that the difference in 776GG genotype was not due to crossbreeding.

The same contrasted groups were evidenced when considering the allele frequencies of *TCN2* polymorphism, instead of *TCN2* 776GG genotype. The 776G allele was the most predominant allele in the Chinese population (*P<0.0001*), the less frequent allele in West Europe, Mediterranean areas and Mexico, and its frequency was dramatically lower in Afro-African subjects than in any other ethnicity (Table 2, P<0.0001). The 776G allele frequency was 2-fold lower in Afro-Africans, compared with Afro-Americans from New York (Table 2, p= *0.0012*) and with the sum of Afro-Americans from New York and from the study of Bowen et al. (2004) (Table 2, *P<0.0001*). The allele frequency in France was not different from that previously reported in Sweden, in the Netherlands, in Ireland, in Greece and in the Caucasian populations from North America [13,19,20,21,22,23,24]. In contrast, it was significantly higher than that reported in Sicily (p=*0.0084*) and in Mexico (p=*0.0161*). The distribution of *MTHFR* 677T allele frequency among the 7 populations was similar to previous reports of the literature [7,8]. In particular, it was in the same order of magnitude in the Afro-African from West Africa and Afro-American from New York. In addition, similar results were reported in the Afro-African population from Atlanta [9]. We investigated the hypothesis of a gene-gene influence of *MTHFR* on the distribution of *TCN2* polymorphism

and vice versa. We failed to find any difference in *776G* allele frequency between the subjects carrying the MTFHR 677T allele and those carrying the 677C allele, in any of the 7 populations. There was no evidence of Hardy-Weinberg disequilibrium of the genotype distribution in West Africa, North eastern United States, Marocco, France and Italy (Chi-square: 0.0002, p=*0.989*, 2.619, p=*0.106*, 0.049, p=*0.825*, 1.869, p=*0.169*, 0.001, p=*0.920*; 0.350, p=*0.554*, respectively). In contrast, the frequency of observed *TCN2* 776CG genotypes was slightly higher than expected in the population samples from China and Mexico and as much as two-fold higher than expected in the African patients with severe malaria (Table 3 and figure 1).

Table 3. Hardy-Weinberg disequilibrium in healthy volunteers from Mexico, China and in patients with severe malaria from Benin.

Geographical area	***Genotype***	***Observed***	***Expected***	***Chi-square***	P-*value (two-tailed)*
Mexico	CC	102	93.5	0.769	
	CG	95	112.0	2.572	
	GG	42	33.5	2.151	
Total				5.492	0.019
China	CC	34	25.7	2.696	
	CG	63	79.6	3.473	
	GG	70	61.7	1.119	
Total				7.288	0.007
Benin - Severe Malaria	CC	172	165.8	0.232	
	CG	64	76.4	2.018	
	GG	15	8.8	4.367	
Total				6.617	0.010
Benin – Healthy Volunteers					
	CC	142	144.7	0.052	
	CG	68	62.5	0.482	
	GG	4	6.7	1.114	
Total		214		1.648	0.199

The homocysteine plasma level was the highest in Africa, intermediate in Morocco, France and the lowest in Italy and Mexico, with respective values of 18.2±13.9, 11.4±3.2, 10.7±3.3, 9.9±5.9 and 10.3±3.7 µmol/L. The serum folate was the highest in Mexico, intermediate in Italy, France and Morocco and the lowest in Africa with respective values of 19.5±9.4, 13.4±6.3, 12.8±5.0, 11.3±5.1 and 10.8±6.8 nmol/L. By comparison, the vitamin B12 level was the highest in Africa and Morocco, intermediate in Mexico and Italy and the lowest in France, with respective values of 662.4±506.4, 590.7±428.5, 422.7±658.8, 347.5±158.7 and 296.8±191.4 pmol/L. We could evaluate the phenotypic influence of *TCN2* on homocysteine plasma concentration in the study groups, except for the subjects recruited in China and New York. An association between *TCN2* polymorphism and homocysteine was observed in the subjects from Mexico and France, but not in those from Italy, Morocco and Africa (figure 2).

DISCUSSION

Our study demonstrate a heterogeneity in the allele distribution of *TCN2* 776 C>G polymorphism worldwide, with the highest 776G allele frequency reported in China. By comparison, the allele frequency was respectively 3.4-fold lower and 1.5-fold lower respectively, in African and Caucasian populations. The 776G allele was even more frequent than the 776C allele in China. We did not observed any difference in 776G allele frequency among Caucasian populations from West Europe and the Mediterranean area. These frequencies were also in the same order of magnitude than those reported in Caucasian populations from North Europe and North America. In contrast, a dramatic difference of 776G allele frequency was observed between populations that have the same ethnicity. The Afro-African populations from New York and the North-East of United States originated from the coastal regions of West Africa as the population from Benin and Togo. However, they had a 2-fold higher frequency of *TCN2* 776G allele. It is noteworthy that such a difference was not observed with the *MTHFR* 677T allele, showing the absence of crossbreeding in the group of Afro-Americans. The *TCN2* 776G allele frequency was not related to its phenotypic influence on homocysteine, as the lowest frequency was observed in the African countries and in Sicily, e.g. in areas where this polymorphism had no effect on homocysteine plasma concentration.

TCN2 polymorphism may have a deleterious influence on very early events of life and on the normal development of the embryo. The prevalence of the mutated *TCN2* 776G allele is significantly higher in spontaneous abortion of embryos and the frequency of wild-type *TCN2* 776CC embryos is much lower among spontaneously aborted embryos, compared to a reference adult population [1,16]. Embryos that had combined *MTHFR* 677TT and TN2 776CG or 776GG genotypes are at increased risk for spontaneous abortion compared to embryos that had only one of these genotypes [17]. In addition, *TCN2* 776C>G was the single polymorphism associated with the onset of non-syndromic cleft lip with or without cleft palate, in a recent study [18]. Contrasting results have been observed with regards to the association between *TCN2* 776C>G and the risk of neural tube defect, with no association reported in Ireland and the Netherlands and an increased risk in the cases with the predominant 677CC genotype of *MTHFR* polymorphism, in Sicily [20,24,25].

In regard to its worldwide distribution, the phenotypic influence of the *TCN2* polymorphism seems not to be primarily associated with its effect on homocysteine metabolism. An effect of the 776G allele on homocysteine concentration was observed only in Mexico and in France, in the two populations that had the highest rate of low concentration of vitamin B12. In fact, we have previously evidenced in different cell lines that this polymorphism was a determinant of the intracellular concentration of transcobalamin [12,13]. This polymorphism had a negative influence on the transcription level, leading to a dramatically lower concentration of the transcript in the 259RR variant than in the 259PP variant. [12,13].

Environmental factors may confer a selective advantage or exert a selective pressure on the frequency of the *TCN2* 776G allele, in regard to the dramatic differences observed in Africa, in the Afro-American populations and in China. One of the factors that may exert a pressure could be the dietary intake of vitamin B12, which is one of the strongest nutritional determinants of homocysteine metabolism. However, this hypothesis is not sustained by the lack of association between the allele frequency and the vitamin B12 status of the study groups. In addition, the lowest frequency of *TCN2* 776GG was observed in Benin and Togo, two countries where the polymorphism had no influence on plasma homocysteine level. Another hypothesis could be the influence of this polymorphism on the gravity of pathologies such as infections diseases in young populations. Among the pathologies, malaria is a good candidate for such an investigation. *Plasmodium Falciparum* requires substrates of the one

carbon metabolism that are provided by the host [26,27]. In addition, cellular vitamin B12 is involved in the synthesis of succinyl-CoA [28], the first substrate of the heme pathway, and haemoglobin is a molecule that is crucial for the growth of the parasite within the erythrocyte. The prevalence of Malaria is very high in West Africa and null in West-North America. We reported a two fold increased frequency of the 776GG genotype in patients with severe malaria, compared with the apparently healthy sample of population from the same urban area of Cotonou, in Benin. The criteria of severity were those defined by WHO. In addition, a disequilibrium of genotype distribution was recorded in these patients, with a 2-fold higher rate of GG genotype than expected (Table1). These data indicated therefore that malaria may exert a selective pressure on the frequency of *GG* genotype in Afro-Africans, considering that the mortality for malaria in Benin is one of the highest in the world, with a rate of complicated malaria estimated at 4.2% of children [29]. By comparison the prevalence of mortality related to malaria is very low in the province of Hubei (central China) [30] and it is null in Mexico city and in Europe. In addition it is noteworthy that the lowest frequency of the *TCN2* 776G allele in Europe was reported in Sicily, a region where the prevalence of Malaria was high until the middle of the 20th century. Finally, the dramatic difference in GG genotype frequency between Afro-African and Afro-American suggest that this variant may confer a selective advantage when the selective pressure is removed or absent. This could explain the Hardy-Weinberg disequilibrium that was observed in healthy volunteers from Mexico, with a frequency of GC and GG genotypes higher than expected (Table 3).

In conclusion, we have observed a worldwide heterogeneity of the distribution of the *TCN2* 776C>G polymorphism that provides evidences for its association with environmental factors in early events of life. One of the environmental factors that may exert a selective pressure could be malaria as the *TCN2* 776GG genotype was associated with the severity of this disease.

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FIGURE LEGENDS

Figure 1: Comparison (by Fisher test) of the frequency of *TCN2* 776GG and *MTHFR* 677TT in healthy volunteers from New York, Cotonou (Benin) and patients with severe malaria from Cotonou. 95% confidence intervals are indicated in brackets.
Figure 2: Plasma homocysteine level (box plots represent median, inter-quartiles and range) according to the genotypes of *TCN2* 776 C>G in West Africa, Italy, France, Marocco and Mexico.

REFERENCES

1. Zetterberg H. Methylenetetrahydrofolate reductase and transcobalamin genetic polymorphisms in human spontaneous abortion: biological and clinical implications. *Reprod Biol Endocrinol 2*004;**2**:7.

2. Fowler B. Homocysteine: overview of biochemistry, molecular biology, and role in disease processes. *Semin Vasc Med* 2005;**5**:77-86.

3. Bollander-Gouaille C. Focus on Homocysteine and the Vitamins involved in its metabolism. Paris : Spinger-Verlag , 2nd Edition, 2002

4. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuve LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;**10**:111-113.

5. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the *MTHFR* C677T polymorphism. *Trends Pharmacol Sci* 2001;**22**:195-201

6. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction in the folate status. *Proc Natl Acad Sci USA* 2002;**99**:5606-5611.

7. Pepe G, Camacho Vanegas O, Giusti B, Brunelli T, Marcucci R, Attanasio M, Rickards O, De Stefano GF, Prisco D, Gensini GF, Abbate R. 1998. Heterogeneity in word distribution of the thermolabile C677T mutation in 5,10-methylenetetrahydrofolate reductase. *Am J Hum Genet* 1998;**63**:917-920.

8. Amouzou EK, Chabi NW, Adjalla CE, Rodriguez-Guéant RM, Feillet F, Villaume C, Sanni A, Guéant JL. High prevalence of hyperhomocysteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa. *Am J Clin Nutr* 2004;**79**:619-624.

9. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M, Stoll C, alembic Y, Dott B, Czeizel AE, Gelman-Kohan Z, Scarano G, Bianca S, Ettore G, Tenconi R, Bellato S, Scala I, Mutchinick OM, Lopez MA, de Walle H, Hofstra R, Joutchenko L, Kavteladze L, Bermejo E, Martinez-Frias ML, Gallagher M, Erickson JD, Vollset SE, Mastroiacovo P, Andria G, Botto LD. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (*MTHFR*): findings from over 7000 newborns from 16 areas world wide. *J Med Genet* 2003;**40**:619-625.

10. Munoz-Moran E, Dieguez-Lucena JL, Fernandez-Arcas N, Peran-Mesa S, Reyes-Engel. Genetic selection and folic acid intake during pregnancy. *Lancet* 1998;**352**:1120-1121.

11. Guéant-Rodriguez RM, Guéant JL, Debard R, Thirion S, Xiao Hong L, Bronowicki JP, Namour F, Chabi NW, Sanni A, Anello G, Bosco P, Romano C, Amouzou E, Heidy R. Arrieta B, Sánchez BE, Romano A, Herbeth B, Guilland JC, Mutchinick OM. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study among Mexican, West African and West European populations. *Am J Clin Nutr* 2006;**83**:701-707.

12. Namour F, Guy M, Aimone-Gastin I, de Nonancourt M, Mrabet N, Gueant JL. Isoelectrofocusing phenotype and relative concentration of transcobalamin II isoprotein related to the codon 259 Arg/Pro polymorphism. *Biochem Biophys Res Commun* 1998;**251**:769–774.

13. Namour F, Olivier J, Abdelmouttaleh I, Adjalla C, Debard R, Salvat C, Guéant JL. Transcobalamin codon 259 polymorphism in HT-29 and Caco-2 cells and in Caucasians:

relation to transcobalamin and homocysteine concentration in blood. *Blood* 2001;**97**:1092–1098.
14. Miller JW, Ramos MI, Garrod MG, Flynn MA, Greeen R. Transcobalain Ii 775G>C polymorphism and indices of vitamin B12 status in healthy older adults. *Blood* 2002;**100**:718-720.
15. Zetterberg H, Nexo E, Regland B, Minthon L, Boson R, Palmer M, Rymo L, Blennow K. The transcobalamin (TC) codon 259 genetic polymorphism influences holo-TC concentration in cerebrospinal fluid from patients with Alzheimer disease. *Clin Chem* 2003;**49**:1195-1198.
16. Zetterberg H, Regland B, Palmer M, Rymo L, Zafiropoulos A, Arvanitis DA, Spandidos DA, Blennow K. The transcobalamin codon 259 polymorphism influences the risk of human spontaneous abortion. *Hum Reprod* 2002;**17**:3033-3036.
17. Zetterberg H, Zafiropoulos A, Spandidos DA, Rymo L, Blennow K. Gene-gene interaction between fetal MTHFR 677C>T and transcobalamin 776C>G polymorphisms in human spontaneous abortion. Hum Reprod 2003;**18**:1948-1950.
18. Martinelli M, Scapoli L, Palmieri A, Pezzetti F, Baciliero U, Padula E, Carinci P, Morselli PG, Carinci F. Study of four genes belonging to the folate pathway: transcobalamin 2 is involved in the onset of non-syndromic cleft lip with or without cleft palate. *Human Mutation* 2006; **27**:27:294.
19. Bowen RA, Wong BY and Cole DE. Population-based differences in frequency of the transcobalamin II Pro259Arg polymorphism. *Clin Biochem* 2004;**37**:128-133.
20. Swanson DA, Pangilinan F, Mills JL, Kirke PN, Conley M, Weiler A, Frey T, Parle-McDermott A, O'Leary VB, Seltzer RR, Moynihan KA, Molloy AM, Burke H, Scott JM, Brody LC. Evaluation of transcobalamin II polymorphisms as neural tube defect risk factors in an Irish population. *Birth Defects Res A Clin Mol Teratol* 2005;**73**:239-244.
21. Lievers KJA, Afman LA, Kluitmans LAJ, Boers GH, Verhoef P, den Heijer M, Trijbels FJ, Blom HJ. Polymorphism in the transcobalamin gene: association with plasma homocysteine in healthy individuals and vascular disease patients. *Clin Chem* 2002;**48**:1383–1389.
22. McCaddon A, Blennow K, Hudson P, Regland B, Hill D. Transcobalamin polymorphism and homocysteine. *Blood* 2001;**98**:3497–3499.
23. Namour F, Guéant JL. Transcobalamin polymorphism, homocysteine and ageing. *Blood* 2001;**98**:3497-3499.
24. Afmann LA, Lievers KJ, van der Put NM, Trijbels FJ, Blom HJ. Single nucleotide polymorphisms in the transcobalamin gene: relationship with transcobalamin concentrations and risk for neural tube defects. *Eur J Hum Genet* 2002;**10**:433-438.
25. Guéant-Rodriguez RM, Rendeli C, Namour B, Venuti L, Romano A, Anello G, Bosco P, Debard R, Gérard P, Viola M, Salvaggio E, Guéant JL. Transcobalamin and methionine synthase reductase mutated polymorphisms aggravate the risk of neural tube defects. *Neurosciences Lett* 2003;**344**:189-192.
26. Nzila A, Ward SA, Marsh K, Sims P, Hyde JE. Comparative folate metabolism in humans and malaria parasites (part I): pointers for malaria treatment from cancer chemotherapy. *Trends in Parasitology* 2005;**21**:292-298.
27. Nzila A, Ward SA, Marsh K, Sims P, Hyde JE. 2005. Comparative folate metabolism in humans and malaria parasites (part II): activities as yet untargeted or specific to Plasmodium. *Trends in Parasitology* 2005;**21**:334-339.
28. Rosenblatt DS, Fenton W. 2001. Inherited disorders of folate and cobalamin transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill, p 3897–3933.

29. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Akogbeto M, Tanner M (2006) Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin). *Malar* 2006;**5**:45-54.
30. Zhou SS, Tang LH, Sheng HF, Wang Y. Malaria situation in the People' s Republic of China. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 2004;**24**:1-3.