

Short Communication

Methylenetetrahydrofolate Reductase (*MTHFR*) Allele Frequencies in Amerindians

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Summary

Neural tube defects (NTDs) have been associated with abnormalities of folate metabolism. Methylenetetrahydrofolate reductase (*MTHFR*) is the regulatory enzyme for the conversion of homocysteine to methionine. The C677T mutation in the *MTHFR* gene affects folate distribution, and homozygosity for the T allele may be associated with an increased risk of NTDs. A second mutation, an A1298C transversion in this same gene, is also associated with an increased risk for NTDs but only in conjunction with the 677T allele. A low incidence of NTDs has been observed in high-altitude populations; however, these studies did not provide information about the allele distribution of genes involved in folate metabolism. This investigation compares allele frequencies of the C677T and A1298C polymorphisms between Quechua people living at 3200–4200 m in the Peruvian Central Andes and an Aché group living at low altitude. Allele frequencies at both loci were not significantly different between the two populations. The absence of the 677T/677T genotypes and of the 677T/1298C arrangement in both groups may indicate a genetic contribution to reduced risk for NTDs; however, factors other than altitude are likely responsible for the low variant allele frequencies in these populations.

Introduction

A recent epidemiological study indicated association between congenital anomalies and residence at high altitude. Residents with Amerindian background born in three high-altitude Andean cities (between 2600 m and 3600 m) and in 38 low-altitude cities (between 5 m and 905 m) were surveyed for a number of conditions, and relatively low frequencies of anencephaly [relative risk (RR) 0.33], spina bifida (RR 0.57), hydrocephaly (RR 0.41) and pes equinovarus (RR 0.70) were found in the high altitude samples (Castilla *et al.* 1999). Although the data were not stratified according to ethnic

origin, the high frequency of aboriginal people in the high-altitude sample suggests that the difference in susceptibility to neural tube defects (NTDs) may be characteristic of these populations. This could be due to environmental differences (such as diet) and/or to a reduced frequency of alleles associated with these conditions.

Animal experimentation and epidemiological studies have shown that deficiency in folic acid may be associated with neural tube defects (Luckock *et al.* 1994). Although it is unclear how disturbances of folate metabolism may cause abnormalities in the embryo, both insufficient methylation of crucial metabolites and direct toxicity of homocysteine have been suggested as possible mediators of teratogenesis (Rosenquist *et al.* 1996). The 677T allele of the C677T locus in exon 4 of the *MTHFR* gene, which substitutes an alanine for a valine residue within the catalytic domain of the

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MTHFR enzyme (Frosst *et al.* 1995; van der Put *et al.* 1998), has been suggested as a risk factor for NTDs (van der Put *et al.* 1995). Homozygosity for the thermolabile MTHFR variant predisposes individuals to the development of hyperhomocysteinemia, especially during times of folate insufficiency (Frosst *et al.* 1995; van der Put *et al.* 1998).

A second variant in the *MTHFR* gene, an A1298C transversion in exon 7 located at the C-terminal regulatory domain, results in a glutamate to alanine substitution (van der Put *et al.* 1998; Weisberg *et al.* 1998). The C allele is associated with decreased enzyme activity, although to a lesser extent than the C677T mutation; however, neither the homozygous nor the heterozygous state for the 1298 mutation is associated with the higher plasma homocysteine or lower plasma folate concentrations that appear in the homozygotes for the C677T mutation. Combined heterozygosity of the *MTHFR* 677 and 1298 variants has been reported to be more common in children with NTDs (van der Put *et al.* 1998), although not all studies support this association (Stegmann *et al.* 1999), and only a proportion of spina bifida cases are attributable to known *MTHFR* alleles (Botto & Yang, 2000).

The aim of the present study is to determine if an Amerindian high-altitude Quechua community of the Peruvian Central Andes, representing a population in which the frequency of NTDs appears to be low, has different allele frequencies at the *MTHFR* locus than an Amerindian Aché low-altitude community. Distribution of allele frequencies in these two groups was compared with data from other populations to place the Quechua and the Aché in a wider genetic-epidemiologic context.

Subjects and Methods

Blood samples were obtained with informed consent from 56 unrelated Quechua people born or living in the villages of Huilloc, Patacancha and Qqelcqanca in the District of Cuzco, Peru. DNA was prepared from peripheral blood leukocytes using chloroform extraction (Mullenbach *et al.* 1986, with some modifications). Sixteen additional Quechua DNA samples previously obtained from buccal material (Rupert *et al.* 1999) were also considered. The second group studied was the Aché

of the Tupi linguistic subgroup living at the Arroyo Bandera and Chupa Pou reservations in the Alto Paraná area of eastern Paraguay (Hill & Hurtado, 1996; Demarchi *et al.* 1999; Battilana *et al.* 2002). Thirty samples from peripheral blood were secured and processed in a similar way.

One hundred ng of genomic DNA was PCR amplified in a 25 μ l reaction volume containing 200 mM each of dNTPs, 20 pmol of each primer and 0.5 units of *Taq* polymerase (BRL). Amplification was accomplished with 30 and 40 cycles for the blood and buccal samples, respectively. The denaturation step of the first cycle was lengthened to 5 min to ensure complete denaturation of the template; the final extension step was 7 min. Fifteen μ l of the amplification reaction with the samples to be studied were digested with 10 units of the appropriate restriction enzyme.

The presence of the C677T mutation was detected by amplifying an aliquot of DNA using the forward primer 5' TGAAGGAGAAGGTGTCTGCGGGA 3' and the reverse primer 5' AGGACGGTGC GG TGA-GAGTG 3' (Frosst *et al.* 1995). The concentration of MgCl₂ was 1.5 mM and the annealing temperature was 66°C. An aliquot of the PCR product was then digested with the restriction enzyme *Hinf*I and the material was electrophoresed with a standard control in 1.8% Synergel (Diversified Biotech, Newton, MA) and 0.8% agarose (BRL).

The presence of the A1298C mutation was detected using the forward primer 5' CTTTGGGAG-CTGAAGGACTACTAC 3' and the reverse primer 5' CACTTTGTGACCATTCGGTTTG 3' (van der Put *et al.* 1998). In this case, the MgCl₂ concentration was 3.0 mM and annealing temperature was 51°C. Aliquots of the samples to be studied were digested with the restriction enzyme *Mbo*I and the product was electrophoresed in a 20% acrylamide gel (BioRad).

Allele frequencies were determined by gene counting. The differences in gene frequencies among the Quechua group (and subgroups) and the Aché were considered using the chi-square test. Contingency table chi-square analysis with Yates' correction for continuity (Sokal & Rohlf, 1981) was used to compare these allele frequencies with other Amerindian populations. Maximum likelihood haplotype frequencies were computed using an

Expectation–Maximization (EM) algorithm (Excoffier & Slatkin, 1995) using the ARLEQUIN program (Schneider *et al.* 2000).

Results and Discussion

Table 1 presents genotype and allele frequencies for the two loci, and compares them with selected additional populations. No deviations from the Hardy–Weinberg equilibrium were detected in the samples studied here. No 677T/677T or 1298C/1298C subjects were observed among the Quechua or Aché. In fact, with just one exception (Huiloc), all samples proved to be monomorphic for the 1298A/1298A genotype. The two 1298A/1298C heterozygotes had the 677C/677C genotype at the other locus. Examination of the prevalences at the 677 locus indicated almost identical frequency of the 677T allele for the Quechua as a whole and the Aché. Within the Quechua populations, however, the group living at the highest altitude

(Qqelcqañca, 4,200 m) showed a significantly lower prevalence of this allele (0.02), as compared to the other two groups (0.14–0.17; $p < 0.05$). Comparison with other Amerindian populations disclosed similar prevalences, exceptions being the Amazonian sample studied by Schneider *et al.* (1998) (0.45) and the Cayapa investigated by Pepe *et al.* (1998) (0.43). No indications of a geographic gradient were found. Examination of the prevalence of the 677 alleles indicated similar frequency of the 677T allele for all the European and European-derived populations, with the exception of the Ashkenazi Jewish population, who presented with the highest frequency (0.48).

Since there is almost no variability at the 1298 locus, the haplotype prevalences obtained considering both loci are completely different from those obtained in other ethnic groups (data not shown). Gene diversity analyses for these two genetic regions also demonstrated marked differences from European or European-derived populations (Quechua: 0.23 ± 0.04 ; Aché: 0.18 ± 0.07 ;

Table 1 MTHFR C677T and A1298C genotype and allele frequencies in Amerindian and non-Amerindian populations

Populations ^a	C677T					A1298C					Ref.
	No. indiv. studied	Genotypes (%)			T allele freq.	No. indiv. studied	Genotypes (%)			C allele freq.	
		CC	CT	TT			AA	AC	CC		
Nu-Chah-Nulth	37	68	27	5	0.19	ND	ND	ND	ND	-	1
Cayapa	57	ND	ND	ND	0.43	ND	ND	ND	ND	-	2
Amazonian 1 ^b	39	31	49	20	0.45	ND	ND	ND	ND	-	1
Amazonian 2 ^b	129	60	32	8	0.24	ND	ND	ND	ND	-	3
Parakanã	83	79	20	1	0.11	ND	ND	ND	ND	-	4
Quechua	72	76	24	0	0.11	72	97	3	0	0.01	5
Qqelcqañca	22	95	5	0	0.02	22	100	0	0	0.00	5
Patacancha	18	72	28	0	0.14	18	100	0	0	0.00	5
Huiloc	32	66	34	0	0.17	32	94	6	0	0.03	5
Aché	30	80	20	0	0.10	25	100	0	0	0.00	5
Dutch	403	50	41	9	0.29	403	45	46	9	0.33	6
German	174	52	37	11	0.30	174	51	39	10	0.30	7
USA,	155	31	43	26	0.48	149	54	38	8	0.29	8
Ashkenazi Jews											
USA,	200	55	33	12	0.28	187	41	48	11	0.35	8
European-derived											
Polish	211	56	35	9	0.26	211	64	32	4	0.20	9

^aThe Amerindian populations are listed according to a north–south gradient.

References: 1. Schneider *et al.* (1998); 2. Pepe *et al.* (1998); 3. Franco *et al.* (1998); 4. Arruda *et al.* (1998); 5. Present communication; 6. van der Put *et al.* (1998); 7. Stegmann *et al.* (1999); 8. Rady *et al.* (1999); 9. Szczeklik *et al.* (2001).

ND: Not Determined.

^bWhile Schneider *et al.* (1998) did not specify the origin of the groups studied, since the samples had been provided by J.F. Guerreiro they should be of the Amazonian region; Franco *et al.* (1998) indicated that samples from the Wayampi, Wayana–Apalai, Kayapo, Arara, and Yanomami tribes had been pooled for their study.

range in five samples of the indicated continental derivation: 0.58 ± 0.02 – 0.70 ± 0.02).

What conclusions can be obtained from our data? Although there is some variability in the allele prevalences of the two loci among Amerindians, these people are clearly at an advantage in relation to non-Indians concerning the possible association of variables at these loci and risk for NTDs. This reduced risk, however, is apparently not related to long-term residence at high altitude, since the allele frequencies for high-altitude Quechua and low-altitude Aché are not significantly different.

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