J Hum Genet (2003) 48:1–7

ORIGINAL ARTICLE

Shuji Ogino · Robert B. Wilson

Genotype and haplotype distributions of *MTHFR* 677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis

Received: August 22, 2002 / Accepted: October 7, 2002

Abstract Common single nucleotide polymorphisms (SNPs; 677C>T and 1298A>C) in the methylenetetrahydrofolate reductase gene (*MTHFR*) decrease the activity of the enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states. We calculate herein the haplotype frequencies of the *MTHFR* 677 and 1298 polymorphisms in pooled general populations derived from published data. We selected 16 articles that provided reliable data on combined *MTHFR* genotypes in general populations (n = 5389). The combined data comprised the following totals for each genotype at nucleotide positions 677 and 1298: 838 CC/AA (i.e., 677CC/1298AA), 1225 CC/AC, 489 CC/CC, 1120 CT/AA, 1093 CT/AC, 8 CT/CC, 606 TT/AA, 10 TT/AC, and 0 TT/CC. The estimated haplotype frequencies, and the fractional contribution of each, were 677C/1298A, 0.37; 677C/1298C, 0.31; 677T/1298A, 0.32; and 677T/1298C, 0.0023 to 0.0034. Thus, a vast majority of 677T alleles and 1298C alleles are associated with 1298A alleles and 677C alleles, respectively. There may be an increased frequency of the very rare *cis* 677T/1298C haplotype in some parts of the United Kingdom and Canada, possibly due to a founder effect. Further studies on both SNPs are needed to determine their exact role in various clinical settings.

S. Ogino (✉)
Department of Pathology, Brigham and Women's Hospital, 75 Francis St., Amory 3rd floor, Boston, MA 02115, USA
Tel. +1-617-632-3978; Fax +1-617-632-5762
e-mail: sogino@partners.org

S. Ogino
Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

S. Ogino
Harvard Medical School, Boston, MA, USA

R.B. Wilson
Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA, USA

Key words *MTHFR* · 677C>T · 1298A>C · SNP · *cis* · Haplotype · Frequency · Meta-analysis

Introduction

A common single nucleotide polymorphism (SNP), a C-to-T change at position 677 (677C>T, commonly referred to as C677T), which corresponds to nucleotide 665 of the open reading frame, in the 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*; OMIM, 236250; GDB, 370882; GenBank, XM_030156 and NM_005957) causes an amino acid substitution (A222V), rendering the enzyme thermolabile (Frosst et al. 1995; Kang et al. 1991). This thermolabile enzyme may exhibit decreased enzymatic activity, leading to mild hyperhomocysteinemia in homozygous 677C>T individuals (Frosst et al. 1995; Kang et al. 1991). Another common SNP of *MTHFR* is an A-to-C change at position 1298 (1298A>C, commonly referred to as A1298C), corresponding to nucleotide 1286 of the open reading frame, which results in a Glu-to-Ala substitution (E429A) (van der Put et al. 1998). Although the 1298A>C polymorphism by itself does not appear to cause hyperhomocysteinemia in either the heterozygous or homozygous state, combined heterozygosity for both 677C>T and 1298A>C mutations can result in hyperhomocysteinemia (van der Put et al. 1998).

Although these two polymorphisms are usually not present in the same allele (i.e., in "*cis*"), studies have shown that very rare *MTHFR* alleles have both polymorphisms (Isotalo et al. 2000; Weisberg et al. 1998). Such *cis MTHFR* 677T/1298C alleles (haplotypes) were seen more frequently in spontaneous abortions than in healthy neonates in a Canadian study (Isotalo et al. 2000). For the most accurate determination of the *cis* 677T/1298C haplotype frequency, all available published data should be combined. We meta-analyze herein *MTHFR* genotype and haplotype frequencies from published population data.

Materials and methods

Selection of populations for meta-analysis of *MTHFR* genotype

For calculation of *MTHFR* 677 and 1298 genotype and haplotype frequencies, we collected data from the literature that showed *MTHFR* 677 and 1298 genotype distributions. For those manuscripts in which the genotype distribution was not clearly stated, we requested the genotype distribution from the corresponding authors. We included data obtained by these personal communications (indicated by PC, for "personal communication," in Table 1). As a result, the *MTHFR* 677 and 1298 genotype distribution was available in a total of 22 manuscripts (Akar et al. 2001; Barber et al. 2000; Chango et al. 2000; Dekou et al. 2001; Fodinger et al. 2000; Friedman et al. 1999; Hanson et al. 2001; Isotalo and Donnelly 2000; Isotalo et al. 2000; Kaiser et al. 2000; Lachmeijer et al. 2001; Meisel et al. 2001; Rady et al. 1999; Richter et al. 2001; Shen et al. 2001; Skibola et al. 1999; Song et al. 2001; Szczeklik et al. 2001; van der Put et al. 1998; Weisberg et al. 1998; Wiemels et al. 2001; Zusterzeel et al. 2000) (Table 1). To obtain the *MTHFR* genotype distribution in the general population, we used 19 different control populations, which included healthy adults, infants, and neonates, from 16 manuscripts (population "W1") (Akar et al. 2001; Barber et al. 2000; Chango et al. 2000; Fodinger et al. 2000; Friedman et al. 1999; Kaiser et al. 2000; Lachmeijer et al. 2001; Meisel et al. 2001; Rady et al. 1999; Richter et al. 2001; Shen et al. 2001; Skibola et al. 1999; Szczeklik et al. 2001; van der Put et al. 1998; Wiemels et al. 2001; Zusterzeel et al. 2000). We excluded data derived from Chinese populations (Song et al. 2001) because only 17% (123/724) of *MTHFR* alleles in the control northern Chinese population had the 1298C allele (Table 1). The data of Weisberg et al. (1998) did not include controls from the general population and we excluded these data. We also excluded the data of Hanson et al. (2001) from control population W1 because they did not discriminate between their vascular disease populations and controls.

The silent 1317T>C polymorphism is known to affect genotyping of 1298A>C by *Mbo*II digestion because it creates an almost identical *Mbo*II restriction pattern as that of the 1298A allele, even in the presence of a 1298C allele (Donnelly 1999; Weisberg et al. 1998). However, the reported frequency of the 1317C allele is low in the Caucasian population (Meisel et al. 2001; Weisberg et al. 1998). Weisberg et al. (1998) found that 4 of 76 Canadian Caucasian alleles had the 1317C allele, and 7 of 18 alleles in African-American females had the 1317C allele. Meisel et al. (2001) found that the 1317C allele was present only in 1 of 1962 alleles among patients with coronary artery diseases, and in none of 1962 control alleles, in Germany. Because the 1317T>C polymorphism is rare in Caucasian populations, it was not taken into account for further analysis.

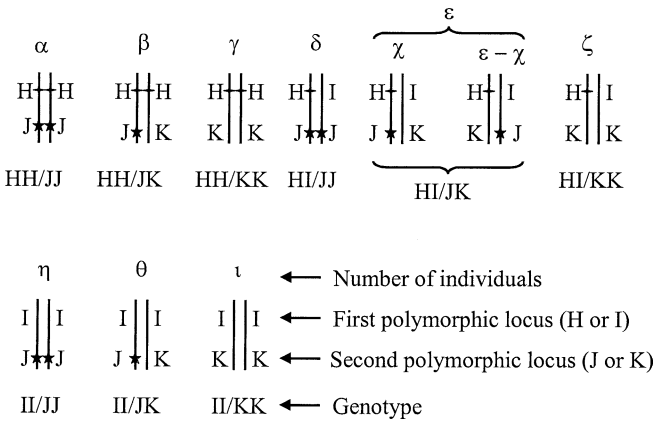

Fig. 1. Two linked polymorphic loci (X and Y) and their geotype (e.g. HI/JK) distribution in a population

Haplotype distributions of two linked polymorphisms

We designate two alternative polymorphic bases in the first locus ("X") as "H" and "I," and those in the second locus ("Y") as "J" and "K." We designate the number of individuals with each possible X/Y genotype in a population as follows: HH/JJ, α; HH/JK, β; HH/KK, γ; HI/JJ, δ; HI/JK, ε; HI/KK, ζ; II/JJ, η; II/JK, θ; and II/KK, ι (Fig. 1). If we designate χ as the number of individuals who have one chromosome with the HJ haplotype and the other chromosome with the IK haplotype, then the number of individuals who have one chromosome with the HK haplotype and the other chromosome with the IJ haplotype is $\varepsilon - \chi$, because both groups of individuals have the HI/JK genotype (Fig. 1). We can calculate the number of chromosomes with each possible haplotype in the population as follows: HJ, $2\alpha + \beta + \delta + \chi$; HK, $\beta + 2\gamma + (\varepsilon - \chi) + \zeta$; IJ, $\delta + (\varepsilon - \chi) + 2\eta + \theta$; IK, $\chi + \zeta + \theta + 2\iota$.

Results

MTHFR 677 and 1298 genotype and haplotype frequencies

We designated the two alternative polymorphic bases 677C and 677T as H and I, and 1298A and 1298C as J and K, as described in Materials and Methods. We refer to the *MTHFR* 677 and 1298 genotype (or haplotype) as two bases (or one base) in the 677 position followed by "/" and two bases (or one base, respectively) in the 1298 position; e.g., CC/AC represents homozygous 677C and heterozygous 1298A and 1298C, and T/A represents the *MTHFR* haplotype 677T/1298A.

At present, there is no convincing evidence that any of the *MTHFR* 677/1298 genotypes decrease fitness and thus skew the genotype distribution. Isotalo et al. (2000) reported increased CT/CC, TT/AC, and TT/CC genotype frequencies in spontaneous and therapeutic abortions (H1 in Table 1). However, the same group also reported unusually

Table 1. *MTHFR* 677/1298 genotype distributions in the literature

Authors	PC	CC/AA		CC/AC		CC/CC		CT/AA		CT/AC		CT/CC		TT/AA		TT/AC		TT/CC		Total	Population [assigned identification no.]
		No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	
Akar et al. 2001	PC	7	0.15	12	0.26	5	0.11	5	0.11	13	0.28	0	0	1	0.022	2	0.043	1	0.022	46	Pediatric stroke, Turkey [A1]
		20	0.18	40	0.35	6	0.053	19	0.17	18	0.16	0	0	11	0.096	0	0	0	0	114	Control healthy infants, Turkey [A2]
Barber et al. 2000	PC	18	0.15	14	0.11	0	0	46	0.37	21	0.17	0	0	23	0.19	1	0.0081	0	0	123	Spontaneous/therapeutic abortions and NTD, Texas Hispanic [B1]
		17	0.10	12	0.071	3	0.018	72	0.43	33	0.20	1	0.0060	29	0.17	1	0.0060	0	0	168	Healthy newborns, Texas Hispanic [B2]
Chango et al. 2000		6	0.095	14	0.22	7	0.11	14	0.22	10	0.16	0	0	12	0.19	0	0	0	0	63	Mild hyperhomocysteinemia, France [C1]
		9	0.14	12	0.18	8	0.12	10	0.15	15	0.23	0	0	12	0.18	0	0	0	0	66	Healthy control, France [C2]
Dekou et al. 2001		134	0.18	159	0.22	43	0.058	185	0.25	137	0.19	10	0.014	56	0.076	13	0.018	2	0.0027	739	General population, British Regional Heart Study [D1]
Fodinger et al. 2000		87	0.12	190	0.26	67	0.091	157	0.21	152	0.21	1	0.0014	79	0.11	0	0	0	0	733	Stable kidney allograft recipients, Austria [E1]
		44	0.12	76	0.21	36	0.099	82	0.23	75	0.21	1	0.0028	49	0.13	0	0	0	0	363	Healthy normal control, normal blood pressure, Austria [E2]
Friedman et al. 1999		40	0.11	62	0.17	46	0.12	73	0.20	97	0.26	1	0.0027	55	0.15	0	0	0	0	374	Jewish population, Israel [F1]
Hanson et al. 2001		148	0.12	235	0.19	129	0.10	298	0.24	285	0.23	2	0.0016	138	0.11	3	0.0024	0	0	1238	CAD (n = 772), DVT (n = 137), control (n = 329), USA [G1]
Isotalo et al. 2000		25	0.16	54	0.34	17	0.11	28	0.17	27	0.17	5	0.031	2	0.012	2	0.012	1	0.0062	161	Fetus (spontaneous and therapeutic abortions), Canada [H1]
		17	0.14	42	0.35	9	0.076	14	0.12	23	0.19	0	0	12	0.10	2	0.017	0	0	119	Healthy neonates, Canada [H2]
Isotalo and Donnelly 2000		2	0.031	17	0.26	6	0.092	7	0.11	21	0.32	0	0	6	0.092	6	0.092	0	0	65	Venous thrombosis, Canada [I1]
		2	0.031	18	0.28	1	0.016	1	0.016	34	0.53	0	0	0	0	8	0.13	0	0	64	Healthy volunteers, Canada [I2]
Kaiser et al. 2000		10	0.068	42	0.29	13	0.088	30	0.20	38	0.26	0	0	13	0.088	1	0.0068	0	0	147	Preeclampsia and eclampsia, Anglo-Saxon whites, Australia [J1]
		13	0.12	22	0.20	11	0.10	18	0.17	30	0.28	1	0.0092	13	0.12	1	0.0092	0	0	109	Normal blood pressure, Anglo-Saxon whites, Australia [J2]
Lachmeijer et al. 2001	PC	2	0.043	13	0.28	7	0.15	12	0.26	9	0.19	0	0	4	0.085	0	0	0	0	47	History of preeclampsia, The Netherlands [K1]
		10	0.083	37	0.31	11	0.092	24	0.20	27	0.23	0	0	11	0.092	0	0	0	0	120	Healthy blood donor, The Netherlands [K2]
Meisel et al. 2001		145	0.15	220	0.22	93	0.095	204	0.21	238	0.24	0	0	81	0.083	0	0	0	0	981	CAD, Germany [L1]
		120	0.12	218	0.22	105	0.11	217	0.22	225	0.23	0	0	96	0.098	0	0	0	0	981	Healthy controls, Germany [L2]
Rady et al. 1999		33	0.18	50	0.27	20	0.11	19	0.10	39	0.21	0	0	25	0.13	0	0	0	0	186	Blood donors, Texas [M1]
		12	0.081	23	0.16	12	0.081	29	0.20	33	0.22	0	0	39	0.26	0	0	0	0	148	Ashkenazi Jewish [M2]
Richter et al. 2001		19	0.10	42	0.23	12	0.065	38	0.21	45	0.24	0	0	28	0.15	0	0	0	0	184	Nonsyndromic spina bifida aperta (NTD), Germany [N1]
		41	0.19	47	0.22	25	0.12	35	0.16	38	0.18	0	0	27	0.13	0	0	0	0	213	General control population, Germany [N2]
Shen et al. 2001		76	0.14	122	0.22	43	0.078	128	0.23	124	0.23	0	0	57	0.10	0	0	0	0	550	Lung cancers, Texas non-Hispanic whites [O1]
		76	0.14	129	0.23	40	0.072	132	0.24	120	0.22	0	0	57	0.10	0	0	0	0	554	Control, Texas non-Hispanic whites [O2]

Table 1. Continued

Authors	PC	CC/AA		CC/AC		CC/CC		CT/AA		CT/AC		CT/CC		TT/AA		TT/AC		TT/CC		Total	Population [assigned identification no.]
		No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	
Skibola et al. 1999		16	0.24	18	0.26	1	0.015	23	0.34	5	0.074	0	0	5	0.074	0	0	0	0	68	ALL adult patients, British [P1]
		39	0.18	66	0.31	20	0.095	33	0.16	27	0.13	3	0.014	20	0.095	3	0.014	0	0	211	AML adult patients, British [P2]
		15	0.13	35	0.31	11	0.096	22	0.19	17	0.15	0	0	12	0.11	2	0.018	0	0	114	Age-sex-matched control to ALL group, adult British [P3]
		58	0.16	95	0.27	36	0.10	65	0.18	60	0.17	2	0.0056	37	0.10	3	0.0084	0	0	356	Age-sex-matched control to AML group, adult British [P4]
Song et al. 2001		15	0.063	9	0.0375	5	0.021	75	0.31	41	0.17	2	0.0083	89	0.37	4	0.017	0	0	240	Esophageal squamous cell carcinoma, northern China [Q1]
		59	0.16	62	0.17	5	0.014	123	0.34	49	0.14	0	0	62	0.17	2	0.0055	0	0	362	Control, northern China [Q2]
Szczeklik et al. 2001		42	0.26	45	0.28	18	0.11	23	0.14	16	0.099	0	0	17	0.11	0	0	0	0	161	CAD, Poland [R1]
		68	0.32	42	0.20	8	0.038	49	0.23	26	0.12	0	0	18	0.085	0	0	0	0	211	Healthy control, negative stress test, Poland [R2]
		113	0.36	71	0.23	17	0.055	53	0.17	41	0.13	0	0	15	0.048	0	0	0	0	310	Population, Poland [R3]
van der Put et al. 1998		9	0.10	17	0.20	8	0.093	18	0.21	24	0.28	0	0	10	0.12	0	0	0	0	86	NTD spina bifida patients, The Netherlands [S1]
		13	0.13	25	0.25	6	0.060	20	0.20	18	0.18	0	0	18	0.18	0	0	0	0	100	Mothers of an NTD patient, The Netherlands [S2]
		9	0.10	19	0.22	10	0.12	20	0.23	20	0.23	0	0	8	0.093	0	0	0	0	86	Fathers of an NTD patient, The Netherlands [S3]
		62	0.15	105	0.26	38	0.094	81	0.20	81	0.20	0	0	36	0.089	0	0	0	0	403	Control, volunteers, The Netherlands [S4]
Weisberg et al. 1998		24	0.17	27	0.19	13	0.092	32	0.23	26	0.18	0	0	19	0.13	0	0	0	0	141	Mother (of spina bifida and control), Canada [T1]
		23	0.17	20	0.15	13	0.098	43	0.32	15	0.11	0	0	18	0.14	1	0.0075	0	0	133	Fetus (with spina bifida and control), Canada [T2]
Wiemels et al. 2001	PC	5	0.15	14	0.41	5	0.15	3	0.088	2	0.059	0	0	5	0.15	0	0	0	0	34	Childhood leukemia with MLL mutations, UK [U1]
		11	0.15	12	0.17	5	0.069	20	0.28	11	0.15	0	0	13	0.18	0	0	0	0	72	Childhood leukemia with TEL-AML translocation, UK [U2]
		22	0.16	40	0.29	5	0.036	29	0.21	27	0.20	0	0	14	0.10	0	0	0	0	137	Childhood leukemia with hyperdipoidy, UK [U3]
		25	0.13	44	0.22	18	0.092	39	0.20	37	0.19	2	0.010	28	0.14	3	0.015	0	0	196	Healthy newborns, UK [U4]

Table 1. Continued

Authors	PC	CC/AA		CC/AC		CC/CC		CT/AA		CT/AC		CT/CC		TT/AA		TT/AC		TT/CC		Total	Population [assigned identification no.]
		No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	/Total	No.	
Zusterzeel et al. 2000		23	0.14	33	0.20	17	0.10	40	0.24	33	0.20	0	0	21	0.13	0	0	0	0	167	Preeclampsia, whites, The Netherlands [V1]
		62	0.15	105	0.26	38	0.094	81	0.20	81	0.20	0	0	36	0.089	0	0	0	0	403	General population, The Netherlands [V2]
Pooled control populations[a]		838	0.16	1225	0.23	489	0.091	1120	0.21	1093	0.20	8	0.0015	606	0.11	10	0.0019	0	0	5389	W1
Total		1846	0.15	2826	0.22	1072	0.085	2789	0.22	2584	0.20	31	0.0025	1437	0.11	58	0.0046	4	0.00032	12 647	

An identification number is assigned to each population (far right column). A blank in the "PC" column indicates data were derived solely from the manuscript. For each genotype, a column on the left is used for the number of individuals and one on the right (indicated by "/Total") for the fraction of individuals in each population. Populations D1, H1, H2, I1, and I2 have exceptionally high percentages of individuals with CT/CC, TT/AC, and/or TT/CC genotypes, indicating that there may be founder effects in these populations

PC, Personal communication; CC/AA, *MTHFR* 677CC/1298AA, etc.; NTD, neural tube defect; CAD, coronary artery disease; DVT, deep venous thrombosis; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia

[a] Including A2, B2, C2, E2, F1, J2, K2, L2, M1, M2, N2, O2, P3, P4, R2, R3, S4, U4, and V2, but excluding D1, G1, H2, I2, Q2, T1, and T2

high frequencies of the TT/AC genotype in their population with venous thrombosis and in their control population (I1, I2 in Table 1) (Isotalo and Donnelly 2000), indicating that there might be founder chromosomes with the *cis* T/C allele in the Canadian populations they studied.

A few investigators determined that individuals of the CT/AC genotype had 677T and 1298C in *trans*, i.e., that 677T and 1298C are located on different alleles (chromosomes). These individuals comprise 38 in a control population in Germany (Richter et al. 2001), 32 from coronary artery disease patients and healthy individuals in Germany (Meisel et al. 2001), and 5 randomly selected individuals from Ashkenazi-Jewish and Texas populations (Rady et al. 1999). However, the total number of individuals ($n = 75$) shown to have 677T and 1298C in *trans* among our meta-analysis populations is very small compared with the total number of individuals in the general population who had the CT/AC genotype (1093, see following). Therefore, we performed calculations as if precise haplotypes of all individuals with the CT/AC were unknown.

We excluded the data of Dekou et al. (2001; D1 in Table 1), Isotalo et al. (2000, H2 in Table 1), and Isotalo and Donnelly (2000; I2 in Table 1) from pooled control population W1. This is because these data showed obviously much higher frequencies of the 677CT/1298CC, 677TT/1298AC, and/or 677TT/1298CC genotypes compared with other populations, indicating higher frequencies of the *cis* 677T/1298C haplotype, possibly due to founder effects. Statistical analyses (χ^2 tests) showed that the fractions of individuals with CT/CC, TT/AC, or TT/CC were significantly higher in populations D1 ($P < 0.0001$), I1 ($P < 0.0001$), or I2 ($P < 0.0001$), respectively, compared with population W1.

Statistical analyses (χ^2 tests) with comparison to W1 also showed that there was a significant increase in the CT/AA ($P < 0.001$) and TT/AA ($P < 0.025$) genotype frequencies, and a decrease in the CC/AC ($P < 0.001$) and CC/CC ($P < 0.005$) genotype frequencies, indicating an increase in the T/A haplotype frequency and a decrease in the C/C haplotype frequency, in a Texas Hispanic population (Barber et al. 2000). There was a significant increase in the TT/AA ($P < 0.0001$) genotype frequency, and a decrease in the CC/AA ($P < 0.025$) and CC/AC ($P < 0.05$) genotype frequencies, indicating an increase in the T/A haplotype frequency and a decrease in the C/A haplotype frequency, in an Ashkenazi Jewish population (Rady et al. 1999). There was a significant increase in the CC/AA ($P < 0.0001$) genotype frequency, and a decrease in the CC/CC ($P < 0.005$), CT/AC ($P < 0.001$), and TT/AA ($P < 0.001$) genotype frequencies, indicating an increase in the C/A haplotype frequency and a decrease in the T/A and C/C haplotype frequencies, in Poland (R2 and R3 in Table 1 combined) (Szczeklik et al. 2001). There was also a significant increase in the CC/AC genotype frequency in Turkey (Akar et al. 2001) ($P < 0.005$). We excluded only the data of Dekou et al. (2001), Isotalo et al. (2000), and Isotalo and Donnelly (2000) from W1 because these data disproportionally affect the T/C haplotype allele frequency because of the very small numbers of the T/C haplotype. Individually, other data have little effect on the genotype and haplotype frequencies in W1.

The *MTHFR* genotype distribution in the control populations of 5389 individuals (W1 in Table 1) is as follows: CC/AA, 838 (= α); CC/AC, 1225 (= β); CC/CC, 489 (= γ); CT/AA, 1120 (= δ); CT/AC, 1093 (= ε); CT/CC, 8 (= ζ); TT/AA, 606 (= η); TT/AC, 10 (= θ); and TT/CC, 0 (= ι). If χ individuals in the CT/AC genotype have a C/A allele plus a T/C (*cis*) allele, 1093 − χ individuals in the CT/AC genotype have a C/C allele plus a T/A allele. Thus, the total number of each allele is as follows: C/A, 4021 + χ; C/C, 3304 − χ; T/A, 3435 − χ; and T/C, 18 + χ. Because there is no individual with the TT/CC genotype in these populations, and because relatively few individuals with the CT/CC and TT/AC genotypes have been described, the T/C allele must be rare. Therefore, χ must be very small compared with the total number of alleles in the populations we analyzed. Although we calculated these results manually as described below, one could also use the expectation-maximization algorithm (Long et al. 1995; Stephens et al. 2001).

Assuming Hardy-Weinberg equilibrium, the ratio of the number of individuals with the CC/AC genotype to that of the CT/CC genotype equals the ratio of the frequency of the haplotype C/A to that of the T/C haplotype. Thus, $1225/8 = (4021 + \chi)/(18 + \chi)$. $\therefore\chi = 8.3$.

Similarly, the ratio of CT/AA to TT/AC equals that of C/A to T/C. Thus, $1120/10 = (4021 + \chi)/(18 + \chi)$. $\therefore\chi = 18.1$.

The ratio of CC/AC to CT/AC with haplotypes C/A and T/C equals that of C/C to T/C. Thus, $1225/\chi = (3304 - \chi)/(18 + \chi)$. $\therefore\chi = 10.7$.

The ratio of CT/AA to CT/AC with haplotypes C/A and T/C equals that of T/A to T/C. Thus, $1120/\chi = (3435 - \chi)/(18 + \chi)$. $\therefore\chi = 8.7$.

The ratio of CT/CC to CT/AC with haplotypes C/A and T/C equals that of C/C to C/A. Thus, $8/\chi = (3304 - \chi)/(4021 + \chi)$. $\therefore\chi = 9.8$.

The ratio of CT/CC to CT/AC with haplotypes C/C and T/A equals that of T/C to T/A. Thus, $8/(1093 - \chi) = (18 + \chi)/(3435 - \chi)$. $\therefore\chi = 7.3$.

The ratio of TT/AC to CT/AC with haplotypes C/A and T/C equals that of T/A to C/A. Thus, $10/\chi = (3435 - \chi)/(4021 + \chi)$. $\therefore\chi = 11.8$.

The ratio of TT/AC to CT/AC with haplotypes C/C and T/A equals that of T/C to C/C. Thus, $10/(1093 - \chi) = (18 + \chi)/(3304 - \chi)$. $\therefore\chi = 12.5$.

Estimated χ in the population W1 ranges from 7.3 to 18.1. Since χ is an integer number, we took 7 as a lower estimate and 19 as a higher estimate. Deduced haplotype frequencies are C/A, 37%; C/C, 30%; T/A, 32%; and T/C, 0.23% to 0.34%. Therefore, the frequencies of the 677T allele and of the 1298C allele in the populations we included were 32% and 31%, respectively. Reported 677T allele frequencies (mostly ranging from 25% to 40%; reviewed by Botto and Yang 2000) match our data.

Validation of the method by deduction of *MTHFR* genotype frequencies

Using our 677/1298 haplotype frequency estimates, we calculated theoretical genotype frequencies as follows: For the *cis* T/A haplotype frequency of 0.0023 (χ = 7 in W1): CC/AA, 0.14; CC/AC, 0.23; CC/CC, 0.094; CT/AA, 0.24; CT/AC, 0.20; CT/CC, 0.0014; TT/AA, 0.10; TT/AC, 0.0015; and TT/CC, 0.0000054. For the *cis* T/A haplotype frequency of 0.0034 (χ = 19 in W1): CC/AA, 0.14; CC/AC, 0.23; CC/CC, 0.093; CT/AA, 0.24; CT/AC, 0.20; CT/CC, 0.0021; TT/AA, 0.10; TT/AC, 0.0022; and TT/CC, 0.000012. These figures match well with actual *MTHFR* 677/1298 genotype frequencies observed in W1 (Table 1), validating our method.

Discussion

Our *MTHFR* 677/1298 haplotype frequency estimates are based on a large number of Caucasian populations and can be used as standards, to which *MTHFR* 677/1298 haplotype frequencies in various study populations, such as those with particular diseases or responses to medication, can be compared. A significant difference between our estimates and the observed frequencies in populations under study would merit further investigation to test the hypothesis that a cause-and-effect relationship exists, versus, for example, a founder effect or other cause of linkage disequilibrium. One should keep in mind that the number of individuals with the CT/CC, TT/AC, or TT/CC genotype in a given study was always small, and therefore, a small error in genotyping, either false positive or false negative, can affect an *MTHFR* T/C haplotype frequency estimate significantly.

Considerably higher frequencies of CT/CC, TT/AC, and/or TT/CC *MTHFR* genotypes in the control populations of studies in the United Kingdom and Canada (Dekou et al. 2001; Isotalo and Donnelly 2000; Isotalo et al. 2000), as well as slightly higher frequencies of the CT/CC and TT/AC genotypes in other control populations in the United Kingdom (Skibola et al. 1999; Wiemels et al. 2001), may be due to an increased frequency of the *cis* T/C haplotype in those areas, possibly due to a founder effect.

Isotalo et al. (2000) found that the T/C allele was more common in spontaneous and therapeutic abortions compared with their neonatal control population. However, the number of individuals with the CT/CC, TT/AC, or TT/CC genotype was small and there might be a founder effect in their population, as stated earlier. Further study is necessary to determine the role of *MTHFR* genotypes in the pathogenesis of spontaneous abortions.

There is increasing interest in the effects of polymorphisms in *MTHFR*, and other gene encoding proteins involved in folate metabolism, on susceptibility or resistance to cancer development. In a study of British adults (Skibola et al. 1999), 677TT, 1298AC, and 1298CC *MTHFR* genotypes were less frequent in acute lymphoblastic leukemia. In a Chinese study (Song et al. 2001), the 677T allele and 1298CC genotype were more common in patients with esophageal squamous cell carcinoma. Further study is necessary to determine the precise role of the *MTHFR* 677C>T and 1298A>C SNPs in the pathogenesis of cancers.

In conclusion, we estimated *MTHFR* 677/1298 haplotype frequencies in the general population. A vast majority of

677T alleles and 1298C alleles are associated with 1298A alleles and 677C alleles, respectively. There may be an increased frequency of the very rare *cis* 677T/1298C haplotype in some parts of the United Kingdom and Canada, possibly due to a founder effect. Further studies on both SNPs are needed to determine their exact role in various clinical settings.

References

Akar N, Akar E, Ozel D, Deda G, Sipahi T (2001) Common mutations at the homocysteine metabolism pathway and pediatric stroke. Thromb Res 102:115–120

Barber R, Shalat S, Hendricks K, Joggerst B, Larsen R, Suarez L, Finnell R (2000) Investigation of folate pathway gene polymorphisms and the incidence of neural tube defects in a Texas hispanic population. Mol Genet Metab 70:45–52

Botto LD, Yang Q (2000) 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 151:862–877

Chango A, Potier De Courcy G, Boisson F, Guilland JC, Barbe F, Perrin MO, Christides JP, Rabhi K, Pfister M, Galan P, Hercberg S, Nicolas JP (2000) 5,10-Methylenetetrahydrofolate reductase common mutations, folate status and plasma homocysteine in healthy French adults of the Supplementation en Vitamines et Mineraux Antioxydants (SU.VI.MAX) cohort. Br J Nutr 84:891–896

Dekou V, Whincup P, Papacosta O, Ebrahim S, Lennon L, Ueland PM, Refsum H, Humphries SE, Gudnason V (2001) The effect of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase gene on homocysteine levels in elderly men and women from the British regional heart study. Atherosclerosis 154:659–666

Donnelly JG (1999) The silent T1317C mutation of methylenetetrahydrofolate reductase should not interfere with *MboII* restriction isotyping of the reported A1298C mutation. Mol Genet Metab 68:511–512

Fodinger M, Buchmayer H, Heinz G, Papagiannopoulos M, Kletzmayr J, Rasoul-Rockenschaub S, Horl WH, Sunder-Plassmann G (2000) Effect of MTHFR 1298A→C and MTHFR 677C→T genotypes on total homocysteine, folate, and vitamin B(12) plasma concentrations in kidney graft recipients. J Am Soc Nephrol 11:1918–1925

Friedman G, Goldschmidt N, Friedlander Y, Ben-Yehuda A, Selhub J, Babaey S, Mendel M, Kidron M, Bar-On H (1999) A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. J Nutr 129:1656–1661

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10:111–113

Hanson NQ, Aras O, Yang F, Tsai MY (2001) C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. Clin Chem 47:661–666

Isotalo PA, Donnelly JG (2000) Prevalence of methylenetetrahydrofolate reductase mutations in patients with venous thrombosis. Mol Diagn 5:59–66

Isotalo PA, Wells GA, Donnelly JG (2000) Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet 67:986–990

Kaiser T, Brennecke SP, Moses EK (2000) Methylenetetrahydrofolate reductase polymorphisms are not a risk factor for pre-eclampsia/eclampsia in Australian women. Gynecol Obstet Invest 50:100–102

Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N (1991) Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 48:536–545

Lachmeijer AM, Arngrimsson R, Bastiaans EJ, Pals G, ten Kate LP, de Vries JI, Kostense PJ, Aarnoudse JG, Dekker GA (2001) Mutations in the gene for methylenetetrahydrofolate reductase, homocysteine levels, and vitamin status in women with a history of preeclampsia. Am J Obstet Gynecol 184:394–402

Long JC, Williams RC, Urbanek M (1995) An E-M algorithm and testing strategy for multiple-locus haplotypes. Am J Hum Genet 56:799–810

Meisel C, Cascorbi I, Gerloff T, Stangl V, Laule M, Muller JM, Wernecke KD, Baumann G, Roots I, Stangl K (2001) Identification of six methylenetetrahydrofolate reductase (MTHFR) genotypes resulting from common polymorphisms: impact on plasma homocysteine levels and development of coronary artery disease. Atherosclerosis 154:651–658

Rady PL, Tyring SK, Hudnall SD, Vargas T, Kellner LH, Nitowsky H, Matalon RK (1999) Methylenetetrahydrofolate reductase (MTHFR): the incidence of mutations C677T and A1298C in the Ashkenazi Jewish population. Am J Med Genet 86:380–384

Richter B, Stegmann K, Roper B, Boddeker I, Ngo ET, Koch MC (2001) Interaction of folate and homocysteine pathway genotypes evaluated in susceptibility to neural tube defects (NTD) in a German population. J Hum Genet 46:105–109

Shen H, Spitz MR, Wang LE, Hong WK, Wei Q (2001) Polymorphisms of methylene-tetrahydrofolate reductase and risk of lung cancer: a case-control study. Cancer Epidemiol Biomarkers Prev 10:397–401

Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, Morgan G (1999) Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA 96:12810–12815

Song C, Xing D, Tan W, Wei Q, Lin D (2001) Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 61:3272–3275

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989

Szczeklik A, Sanak M, Jankowski M, Dropinski J, Czachor R, Musial J, Axenti I, Twardowska M, Brzostek T, Tendera M (2001) Mutation A1298C of methylenetetrahydrofolate reductase: risk for early coronary disease not associated with hyperhomocysteinemia. Am J Med Genet 101:36–39

van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 62:1044–1051

Weisberg I, Tran P, Christensen B, Sibani S, Rozen R (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 64:169–172

Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF (2001) Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA 98:4004–4009

Zusterzeel PL, Visser W, Blom HJ, Peters WH, Heil SG, Steegers EA (2000) Methylenetetrahydrofolate reductase polymorphisms in preeclampsia and the HELLP syndrome. Hypertens Pregnancy 19:299–307