Folate Status, Homocysteine Metabolism, and Methylene Tetrahydrofolate Reductase Genotype in Rural South African Blacks With a History of Pregnancy Complicated by Neural Tube Defects

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The birth incidence of neural tube defects (NTDs) in South Africa is threefold to sixfold higher in rural compared with urban blacks. We investigated whether folate deficiency and aberrant homocysteine metabolism could explain the high NTD incidence in rural black populations. Plasma folate and total homocyst(e)ine (tHcy) concentrations were determined in apparently healthy rural black women (n = 107), rural black women with a history of pregnancy complicated by NTDs (n = 54), and urban blacks (n = 101). Methionine load tests were performed on the 54 women with a history of NTD-affected pregnancy and 54 controls matched for age and body mass. The presence of the 677C → T mutation in the methylene tetrahydrofolate reductase (MTHFR) gene was investigated in both groups by a polymerase chain reaction (PCR) of genomic DNA and *Hin*fI digestion of the PCR product. Apparently healthy urban black women (n = 101) had a lower ($P < .001$) plasma folate concentration compared with rural black women (n = 107). Women with a history of NTD-affected pregnancy did not differ significantly from controls with respect to plasma folate, fasting homocyst(e)ine, methionine, and the post–methionine load increase in plasma homocyst(e)ine. More than 50% of both of the latter groups had a post–methionine load increase in plasma tHcy less than the fifth percentile as observed in a healthy white control group. No homozygotes for the 677C → T mutation in the MTHFR gene were found in black mothers with NTD-affected offspring or controls. It is concluded that black urbanization is characterized by a diminished folate status that is paradoxically associated with a lower NTD birth incidence. Homozygosity for the 677C → T mutation in the gene coding for MTHFR does not constitute a genetic risk factor for NTDs in blacks. No aberrant homocysteine metabolism could be demonstrated in black women with NTD-affected pregnancies.

IN SOUTH AFRICA, the prevalence of neural tube defects (NTDs) in the black population varies according to geographical location. Ncayiyana reported that the birth incidence of NTDs in blacks from the rural Transkei was 6.13 per 1,000 live births,[1] while a recent study undertaken on blacks in the rural Northern Province reported a NTD incidence of 3.55 per 1,000 live births.[2] In contrast, the documented birth incidence of NTDs is considerably lower in the black urban populations of South Africa.[3,4] Based on a 20-year observation period, Buccimazza et al[3] reported a NTD incidence of only 0.95 per 1,000 live births in the black population of Cape Town. Delport et al[4] performed a prospective hospital-based study on live-born infants over a 3-year period at Kalafong Academic Hospital, Pretoria, and found a NTD incidence of 0.99 per 1,000 live births in the urban black population group. It remains unexplained as to why urbanization is associated with such a dramatic decline in the NTD incidence.

Maternal folate supplementation in the periconceptional period reduces the frequency of NTD-affected births.[5,6] Although the data are not entirely consistent, there is evidence that maternal folate deficiency in the periconceptional period increases the risk for a NTD-affected pregnancy. Kirke et al[7] demonstrated that early pregnancy serum and red blood cell folate concentrations were significantly lower in 81 women with pregnancies subsequently affected by NTDs compared with a systematic sample of 247 control pregnancies. Further analysis of the data reported by Kirke et al showed a continuous dose-response relation between a woman's risk of having a child with a NTD and red blood cell folate levels.[8] Wald et al[9] reviewed studies published up to 1996 and concluded that maternal folate levels were significantly lower in NTD cases compared with controls during the first trimester of pregnancy.

The mechanism by which reduced folate status may cause a NTD has been suggested to involve impaired homocysteine metabolism. Elevated plasma homocyst(e)ine* concentrations have been observed in mothers who previously gave birth to children with NTDs[10-12] and in the amniotic fluid of mothers pregnant with a NTD fetus.[13] Furthermore, post–methionine load hyperhomocyst(e)inemia was reported in nine of 41 women with a history of a NTD child.[10] Aberrant homocysteine metabolism in parents with NTD-affected offspring may be at least partially explained by a twofold to threefold increase in the prevalence of homozygosity for the 677C → T mutation in the gene coding for the enzyme methylene tetrahydrofolate reductase (MTHFR).[14] A study on fibroblast cultures from NTD-affected fetuses showed that homozygosity for the 677C → T mutation was associated with a 7.2-fold increased risk for NTDs.[15] The 677C → T mutation results in an alanine to valine substitution in MTHFR,[16] renders the enzyme thermolabile,[16]

*Homocyst(e)ine (tHcy) refers to the sum of concentrations of free homocysteine, protein-bound homocysteine, the disulfide homocystine, and the mixed disulfide homocysteine-cysteine.

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and reduces cellular MTHFR activity,[14,16,17] resulting in partially impeded homocysteine remethylation (Fig 1). Therefore, homozygosity for this mutation results in higher plasma total homocyst(e)ine (tHcy) levels,[17-20] especially when dietary folate intake is also low.[18,20]

The remethylation of homocysteine to methionine requires vitamin B_{12} as a cofactor, and a suboptimal vitamin B_{12} status may therefore also result in hyperhomocyst(e)inemia.[21] Mothers with NTD-affected children have lower plasma vitamin B_{12} concentrations compared with controls,[11,22,23] and this relation is independent of folate status.[22]

Considering the etiological role of folate and/or vitamin B_{12} status in the prevention of NTDs, we hypothesized that differences in the folate/vitamin B_{12} nutritional status could explain the differences in NTD incidence in rural and urban black populations. It has been reported previously that folate deficiencies are common in rural pregnant black women[24] and rural black children[25]; however, we are unaware of any data describing the folate status of the South African urban black population. It is also not known whether impaired homocysteine metabolism exists in black women with a history of a NTD pregnancy, or whether homozygosity for the thermolabile variant of MTHFR increases the risk for a NTD-affected pregnancy in blacks.

We now report a cross-sectional population survey in which we compared the folate and vitamin B_{12} nutritional status of rural and urban black women. We also studied folate and vitamin B_{12} status, homocyst(e)ine metabolism, and MTHFR genotypes in 54 rural black women with a history of NTD-affected pregnancy. Our results suggest that neither inadequate folate or vitamin B_{12} nutritional status nor impeded homocyst(e-)ine metabolism can explain the etiology of NTDs in rural black women.

SUBJECTS AND METHODS

Population Survey of Folate Nutritional Status

Two tubes of blood with EDTA as the anticoagulant were obtained from an apparently healthy group of nonpregnant rural black women (age, 31.3 ± 7.5 years [mean ± SD]; n = 107). The women lived from subsistence farming and attended family planning or immunization clinics at various rural hospitals in the Northern Province, South Africa (Fig 2). Blood samples were immediately chilled on ice and centrifuged on site. Plasma samples were frozen in dry ice and sent to the laboratory in Pretoria. An urban sample of women were obtained from black women (age, 25.4 ± 3.9 years; n = 101) of the Pretoria area who visited a family planning or immunization clinic; blood samples were obtained from four clinics to reflect the socioeconomic composition of the local urban black population. Seventeen women were recruited from an inner-city clinic, 23 came from two clinics in middle-class suburbs, and 61 came from a clinic serving a low socioeconomic suburb and adjacent informal settlements. Blood samples were obtained and handled as already described. All blood samples were collected between October 1995 and March 1996.

Follow-up Study of Women With a History of a NTD Birth

Since 1990, a clinical genetic outreach program to seven rural hospitals in the Northern Province has been conducted by the Department of Human Genetics, University of Pretoria. Nursing staff in these rural hospitals were trained in the diagnosis and documentation of congenital anomalies including NTDs. This documentation, which included a clinical description and pregnancy and family history, as well as photographs, was then available for review by the visiting clinical geneticists for genetic counseling. With the help of the genetics-trained nursing staff of five of these rural hospitals, 54 black women who gave birth to a NTD-affected child between 1990 and 1995 were invited to participate in the study. Of 54 participants, 30 had a child with documented spina bifida, 10 with anencephaly, and seven with craniorachischisis, while seven had offspring with an encephalocele. For each woman with a NTD birth history, an age- and body mass–paired control was selected from the rural population sample already described. Controls had no history of children with any birth defects. The mean age

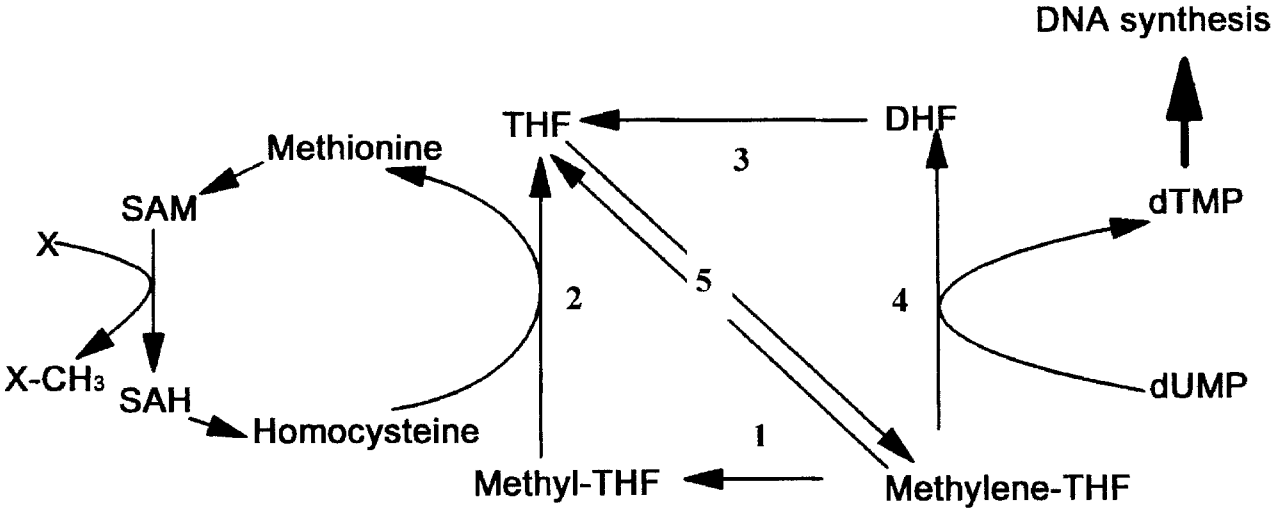

Fig 1. Simplified diagram illustrating the role of folic acid in biological methylation and DNA synthesis. Note that the methyl-THF–dependent remethylation of homocysteine requires vitamin B_{12} as as cofactor. Enzymes: 1, MTHFR; 2, methionine synthase; 3, dihydrofolate reductase; 4, thymidylate synthetase; 5, serine transhydroxymethylase. DHF, 7,8-dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; SAM, *s*-adenosylmethionine; SAH, *s*-adenosylhomocysteine; THF, tetrahydrofolate; X, methyl group acceptor.

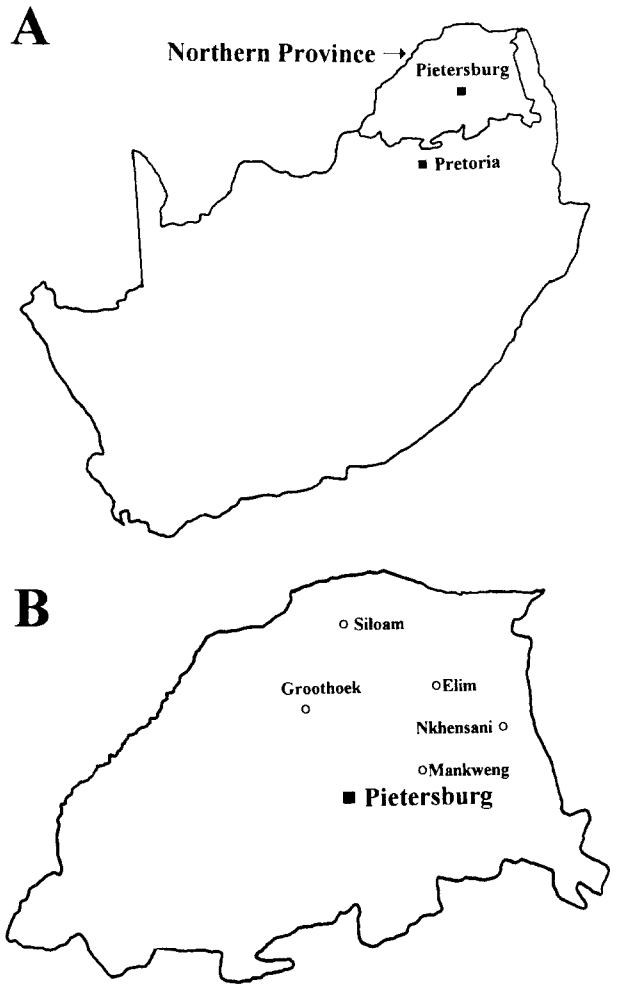

Fig 2. Map showing the (A) area (Northern Province) and (B) rural villages where the study was performed. The population sample consisted of 53, 31, 2, 18, and 3 women from Nkhensani, Groothoek, Siloam, Elim, and Mankweng, respectively, and the number of NTD cases from these centers was 25, 8, 2, 12, and 7, respectively.

of women with a history of a NTD birth was 26.5 ± 9.8 years, which was not significantly different from the mean age of 28.3 ± 9 8 years for the controls. None of the cases or controls were on vitamin supplementation. Fasting blood samples with EDTA anticoagulant were obtained from each woman; the samples were centrifuged on site, and the plasma was frozen in dry ice. The buffy coat and red blood cells were separated and stored frozen. Subsequently, L-methionine (100 mg/kg body weight, dissolved in imitation orange drink) was administered orally. After 2 hours, a light methionine-poor lunch was served; the participants were not allowed to eat until the first 2 hours elapsed, but were allowed to drink water at will. The second blood sample was obtained 6 hours after administration of the methionine load and was processed as already described.

Methionine Load Tests on Apparently Healthy White Women

Oral methionine load tests were also administered to 64 apparently healthy white women (mean age, 35 9 ± 8.1 years) recruited from the major employers in Pretoria. Women who participated were mothers of healthy normal children, and women who reported a previous miscarriage were excluded.

Laboratory Analyses

Plasma concentrations of vitamin B_{12} and folate, as well as red blood cell folate concentrations, were determined by a commercially available radioassay kit (Becton Dickinson, Orangeburg, NY). Plasma pyridoxal 5′-phosphate (PLP) concentrations were measured by high-performance liquid chromatography (HPLC) as described previously.[26]

Plasma homocyst(e)ine was derivatized with ammonium 7-fluorobenzo-2 oxa 1, 3 diazole-4-sulfonate ([SBD-F] Wako, Neuss, Germany) according to the method of Araki and Sako.[27] The method entails complete reduction of homocystine, the mixed disulfide (cysteine-homocysteine), and release of protein-bound homocysteine. This method therefore measures total (free + protein-bound) plasma homocyst(e)ine concentrations. The SBD-derivative of homocysteine was determined by HPLC.[28] Plasma methionine concentrations were measured according to the HPLC method of Potgieter et al.[29] This method quantifies methionine and methionine sulfoxide (an oxidation product of methionine formed during sample processing) and produces a reliable estimate of an individual's methionine status.

DNA was extracted from lymphocytes as described previously.[30] The presence of the 677C → T mutation in the MTHFR gene was investigated by a polymerase chain reaction (PCR) of genomic DNA and *Hin*fI digestion of the PCR product as described by Kluijtmans et al.[31]

Statistics

Student's *t* test was used for all between-group comparisons except plasma tHcy determinations, for which the Mann-Whitney *U* test was used to accommodate the observed nongaussian data distribution.

Ethics

The study was approved by the University Human Ethics Committee and performed according to the guidelines of the Declaration of Helsinki. Subjects for methionine load tests provided informed consent before participation in the study. When appropriate, the informed-consent form was translated into the native language of the participants by a community sister fluent in that language.

RESULTS

Table 1 compares the parameters of vitamin nutritional status for the rural and urban black women. Rural black women had significantly higher plasma folate ($P < .001$) and PLP ($P < .01$) compared with urban women. The better folate nutritional status of the rural women is also reflected by lower plasma tHcy concentrations in the latter group. However, this difference failed to reach the conventional limit of statistical significance ($P = .09$).

The vitamin status of black women with previous NTD-affected pregnancies and controls did not differ significantly

Table 1. Parameters of Vitamin Nutritional Status in Rural and Urban Black Women

Parameter	Rural Blacks (n = 107)	Urban Blacks (n = 101)	*P*
Body mass (kg)	64.6 ± 13.7	65.2 ± 13.0	NS
P-tHcy (μmol/L)	7.52 ± 1.87	8.05 ± 2.26	09
P-PLP (nmol/L)	33.7 ± 16.5	27.5 ± 16.0	<.01
P-vitamin B_{12} (pmol/L)	314.5 ± 163.5	300.3 ± 127.2	NS
P-folate (nmol/L)	9.75 ± 3.83	7.93 ± 3.00	<.001

NOTE. Results are the mean ± SD.
Abbreviation: P, plasma.

Table 2. Vitamin Status and Methionine Load Test Results in Rural Black Women With a History of a NTD Birth and Age-Matched Controls

Parameter	NTD Births (n = 54)	Controls (n = 54)
Age (yr)	26.5 ± 7.9	28.3 ± 9.8
Body mass (kg)	61.1 ± 9.7	64.9 ± 13.8
P-PLP (nmol/L)	30.2 ± 16.1	33.3 ± 15.6
P-vitamin B_{12} (pmol/L)	273.2 ± 172.5	307.4 ± 163.3
P-folate (nmol/L)	11.2 ± 4.7	10.3 ± 4.2
Red blood cell folate (nmol/L)	564.3 ± 186.0	539.8 ± 211.7
Fasting P-methionine (μmol/L)	24.6 ± 5.2	26.9 ± 8.7
P-methionine increase (μmol/L)	686.2 ± 191.1	680.2 ± 209.8
Fasting P-tHcy (μmol/L)	7.17 ± 1.88	7.31 ± 1.63
P-tHcy increase (μmol/L)	10.81 ± 3.91	11.54 ± 4.04

NOTE. Results are the mean ± SD. Plasma methionine is the total methionine concentration, ie, including oxidized methionine. Post–methionine load plasma methionine and homocyst(e)ine concentrations were corrected for the respective fasting concentrations. Differences between the 2 groups were not statistically significant.

(Table 2). There were no significant differences in fasting plasma tHcy and methionine, as well as the increase in post–methionine load tHcy and methionine. Both the black control and NTD groups had considerably lower post–methionine load tHcy concentrations compared with apparently healthy white women (Fig 3): 51% of black controls and 52% of black NTD cases had post–methionine load plasma tHcy concentrations less than the fifth percentile as observed in whites.

The prevalence of the homozygous 677C → T mutation in the MTHFR gene was examined in women with a history of a NTD birth and in controls. No homozygotes were found in either group (Table 3). However, nine of 54 (16.6%) controls and 12 of 58 (20.7%) NTD cases were heterozygous for the mutation. A population genetic test showed that this black population sample was in Hardy-Weinberg equilibrium.

Table 3. MTHFR Genotype Distribution Among Rural Black Women With a History of a NTD Birth and Age-Matched Control

Genotype	NTD Births (n = 53)	Controls (n = 54)	Total (n = 107)	
			Observed	Expected
677C/C (−/−)	42	43	85	86.1
677C/T (+/−)	11	11	22	19.8
677T/T (+/+)	0	0	0	1.1

DISCUSSION

A previous study by Baynes et al[24] at an antenatal clinic in the Northern Province found that folate deficiency was common among black women in the second or third trimester of pregnancy: 48% had plasma folate concentrations less than 3.0 ng/mL (<6.75 nmol/L), and a mean corpuscular volume greater than 100 fL was present in 35% of the study population. Our results suggest that the prevalence of folate deficiency is also high in rural nonpregnant black women. Twenty-one percent of the rural population sample had plasma folate levels less than 6.75 nmol/L. Nevertheless, the rural population sample had a significantly better folate status (as assessed by the plasma folate concentration) than the urban population group (Table 1), in which the prevalence of a low folate status (<6.75 nmol/L) was 46%. This is presumably explained by the fact that the rural population, which lives mainly from subsistence farming, has more ready access to leafy vegetables (a rich dietary source of folate) than urban dwellers. The vitamin B_6 status of rural women was also significantly better compared with urban dwellers, which supports the suggestion that rural women consume a diet containing relatively more vitamins. The fasting plasma tHcy concentration, a sensitive metabolic indicator of folate and vitamin B_{12} nutritional status, tended to be lower in rural women, again indicating a relatively better vitamin nutritional status. Our results support the observations from a dietary study on urban blacks in Cape Town, which indicated a significant trend for lower folate consumption with urbanization.[32]

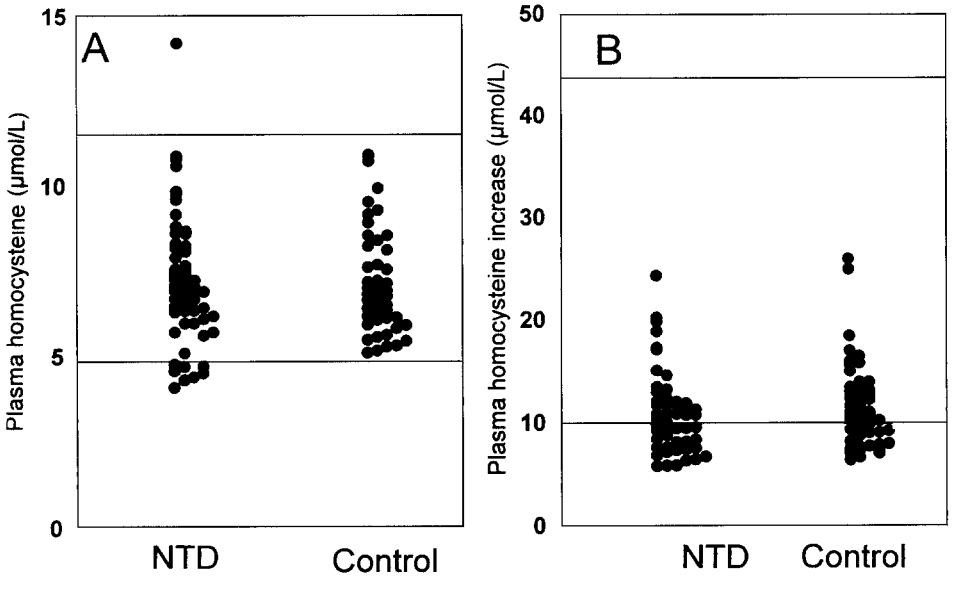

Fig 3. Post–methionine load plasma tHcy concentrations in black women with NTD-affected pregnancy and controls. Lower and upper horizontal lines indicate the 5th and 95th percentiles, respectively, as determined in apparently healthy white women.

The urban population sample is reasonably representative of the various socioeconomic classes among the black urban population and is typical of the population served by the Kalafong Academic Hospital, Pretoria, where a well-documented study reported a NTD incidence of only 0.99 per 1,000 live births.[4] Considering that the NTD prevalence in rural areas is 3.55 to 6.13 per 1,000 live births.[1,2] our data suggest that the substantial decrease in the NTD prevalence with black urbanization cannot be explained by an improved folate or vitamin B_{12} status. In fact, the significantly lower folate status and a tendency for a higher tHcy concentration in urban blacks challenges the notion that folate deficiency increases the risk of a NTD pregnancy in this population group. This possibility has been further investigated in black women with a history of NTD births.

The data presented in Table 2 indicate that black rural women with a history of NTD-affected children do not differ significantly from controls with respect to vitamin nutritional status. This finding supports the general notion that a frank folate deficiency is not a prerequisite for a NTD.[33] It has been suggested that supplemental folate relieves a relative metabolic block[33] that otherwise may have resulted in a failure of the neural tube to close early in fetal development. Evidence from white populations indicates that this presumed metabolic aberration involves homocysteine metabolism,[10-13] but our results do not support a causative role for aberrant homocysteine metabolism in the failure of proper neural tube closure. In contrast to studies performed in a European population,[10] we found no difference in post–methionine load tHcy concentrations in black mothers with NTD-affected offspring compared with age-matched controls. On the contrary, black women with NTD-affected offspring demonstrated highly effective metabolism of the methionine load. More than 50% of these women had post–methionine load plasma tHcy concentrations less than the fifth percentile as observed in healthy whites with normal children (Fig 3). This supports a previous observation that blacks metabolize homocysteine more effectively than whites,[34] and indicates that other metabolic and/or genetic factors contribute to the high rate of NTD-affected pregnancy among rural blacks.

In whites, homozygosity for the 677C → T mutation in the MTHFR gene constitutes a genetic risk factor for a NTD-affected pregnancy.[14,15] However, we found no homozygotes for the 677C → T mutation in black mothers with NTD-affected offspring or controls (Table 3). Based on the frequency of heterozygotes (20.5% for the whole group, ie, cases and controls), we expect the frequency for homozygosity in the black population to be approximately 1%. This corresponds to a study in African-Americans, in which the estimated homozygote frequency is also about 1%.[35] It is clear that the MTHFR polymorphism does not explain the occurrence of NTDs in the South African black population.

In conclusion, the well-documented and considerable decline in the prevalence of NTDs with black urbanization remains unexplained. Homozygosity for the 677C → T mutation in the gene coding for MTHFR is low in blacks and does not constitute a genetic risk factor for NTDs in this group. Our data question the role of folate deficiency and aberrant homocysteine metabolism in the etiology of NTDs in blacks.

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