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Neural tube defects, methylenetetrahydrofolate reductase mutation, and north/south dietary differences in China

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Abstract: There is a well-recognized correlation between methylenetetrahydrofolate reductase (MTHFR) C677T mutation homozygosity, elevated plasma homocysteine, and increased risk of neural tube defects (NTDs). This risk is modulated by maternal and fetal folate levels provided provided by diet or supplement. Although the frequencies of the C677T mutation are nearly identical between north and south China, the incidence of NTDs is nearly 5 times greater in the north than in the south. This dramatic difference appears related to the fact that dietary sources of folate are more plentiful and varied in South China.

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Schneider et al. [1998] recently reported the frequencies of the methylenetetrahydrofolate reductase (MTHFR) C677T mutation in 16 populations worldwide. Based on the now well-recognized correlation between C677T homozygosity, elevated plasma homocysteine, and increased frequency of neural tube defects (NTDs) [Wilcken, 1997; van der Put et al., 1998], they conclude that the correlation of European and sub-Saharan African NTD frequencies with C677T allele frequencies in these populations is consistent with the hypothesis that the C677T mutation is a risk factor for NTDs. Data gathered in China for a study that was prematurely ended by events surrounding the infamous Tiananmen Square incident [Turnley et al., 1989] may serve to further enlighten.

China's classic north/south divide is the Qin Mountain-Huai River Line (Fig. 1). NTD birth incidence data for 1980–1987 was ascertained from 42 hospitals in districts north and south (Fig. 1, Table 1). The mean incidence for North China was 1:137 and for South China 1:637, nearly a 5-fold difference. Previous studies of births prior to 1980 showed an incidence of 1:182 in Beijing [Lei and Yang, 1985] and 1:1,020 in Hong Kong [Ghosh et al., 1981], nearly a 6-fold difference. These remarkable frequency differences should be viewed in light of the Schneider et al. [1998] findings: other than the Brazilian Amerindians (a population of North Asian origin), the Hong Kong Chinese and Mongolia populations had the highest C677T allele frequencies, frequencies that were nearly *identical*!

Nevertheless, C677T homozygosity is clearly correlated with an increased birth incidence of NTDs [van der Put et al., 1998]. A segregation analysis using POINTER for mixed model tests was performed with 102 extended NTD Shanghai kindreds (2,763 total persons) divided into 102 families of probands, siblings, and parents (single incomplete ascertainment) and 685 nuclear families related to probands [ascertainment through pointers (probands)], a total of 787 nuclear families. The model with a recessive major locus was the most parsimonious, with a penetrance of 1.0 and an estimated phenocopy rate of 0.0005. This outcome is consistent with an increased NTD risk with C677T homozygosity but not heterozygosity, and with the recently described increased risk associated with C677T/A1298C compound heterozygosity [van der Put et al., 1998].

In light of the requirements for methionine metabolism, this risk is undoubtedly modulated by folate levels in mother and fetus, and accounts (at

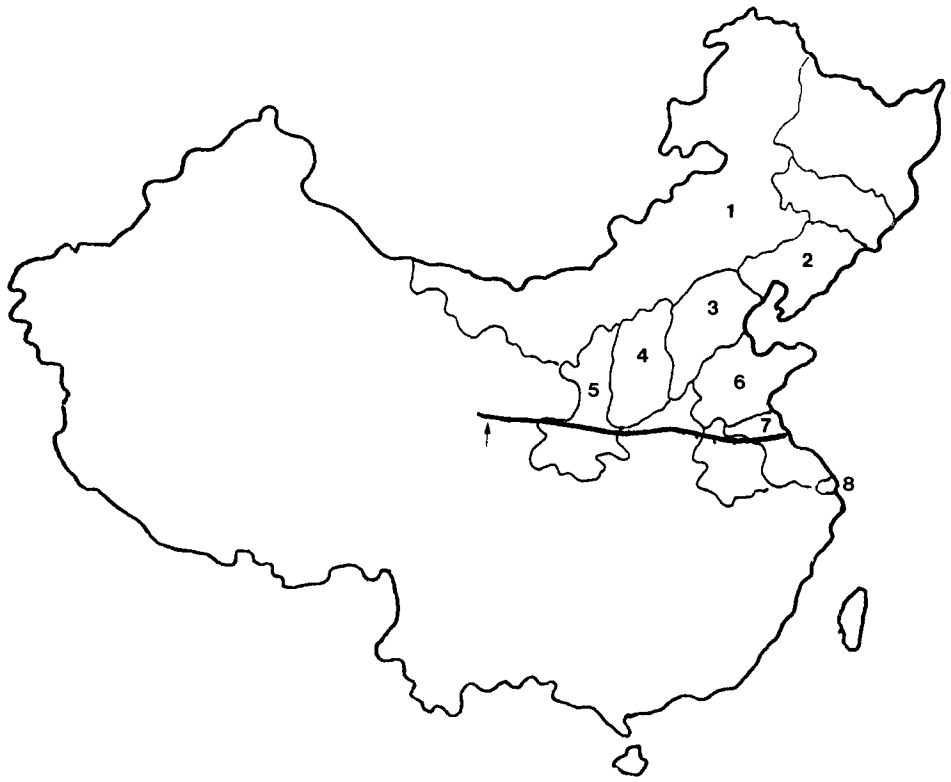

Fig. 1. The black line (arrow) represents the Qin Mountain-Huai River Line, the classical divide of North and South China. Districts: 1) Neimenggu; 2) Liaoning; 3) Heibei; 4) Shānxi Province; 5) Shănxi Province; 6) Shandong; 7) Jiangsu; and 8) Shanghai.

least in part) for the dramatic decline in NTD incidence with periconceptional vitamin supplementation [Czeizel and Dudás, 1992]. Accordingly, it is instructive to look at the 5-fold NTD risk difference between North and South China in the context of climate and diet. South China districts have an average yearly temperature 22% greater than North China (60°F vs. 49°F) and an average yearly rainfall which is 43% greater. This correlates with the availability of plant and animal foodstuffs in South China which are more scarce in North China. Table 2 shows the contents of the average diet in districts of North and South China and the associated NTD frequencies. South China diets, in contrast to North China, contain a larger variety of foods including meat, fish, shellfish, green vegetables, and rice, and much less corn, Chinese sorghum, and potato. The dietary sources of folate are more plentiful in the South China and more varied, and this is associated with a dramatically lowered NTD incidence despite a C677T allele frequency equivalent to that of North China.

Finally, the dietary sources of vitamin A are also more plentiful and varied in South China and deficiencies of vitamin A may also be a risk factor [Sandford et al., 1992], perhaps associated with a different genetic risk. Retinoids are an important

TABLE 1. NTD study participating hospitals

1. Shenyang Maternity Infant Hospital	15. Xian Municipal First Hospital	29. Yangpu District Maternity Hospital
2. Chinese Medical University, Shenyang	16. Xian Medical University	30. Second Textile Hospital
3. Shenyang Medical College	17. Zhengzhou Railway Hospital	31. Yanpu District Central Hospital
4. Dalian Obstretric Hospital	18. Jinan Municipal Second Hospital	32. Huangpu District Maternity Hospital
5. Dalian Institute for Family Planning	19. Liubo Maternity Infant Hospital	33. Third People Hospital
6. Tongliao Municipal Second Hospital	20. Xuzhou Medical College	34. Nanshi District Maternity Hospital
7. Tongliao Maternity Infant Institute	21. Putuo District Maternity Hospital	35. Shanghai Second Municipal Hospital
8. Wulan Chabu District Family Planning Center	22. Railway Central Hospital	36. Reijin Hospital
9. Heibei Medical College	23. Zhabei District Central Hospital	37. Luwa District Maternity Hospital
10. Baoding District Hospital	24. Zhabei District Maternity Hospital	38. Sanghai Sixth Municipal Hospital
11. Changzhi Municipal Hospital	25. Honkou District First Hospital	39. Changning District Maternity Hospital
12. Shanxi Medical College	26. Shanghai First Municipal Hospital	40. Changning District Central Hospital
13. Taiyuan Obstetric Hospital	27. Honku District Maternity Hospital	41. International Peace Maternity Hospital
14. Taiyuan Railway Hospital	28. Xinhua Hospital	42. Wusong District Hospital

TABLE 2. Average diets in representative districts of north and south China (grams/day)

Food	District		
	Hebei (north)	Shānxi (north)	Shanghai (south)
Rice	11	11	459
Wheat flour	346	179	44
Corn/sorghum	265	357	0
Potato	100	331	43
Light leaf vegetable	178	208	651
Green vegetable	106	74	174
Fruit	31	12	9
Meat	9	1	41
Fish/shellfish	1	0.1	33

morphogen, which operate in a comparatively narrow quantitative window; both excess and deficiency have a profound effect on embryogenesis [Hoffman, 1990; Blomhoff et al., 1990]. The curly-tail (CT) mouse is perhaps the best mouse model of human NTD [Embury et al., 1979]. The phenotype (spina bifida and, less often, exencephaly) is due to the recessive gene *ct,* which maps to distal mouse Chromosome 4 [Beier et al., 1995]. Penetrance is incomplete and is reduced further in outcrosses to other strains, suggesting the existence of modifier loci. One such locus, *mct 1,* has been identified and maps to mouse chromosome 17 [Letts et al., 1995]. Vitamin A is normally thought of as teratogenic to neurulation [Shankar et al., 1994]. However, when vitamin A is administered on gestation day 9 to homozygous *ct* mice, the NTD frequency declines below that of untreated homozygous *ct* mice [Seller et al., 1979]. As justifiably enthusiastic as we may be about the C677T/homocysteine/folate pathogenetic pathway, it is critical to recall Holmes' [1992] admonition that there are undoubtedly many causes of NTDs, only some of which may be modified by folate supplementation.

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