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Differentiating between Fetal and Maternal Genotypic Effects, Using the Transmission Test for Linkage Disequilibrium

To the Editor:

The transmission-disequilibrium test (TDT) (Spielman et al. 1993) provides an attractive alternative to case-control studies of the relationship between a complex disease and a genetic marker. The principal advantage of the TDT is that, unlike case-control studies, it is not subject to confounding due to the use of inappropriate control groups. An additional advantage of the TDT over case-control studies, which has not previously been described, is that the TDT can be used to differentiate between fetal and maternal genotypic effects.

As an example, consider the association between the 677T→C mutation in the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR) and the risk of neural tube defects (NTD), which has been noted in several case-control studies (Ou et al. 1996; van der

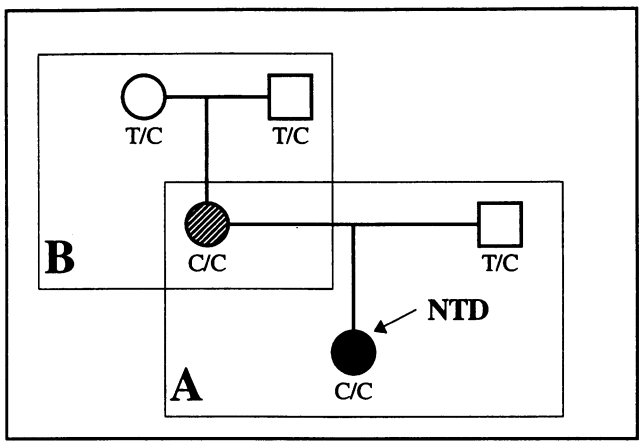

Figure 1 Illustration of how the TDT can be used to distinguish between fetal and maternal genotypic effects. The relationship between the fetal genotype and the risk of NTD is assessed by evaluating the transmission of alleles from heterozygous parents to their NTD-affected offspring (*A*). The relationship between the maternal genotype and the risk of having a child with an NTD is assessed by evaluating the transmission of alleles from heterozygous maternal grandparents to the mothers of NTD-affected individuals (*B*). Individuals with NTD are denoted by darkened symbols; mothers of individuals with NTD are denoted by hatched symbols. T and C refer to the normal and mutant forms, respectively, of the gene for MTHFR.

Put et al. 1996; Whitehead et al. 1996). Compared to controls, the frequency of the MTHFR 677T→C mutation appears to be increased in individuals with NTD as well as the mothers (and possibly the fathers) of affected individuals. There are at least four potential explanations for these findings: (i) differences between cases and controls are due to the use of inappropriate control groups (Posey et al. 1996); (ii) both fetal and maternal genotypes influence the risk of NTD; (iii) only fetal genotypes influence the risk of NTD; or (iv) only maternal genotypes influence the risk of NTD. It is not possible to differentiate among the last three possibilities on the basis of case-control data, since allele frequencies in parents are not independent of those in offspring (i.e., even if the risk of NTD is entirely attributable to the fetal genotype, an increased frequency of the MTHFR 677T→C mutation would be expected in the parents of affected individuals).

By use of the TDT, however, it is possible to distinguish between fetal and maternal genotypic effects by testing the two possibilities separately. The relationship between the *fetal* genotype and the risk of NTD is simply assessed by evaluating the transmission of alleles at the MTHFR locus from heterozygous parents to their NTD-affected offspring (fig. 1*A*). The relationship between the *maternal* MTHFR genotype and the risk of having a child with an NTD is assessed by evaluating the transmission of alleles from heterozygous maternal grandparents of NTD-affected individuals to the mothers of NTD-affected individuals (fig. 1*B*). Therefore, when the fetal genotype is assessed, "being affected with an NTD" is the phenotype of interest, whereas, when the maternal genotype is assessed, "having a child with an NTD" is the phenotype on which the TDT is performed.

Unlike allele frequencies, the transmission of alleles to parents and offspring are independent events. When both tests are significant, both fetal and maternal genes are implicated in disease etiology. Hence, use of this two-step TDT should help to establish whether fetal and/or maternal MTHFR genotypes are associated with the risk of NTD.

The two-step TDT should be considered whenever disease risk may be influenced by the maternal genotype. However, it is not necessary to obtain data for both tests from each pedigree. Data may be obtained from a combination of three-generation pedigrees (as illustrated in fig. 1) and two-generation pedigrees, which include either affected offspring (fig. 1*A*) or mothers of affected children (fig. 1*B*).

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