or 100 genetic tests might be available for many common diseases. If there are numerous predisposing alleles and each has an independent odds ratio of only 1.5–1.7, the overall effect would still be substantial. We simulated models of 10, 15, and 20 genes with a risk of 1.5–1.7 each and found the areas under the ROC curves (AUCs) to be 0.70, 0.74, and 0.77, respectively. The discriminatory ability of 20 gene tests, each with an odds ratio of 1.5–1.7, is comparable with the test of total cholesterol level for prediction of coronary heart disease (Wilson et al. 1998). The effect would be even greater if only 5% or 10% of all alleles tested had odds ratios in the range of 2.5–3.5 or if we could identify combinations of a few genes and/or gene-environment interactions that are strong predictors of the disease.

The comments of Janssens et al. also raise several interesting points regarding different perspectives on multiple genetic testing. Epidemiologic studies, including those on the utility of ROC curves for screening, provide a useful population perspective. In contrast, clinicians usually focus on individual patients rather than on the population as a whole, and this focus will be enhanced by the development of personalized genomic medicine (Roses 2000; Jain 2002). It is true that no more than a few people per million might turn out to have a very high risk defined by positive results for multiple genetic tests for a particular disease. However, it might be very important to these few people to know that they are at high risk if an intervention is available to prevent the disease. Our likelihood-ratio–based method provides an approach that is useful for individual patients and their physicians in predicting the probability of developing disease.

QUANHE YANG,[1] MUIN J. KHOURY,[2] LORENZO BOTTO,[1] J. M. FRIEDMAN,[4] AND W. DANA FLANDERS[3]

[1]*National Center on Birth Defects and Developmental Disabilities and* [2]*Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention (CDC), and* [3]*Department of Epidemiology, School of Public Health, Emory University, Atlanta; and* [4]*Department of Medical Genetics, University of British Columbia, Vancouver*

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Address for correspondence and reprints: Dr. Quanhe Yang, National Center on Birth Defects and Developmental Disabilities (CDC), 1600 Clifton Road, MS E-86, Atlanta, GA 30333. E-mail: qay0@cdc.gov

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Impact of Genotyping Errors on Type I Error Rate of the Haplotype-Sharing Transmission/Disequilibrium Test (HS-TDT)

To the Editor:

In a recent issue of the *Journal,* Zhang et al. (2003) proposed a haplotype-sharing transmission/disequilibrium test (HS-TDT) for the null hypothesis of no linkage or no association between a disease and a chromosomal region in which several tightly linked markers have been typed. Their method is applicable to data of nuclear families without phase information. The general idea of their approach is to compare the similarity of the transmitted haplotypes with the similarity of the nontransmitted haplotypes. If the chromosomal region contains a susceptibility locus, it is expected that the haplotypes being transmitted to affected children are more similar than parental haplotypes that have not been transmitted. This reasoning seems intuitively appealing. However, it may be supposed that a larger observed similarity for transmitted than for nontransmitted haplotypes is not necessarily due to the presence of a disease-susceptibility locus but can be a consequence of undetected genotyping errors. The proportion of genotyping errors that result in a Mendelian inconsistency (MI) is relatively small for family trios (Gordon et al. 1999). More important, in the context of HS-TDT, is the fact that the chance to detect a genotyping error differs for transmitted and nontransmitted haplotypes. Obviously, mistyping of an allele on a nontransmitted parental haplotype can never

Table 1

Estimates (Based on 1,000 Replicated Samples) of the True Type I Error Rate of HS-TDT in the Absence of Genotyping Errors for Nominal Type I Error α and for Two Different AOs to Handle Ambiguous Families

	Estimated True Type I Error Rate			
	AO1		AO2	
N	$\alpha = .05$	$\alpha = .01$	$\alpha = .05$	$\alpha = .01$
100	.049	.009	.045	.006
200	.061	.009	.061	.009
1,000	.045	.010	.045	.010

result in a MI and, therefore, cannot become prominent. Another way to understand this problem is to imagine that transmitted haplotypes are partially checked for their integrity, whereas there is no such checking at all for the nontransmitted haplotypes. A single error occurring at one locus of a haplotype, however, can have a tremendous effect on the measure of similarity of this haplotype with all other haplotypes. Thus, nontransmitted haplotypes can appear less similar than transmitted haplotypes as a result of undetected genotyping errors. In statistical terms, genotyping errors may lead to an inflated type I error rate for the HS-TDT.

To quantify the magnitude of this inflation, we performed a simulation study. Our simulation study assumes that, for 19 tightly linked and equidistant diallelic marker loci, only 29 different haplotypes occur in the population. This set of haplotypes and the corresponding frequencies are shown in table A of the online-only supplemental material. For all family trios, we generate the parents' genotypes according to this haplotype distribution. The haplotype pair in the child is obtained by randomly selecting one of the two haplotypes in each parent. Next, genotyping errors are introduced independently into the alleles according to the stochastic error model, for which ε denotes the probability that, at each marker locus, the allele is changed. We consider the cases for $\varepsilon = 0$ (no genotyping errors), $\varepsilon = 0.001$, $\varepsilon = 0.005$, and $\varepsilon = 0.01$. Sometimes, a genotyping error becomes visible by leading to MI. We consider three different error options (EOs) as strategies for responding to such an inconsistency: (EO1) genotypes of a marker locus with MI are considered to be unknown in all individuals of the family; (EO2) in the presence of MI for at least one marker locus, the whole family is discarded from the analysis; and (EO3) a marker locus showing MI is typed again, and it is assumed that the retyping results in error-free genotypes for this marker locus. The number of family trios in a sample is denoted by N, and we let $N = 100$, 200, or 1,000. Note that for EO2, the number of families used for statistical analysis is generally smaller than N. For each combination of ε, EO, and N, 1,000 samples are generated.

To analyze a simulated sample by the HS-TDT proposed by Zhang et al. (2003), we discard the phase information. The first step for statistical analysis is to obtain haplotype estimates. This is achieved by the program FAMHAP (Becker and Knapp, in press), which applies a locus-iterative mode of the expectation-maximization (EM) algorithm (Dempster et al. 1977) to obtain maximum-likelihood estimates of haplotype frequencies in general nuclear families. Zhang et al. (2003) discussed two different analysis options (AOs) to make use of estimated haplotype frequencies in case of ambiguous phase information in the families of the sample: (AO1) each possible haplotype explanation of an ambiguous family is weighted by its relative likelihood and (AO2) each ambiguous family is assigned its most likely haplotype explanation. Our simulated samples are analyzed by both of these AOs. The HS-TDT requires a permutation procedure to obtain the P value of the test. For each sample, we estimate the P value by 10,000 permutations. The true type I error rate at nominal error rate α is estimated by the fraction of the 1,000 replicated samples resulting in a P value $\leqslant\alpha$.

The results are shown in tables 1 and 2. If there are no genotyping errors (i.e., $\varepsilon = 0$), table 1 reveals a good agreement between nominal and true type I error rate, irrespective of the AO used to handle ambiguous families. (Note that if there are no genotyping errors, no MIs can occur, and, therefore, the EO is irrelevant.) Table 2 gives estimated type I error rates for the three EOs

Table 2

Estimates (Based on 1,000 Replicated Samples) of the True Type I Error Rate of HS-TDT (AO1) in the Presence of Genotyping Errors for Nominal Type I Error α and for Three Different Options to Handle MIs

		Estimated True Type I Error Rate					
		EO1		EO2		EO3	
N	ε	$\alpha = .05$	$\alpha = .01$	$\alpha = .05$	$\alpha = .01$	$\alpha = .05$	$\alpha = .01$
100	.01	.576	.297	.471	.228	.540	.264
100	.005	.215	.079	.219	.075	.208	.075
200	.005	.389	.164	.393	.146	.383	.164
1,000	.001	.146	.039	.167	.045	.147	.039

and AO1. Results are virtually identical when the most likely haplotype explanation is assigned to ambiguous families (see table B in the online-only supplemental material). As is obvious from table 2, the agreement between nominal and true type I error rate is disastrous in the presence of genotyping errors. Even quite small probabilities of genotyping errors lead to a dramatic inflation of the type I error. For fixed values of ε, the extent of this inflation increases with increasing sample size (N), as can be seen by comparing the second and third row in table 2. For a large sample size of $N = 1{,}000$ family trios, an error probability of $\varepsilon = 0.1\%$ is sufficient to falsely reject the null hypothesis at $\alpha = 0.05$ in almost every sixth study. For small values of N and large values of ε, the inflation of type I error is slightly less pronounced for EO2 than for EO1, which is explained by noting that EO2 leads to a decrease of the sample size used for the analysis. At first sight, it may be surprising that no essential decrease of the inflation of type I error is obtained by employing EO3. However, correcting genotypes leading to MIs does not affect errors in the nontransmitted haplotypes.

What are possible limitations of our simulation study? We assume a specific haplotype structure in the population, such that only 29 different haplotypes are present. Indeed, we conjecture that with larger haplotype diversity, the effect of genotyping errors on the type I error rate of the HS-TDT will be less pronounced than in the example considered here. On the other hand, however, it does not seem very realistic to expect that the HS-TDT will have substantial power to detect a disease locus in a region in which the markers are in complete or nearly complete linkage equilibrium in the population. Thus, although our example describes a specific situation, it does not seem to be unrealistic for the genetic structure of a region for which the HS-TDT may have a good chance of detecting a disease locus. A second possible limitation is that we employed a quite simple error model that assumes the independence of genotyping errors from factors such as marker locus, true allele, etc. However, we see no reason why the behavior of the type I error rate of the HS-TDT should be qualitatively different for more complex models of genotyping errors. Additionally, we are convinced that the range 0.1%–1% for the probability (ε) of a genotyping error considered here is not too pessimistic for currently available methods of high-throughput genotyping.

In summary, we have shown that the correctness of genotypes is crucial for obtaining meaningful results by the HS-TDT. We have also demonstrated that the retyping of only those marker loci that show MIs within a family is useless. A more extreme approach is to genotype all marker loci in all families in duplicate, which is very expensive and certainly not very popular with geneticists responsible for generating genotypes. However, unless extreme care is taken to guarantee the integrity of the data analyzed by the HS-TDT, this interesting and appealing method has the potential of becoming a mighty tool for the enlargement of the heap of false-positive association results in human genetics.

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MICHAEL KNAPP AND TIM BECKER
Institute for Medical Biometry, Informatics, and Epidemiology
University of Bonn
Bonn

Electronic-Database Information

The URL for data presented herein is as follows:

FAMHAP: Haplotype Frequency Estimation, http://www.uni-bonn.de/~umt70e/becker.html

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Address for correspondence and reprints: Dr. Michael Knapp, Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany. E-mail: knapp@uni-bonn.de

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Reply to Knapp and Becker

To the Editor:

Knapp and Becker (2004 [in this issue]) have argued that genotyping errors may lead to an inflated type I