Parent-of-Origin Effect and Risk for Attention-Deficit/Hyperactivity Disorder: Balancing the Evidence against Bias and Chance Findings

To the Editor: In the December 2005 issue of *The American Journal of Human Genetics,* Hawi et al. made the intriguing observation that a group of catecholamine-related genes, shown elsewhere[1] to be associated with attention-deficit/hyperactivity disorder (ADHD [MIM 143465]), further demonstrate a "consistent pattern of preferential paternal transmission of risk alleles to affected children with ADHD."[2(p.959)] The hallmark of this article is that it collated transmission/disequilibrium information from several genes and tested a combined genetic hypothesis, which led to the identification of remarkably significant statistical effects. We believe that the conclusions of this article are flawed, for the following reasons.

1. Hawi et al.[2] used two alternative criteria to determine whether a gene/locus is associated with ADHD and, therefore, whether it should be retained in the main analysis comparing paternal and maternal transmissions. The first criterion was that association with ADHD must have been demonstrated in their sample of Irish children (significant overtransmission of a specific allele with $P \leqslant .1$). Of the 17 genes listed in table 1 of their article,[2] 6 genes (*DRD4* [MIM 126452], *DRD5* [MIM 126453], *TH* [MIM 191290], *DDC* [MIM 107930], *SERT* [MIM 182138], and *TPH2* [MIM 607478]) fulfilled this criterion and were included in the parent-of-origin analysis. Alternatively, Hawi et al. chose genes/loci that "have been confirmed (by several groups) to be associated with ADHD."[2(p.960)] With the use of this alternative criterion, three additional genes were retained in the parent-of-origin analysis (*DAT1* [MIM 126455], *SNAP-25* [MIM 600322], and *5HT1B* [MIM 182131]), even though they did not show association with ADHD in the sample presented in their study.[2] We believe that both criteria are problematic, for the following reasons:

   a. Had the first criterion been used alone, three genes would have been excluded from the parent-of-origin analysis: *DAT1* ($P = .4$), *SNAP-25* ($P = .12$), and *5HT1B* ($P = .2$). The exclusion of these three genes from the joint analysis of paternal versus maternal transmission would have resulted in a marginal parent-of-origin effect in the remaining six genes ($\chi^2 = 4.07$; $P = .04$). In addition, the claim made by the authors that the lenient threshold ($P \leqslant .1$) would protect against type II error and would "lead to an underestimate of the size of parent-of-origin effects"[2(p.960)] is not a valid one, since the statistic used to test association between ADHD and each allele in a given gene/locus (transmission/disequilibrium test [TDT]) and the one used to test for parent-of-origin effect of this allele (χ^2 test with 1 df) are not necessarily correlated. Indeed, it is possible to observe a significant TDT in the absence of parent-of-origin effect (the risk allele is equally overtransmitted from mothers and fathers) and a nonsignificant TDT in the presence of parent-of-origin effect (paternal overtransmission and maternal undertransmission or vice versa, where the effects are canceled out in a global TDT test). Thus, it is not possible to predict the behavior of one statistic given the behavior of the other one, and, consequently, the decision to set the threshold of the individual TDT at $P \leqslant .1$, to include an allele in the analysis of parent-of-origin effect, is arbitrary. Interestingly—and in contrast to the claim that a lenient threshold of .1 is conservative, with regard to parent-of-origin effect—had the authors chosen a slightly more stringent criterion ($P \leqslant .07$) without invoking any other criterion, a fourth gene (*SERT*) would have been excluded from the analysis, leading to a nonsignificant statistic of the parent-of-origin effect in the joint analysis of the five remaining genes ($\chi^2 = 1.91$; $P = .17$).

   b. The second criterion is also problematic, for at least two reasons. First, the literature still lacks consensus on which genes are implicated in ADHD and which are not. An excellent illustration of this problem is provided by the authors themselves. Indeed, in two earlier publications, they reported that *DAT1*[3] and *DBH*[4] contribute significantly to the risk of ADHD. However, both of these associations have not been confirmed in the extended sample presented in the 2005 study.[2] Second, this criterion seems to reflect a post hoc decision that favors their postulated hypothesis. For example, *DAT1,* which has the most negative effect on sensitivity analysis (*P* value dropped from .0019 to .013), was "rescued" using this criterion.

2. Remarkably, when we used a χ^2 statistic to compare paternal and maternal transmission of the risk allele separately for each of nine individual genes selected by Hawi et al.,[2] *only two* of these alleles in two genes (*DAT1* [$P = .03$] and *SERT* [$P = .009$]) resulted in a significant overtransmission from fathers compared with mothers. Given that the samples used to calculate the χ^2 statistic for each individual allele are quite small (particularly for *SERT* and *DDC,* for which some of the counts are as low as two) and, additionally, that these *P* values need to be corrected for the large number of tests conducted (at least 17 genes, not to mention the markers in each gene), this is really not an impressive obser-

vation and may, in fact, simply reflect chance findings.

3. The likelihood that these results represent chance findings from this data set is clearly illustrated by the simple statistical analysis performed using the data provided in table 1 of Hawi et al.[2] We correlated the number of markers tested in each gene/locus with the strength of the association between the risk allele in that gene/locus and ADHD, as measured by the χ^2 values. A highly significant correlation was observed (Spearman $R = 0.7$; $P = .002$; $N = 17$).
4. The dramatic contrast between the overtransmission of the parental risk allele at $P = 1.5 \times 10^{-10}$, as opposed to the meager overtransmission of the risk allele from the mother's side ($P = .026$), may also be a reflection of the arbitrary nature of inclusion and exclusion criteria. Indeed, if these criteria were biased in a way in which alleles that are overtransmitted from the paternal side are more likely to enter the parent-of-origin analysis, there would be a highly significant difference between the transmission of parental risk alleles summed over the nine loci compared with the transmission of the same maternal risk alleles summed over the nine loci. This impressive contrast between paternal and maternal overtransmission of the so-called risk allele may be observed even if paternal transmission of the risk allele only marginally exceeds maternal transmission of the risk allele at each individual gene/locus selected by the authors.
5. More generally, results showing significant overtransmission of an allele from one but not the other parent could be interpreted in two different ways. First, the overtransmission from one parent could reflect a true-positive result, and its absence from the other parent could be interpreted by invoking a parent-of-origin effect. Alternatively, the absence of overtransmission from one parent could reflect a true-negative result, and its presence from the other parent could be interpreted as a false-positive result. Although Hawi et al.[2] systematically sided with the first interpretation, we believe that the second interpretation should be carefully considered before retaining the first one, for several reasons. First, as mentioned above, this particular data set is likely to contain false-positive results. Second, none of the genes studied by Hawi et al.[2] is known to be associated with any of the molecular mechanisms underlying parent-of-origin effect (e.g., genomic imprinting and trinucleotide-repeat instability), and, to the best of our knowledge, no other studies have reported parent-of-origin effects in any of these genes in relation to ADHD. Third, it has been recognized that true-positive genetic-association results in complex disorders are rather rare.[5] Finally, the second interpretation is simpler and does not invoke any complex mechanisms such as parent-of-origin effect, which makes it more compatible with the principle of parsimony.

For all these reasons, we call into question the validity of the results of the work of Hawi et al.[2]

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Web Resource

The URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ADHD, *DRD4, DRD5, TH, DDC, SERT, TPH2, DAT1, SNAP-25,* and *5HT1B*)

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