LETTER TO THE EDITOR

Reply to Joober and Sengupta

To the Editor: We are grateful for the opportunity to respond to the letter by Dr. Ridha Joober and Dr. Sarojini Sengupta,[1(in this issue)] which criticizes our article[2] published in the December 2005 issue of *The American Journal of Human Genetics*. We believe that the correspondents misunderstood our analysis and have misrepresented our strategy and findings.

First, Joober and Sengupta contend (point 1b in their letter[1]) that there is insufficient evidence of association of any genes with attention-deficit/hyperactivity disorder (ADHD [MIM 143465]) and that our inclusion criteria are therefore arbitrary. Contrary to the authors' opinion, there is a general consensus that *DRD4* (MIM 126452), *DRD5* (MIM 126453), and *DAT1* (MIM 126455) contribute to the development of ADHD, although the odds ratios (ORs) are small and biological mechanisms have not been established. In fact, Dr. Joober and colleagues reviewed the literature and concluded that the association of *DAT1* and *DRD4* with ADHD "appears to be one of the most replicated in psychiatric genetics and strongly suggests the involvement of the brain dopamine systems in the pathogenesis of ADHD."[3(p.27)] Meta-analyses of published data for *DRD4* and *DAT1* support this conclusion,[4,5] and a joint analysis involving 1,980 probands with ADHD and 3,072 of their parents showed association with the *DRD5* locus.[6]

Second, we address their criticism of our inclusion criteria (points 1a and 4 in their letter[1]). Alteration of our inclusion criteria to exclude four genes (*DAT1, SNAP-25,* [MIM 600322], *5HT1B* [MIM 182131], and *SERT* [MIM 182138]) is an arbitrary decision of the sort that we are accused of making and is a false demonstration of the sensitivity of the results. We refute the suggestion that our selection criteria were selected post hoc. Comparison of the paternally versus the maternally transmitted risk alleles from all 17 genes (table 1) reveals a significant difference in paternal versus maternal transmission ($\chi^2 = 9.47$; $P = .0021$). Our inclusion criteria were designed in an attempt to further define this effect in the same data set that generated the hypothesis. Indeed, parent-of-origin analysis of the eight excluded genes ($\chi^2 = 1.15$; $P = .284$) suggests an effect specific to genes "most associated"[2] with ADHD. As we made clear in our original article,[2] initial informal observation of a paternal trend was the motivation for our analysis.

Now we address the statistical questions raised in point 1a of their letter[1]: it is true and relevant that the χ^2 test of paternal versus maternal transmissions is expected to be statistically independent of the association test. Concerning our claim that a lenient genewise threshold of $P < .1$ would reduce type II error and would underestimate the size of parent-of-origin effects, we make the following points:

1. Power to detect such effects admittedly depends both on the threshold and on the magnitude and mechanism of a parent-of-origin effect, and the effect on power in this case is unclear. An excessively low or high threshold will decrease power by dilution or sample-size reduction, respectively, and these factors must be balanced.
2. We expect that a low threshold will dilute the magnitude of any ADHD-specific parent-of-origin effect (because of genes unrelated to ADHD being included in the analysis, which is a scenario Joober and Sengupta[1] feel is likely). On the other hand, a high threshold is, on average, unlikely to change the magnitude of a parent-of-origin effect.

The authors claim (point 3 in their letter[1]) that correlation between the number of markers tested at each gene and the transmission/disequilibrium test (TDT) statistic is evidence that our finding of a parent-of-origin effect is a chance finding. This is untrue, since the TDT and parent-of-origin statistics are not correlated. It is our view that, even if the associations at these genes amount to type I error, this situation should have no bearing on the comparison of maternal and paternal transmissions.

In agreement with Joober and Sengupta, but for different reasons, we also find it remarkable that two of the genes demonstrate significant parent-of-origin effects when analyzed individually (point 2 in their letter[1]), since the effects are more significant than would be expected by chance. These tests are amenable to a Bonferroni correction for the number of genes. The tests for association at each gene also require this correction, as well as correction for the number of markers at each gene. The latter has not been performed and is complicated by our candidate-gene strategy of pursuing initial findings with extra markers across the gene. We presented the statistics for individual genes, to facilitate exploration of the data by readers.

The authors' alternative explanations of the results (point 5 in their letter[1]) are equally invalid. Our results indicate that joint transmissions of risk alleles from each parent separately are significant. Our subsequent test was for a differential rate of overtransmission. The probability of a false-positive finding is given by the *P* value—in this case, .0019—which does not require adjustment for multiple testing, is independent of the significance of the TDT statistics for individual genes, and should not be influenced by the likelihood of potential biological explanations.

The authors' comments[1] regarding molecular mechanisms suggest that they did not carefully read our article.[2] We draw attention to the paragraph containing this sentence: "Since ADHD-associated genes map to many dif-

Table 1. Alleles in All 17 Genes Considered for Our Analysis

Gene	Database Identification	Paternal Alleles					Maternal Alleles					All Alleles				
		No. T	No. NT	χ^2	*P*	OR	No. T	No. NT	χ^2	*P*	OR	No. T	No. NT	χ^2	*P*	OR
Excluded:																
DRD1	*rs265981*[a]	10	9	.05	1	1.1	11	8	.47	.65	1.4	28	25	.17	.78	1.1
DRD2	*rs1800497*[a]	8	7	.06	1	1.1	7	3	1.6	.34	2.3	16	12	.57	.57	1.3
DRD3	*rs6280*[a]	6	6	0	1	1.0	9	7	.25	.84	1.3	21	19	.1	.87	1.1
COMT	*rs4680*[a]	38	24	3.2	.1	1.6	27	35	1	.77	.8	82	79	.06	.87	1.0
DBH (*Taq*I)	*rs2519152*[a]	27	24	.18	.78	1.1	31	23	1.2	.34	1.3	77	64	1.2	.31	1.2
5HT2A 102C	*rs6313*[a]	22	17	1.64	.5	1.3	26	22	.3	.66	1.2	72	63	.6	.5	1.1
NET	*rs5568*[a]	17	5	6.5	.017	3.4	8	10	.2	.82	.8	32	21	2.3	.17	1.5
GRIN2A	*rs8049651*[a]	21	21	0	1	1.0	29	27	.07	.89	1.1	69	67	.03	.93	1.0
Total[b]	…	149	113	…	…	…	148	135	…	…	…	397	350	…	…	…
Included:																
DRD4 (−616)	*rs12720373*[a]	40	23	4.6	.043	1.17	32	19	3.3	.09	1.7	80	49	7.5	.008	1.6
DRD5 $(CA)_n$	270166[c]	57	29	9.1	.0034	2.0	54	36	3.6	.07	1.5	114	69	11.1	.0001	1.7
DAT1 (*VNTR*)	161500[c]	33	18	4.4	.048	1.8	23	30	.9	.4	.8	76	63	1.2	.31	1.2
TH $(TCAT)_n$	180306[c]	21	12	2.5	.16	1.8	28	19	1.7	.24	1.5	55	35	4.4	.04	1.6
DDC (4-bp ins)	M77828[d]	7	2	2.8	.18	3.5	10	7	.53	.63	1.4	20	9	4.2	.06	2.2
SNAP-25 (*Mnl*I)	*rs3746544*[a]	33	22	2.2	.18	1.5	28	33	.4	.6	.8	70	52	2.7	.12	1.3
5HT1B (861G)	*rs6296*[a]	36	23	2.9	.11	1.6	29	23	.73	.46	1.3	85	68	1.9	.2	1.3
SERT (*D17S1294*)	*D17S1294*	15	2	9.9	.002	7.5	9	10	.05	1.0	.9	26	14	3.6	.08	1.9
TPH2 (*rs1843809*)	*rs1843809*[a]	26	7	10.9	.001	3.7	23	12	3.5	.09	1.9	52	22	12.1	.0006	2.4
Total[e]	…	268	138	…	…	…	236	189	…	…	…	578	381	…	…	…
Grand total[f]	…	**417**	**251**	…	…	…	**384**	**324**	…	…	…	**975**	**731**	…	…	…

NOTE.—Sum of paternal and maternal counts do not equal all counts because of the exclusion of trios with two informative parents, where parent-of-origin effect cannot be determined. T = transmitted; NT = not transmitted.

[a] dbSNP accession number.

[b] Maternal versus paternal transmissions in eight excluded genes: $\chi^2 = 1.15$; $P = .284$.

[c] GDB accession number.

[d] GenBank accession number.

[e] Maternal versus paternal transmissions in nine included genes: $\chi^2 = 9.56$; $P = .0019$.

[f] Maternal versus paternal transmissions in all 17 genes: $\chi^2 = 9.47$; $P = .0021$.

ferent chromosomes, it is unlikely, a priori, that all these genes are imprinted."[2(p.963)] Further experiments may help to clarify whether we are observing a true effect, a methodological bias, or a chance finding. The possibility remains that there is a nonmolecular phenomenon, such as selective mating for genetically influenced ADHD-related traits in the male lineage.

Finally, our work is as we described in our article,[2] and the suggestion that our selection criteria reflect a post hoc decision that favored the hypothesis is untrue and unwarranted. We welcome suggestions for further tests to confirm or to invalidate our findings, including exploration of criteria for inclusion of genes, and we look forward to seeing our hypotheses tested in independent ADHD and control samples.

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Web Resources

The accession numbers and URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (see table 1 for accession numbers)

GDB Human Genome Database, http://www.gdb.org/ (for *DRD5* $[CA]_n$ [accession number 270166], *DAT1* [*VNTR*] [accession number 161500], and *TH* $[TCAT]_n$ [accession number 180306])

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *DDC* [4-bp ins] [accession number M77828])

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

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