**Author's response to reviews**

**Title:** Case-control and family-based association studies of candidate genes in autistic disorder and its endophenotypes: TPH2 and GLO1

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**Author's response to reviews:** see over
Deborah Saltman, M.D., Ph.D., Editorial Director

BMC Medical Genetics

Rome, January 5, 2007

Dear Dr. Saltman,

We are hereby resubmitting on-line our manuscript “Case-control and family-based association studies of candidate genes in autistic disorder and its endophenotypes: TPH2 and GLO1” (MS #248980651137726), written in Research Article format for BMC Medical Genetics. The manuscript file name is “Persico_BMCMedGenet_resub_final.doc”. In this revised version, we have significantly increased the number of families genotyped at the TPH2 locus, updated all statistical analyses and the reference list, included also an additional population structure analysis. We also wish to thank both Reviewers for their helpful comments, which have all been addressed as follows:

Reviewer: Maricela Alarcon

A) Major Compulsory Revisions:

We agree with this Reviewer that the same genetic sample should ideally be assessed at all loci analyzed in a single report. However, budget constraints forced us to limit our assessment to a subset of all available families for both loci. For the same reason, we genotyped our 171 normal controls only at the GLO1 locus and had to use published control allelic frequencies for the TPH2 locus (see tables 4 and 5). Our family-based association strategy was to genotype a subsample large enough to unveil a significant genetic association or a trend worth further genotyping, if present. The sample size genotyped at the TPH2 locus was thus significantly increased, to encompass well over 200 trios for both loci. Table 1 summarizes the size of the entire available sample, whereas the following sentence “Out of this global sample, 210 Italian families and 24 Caucasian-American families from Arizona were involved in the TPH2 study, whereas GLO1 was genotyped in a partly-overlapping set including 176 Italian families and all 76 Caucasian-American families available.”, now clearly describes the samples assessed at each locus in the Methods (page 6, par. 1).

B) Minor Essential Revisions:

1. We have removed “n.s.” from the entire manuscript.

2. A more complete definition of “endophenotypes”, namely “heritable clinical, biochemical or morphological traits especially frequent among affected individuals and their unaffected first-degree relatives”, is now provided in the Introduction (page 4, par. 1).
3. The chromosomal regions 12q21.1 and 6p21.3-21.2 are neither supported nor excluded by linkage studies of autism. However, single genes may not necessarily yield a detectable signal in linkage studies of polygenic disorders. Furthermore, studies of TPH2 and GLO1 were initially undertaken by other groups largely because of the functional relevance of these genes or following the results of prior proteomic assessments. These points are now clarified in the Introduction (page 4, end of par. 1), with the sentence: “Following these approaches, and under the assumption that single genes may not necessarily yield a detectable signal in linkage studies of polygenic disorders such as autism, TPH2 and GLO1 were initially assessed despite a relative lack of support by sib-pair analyses for their chromosomal localizations (ch. 12q21.1 and 6p21.3-p21.2, respectively).”

4. The sentence defining macrocephaly, hyperserotoninemia and peptiduria as the best-characterized endophenotypes in autism Literature has been somewhat toned down. This sentence represents our opinion, and no reference can thus be provided for this statement. Immediately after this sentence, in page 5 (end of the last paragraph), the consistent evidence supporting our view has been summarized as follows: “Indeed, macrocephaly (i.e., cranial circumference > 97th percentile) is present in approximately 20% of autistic patients, 5-HT blood levels are elevated in at least 25% of patients, and excessive peptiduria is found in up to 60% of cases depending on ethnicity and country of origin; sizable familiality and heritability is present for all three parameters” [20-23, and A.M. Persico et al, manuscript in preparation for familiality of peptiduria].

5. The discrepancy pointed out by this Reviewer in Table 1 is real, it is not an error. Unfortunately, DNA was not available to us for one of the two affected children from one multiplex family from the AGRE Consortium. This family is still listed as “multiplex” in table 1, but the overall number of patients is lower than expected by one unit. This is now explained in a footnote below table 1.

C) Discretionary Revisions:

1. Family-based association analyses were performed merging together Italian and Caucasian-American families, because we have actually verified the absence of population substructure by genotyping 90 SNPs distributed genome-wide and applying Pritchard’s method (now reference #31), as implemented by the STRUCTURE program. We assessed a large subgroup of 179 autistic patients, including 155 Italian and 24 Caucasian-American individuals randomly chosen one per family, finding no evidence of population substructure. Unfortunately we had to exclude controls from this genotyping effort, again due to budget limitations; therefore, case-control analyses were still prudently performed only on patients and controls of Italian descent, to minimize the risk of ethnic stratification (see table 4). These points are now described in pages 8-9 (“Data analysis”).

2. We agree that the ADI-R represents the gold-standard in autism diagnosis, and we appreciate that this Reviewer understands the difficulties and limitations inherent to working in non English-speaking countries, where these measures need to go through a long translation and back-translation process before becoming publicly available. For the reasons discussed above (see Major Compulsory Revisions), our AGRE families were not genotyped for TPH2, but only for GLO1; therefore, we are not able to run the exploratory analysis suggested by this Reviewer, using ADI-R scores pertaining to stereotyped behaviors. We now acknowledge this limitation in the Discussion (end of page 12 and beginning of page 13), while also underscoring that the presence/absence of
relevant verbal and motor stereotypic behaviors that was assessed in our study by an experienced clinician at intake largely corresponds to the current presence of “stereotyped utterances and delayed echolalia”, “hand and finger mannerisms”, “other complex mannerisms or stereotyped body movements”, and “midline hand movements”, as assessed by ADI-R items n. 33, 77, 78, and 79, respectively.

Reviewer: Sabine Klauck

A) Major Compulsory Revisions:

1. We totally agree with this reviewer about the existence of a discrepancy between our results and those reported Junaid and Colleagues. We believe this discrepancy is driven mainly by (a) spuriously low A419 allelic frequencies in controls recruited for the initial study (please, notice that only 50 “controls” were employed, including normal individuals and patients with Batten disease or fragile-X syndrome…we would all agree that this is not a random selection of population controls), and the fact that unaffected controls of autistic patients were not assessed in the initial study. Our study thus represents the first report that could have picked up protective effects, if present. The entire last paragraph of our revised Discussion (page 13, par. 2) is now devoted to this issue.

B) Minor Essential Revisions:

1. The number of unaffected siblings (N=156) is now reported in the Abstract, under “Methods”.

2. The number of siblings from simplex and multiplex families is reported also in the Methods section (end of page 6, par. “Subjects”).

3. Former table 5 (table 3 in this revised version of our manuscript), is now cited in page 10 (par. “TPH2”).

4. See response C1 to the previous Reviewer, regarding the population structure control statistics.

5. Correct A419 allelic frequencies for controls (0.5439) are now reported in page 13, par. 2.

6. The web address for the LINKUTIL package (ref. 26) has been updated and is now correct.

7. We have clarified that the N below the label “Patients” and “Controls” in the right half of table 5 refers to chromosome numbers, and also the statistics now distinguish between “genotypes” and “alleles” (left and right columns, respectively).

C) Discretionary Revisions:

1. Our selection criteria and the instruments used for diagnosis are now detailed in the Methods section (page 4, par. 1 under “Subjects”).
2. The rare GLO1 polymorphism is present in 1.8% of our controls (3/171), as well as in one father, and in one unaffected sibling. This is now reported in the Methods section (pages 7-8, last sentence in “Markers and genotypes”).

I sincerely hope you will now deem this manuscript acceptable for publication in *BMC Medical Genetics*. Looking forward to the final outcome of your review process, I send you my Best Regards.

Sincerely,

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