Brief Report

Lack of Association Between Autism and *SLC25A12*

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Objective: Autism has a strong, complex genetic component, most likely involving several genes. Multiple genomic screens have shown evidence suggesting linkage to chromosome 2q31–q33, which includes the *SLC25A12* gene. Recently, an association between autism risk and two single nucleotide polymorphisms (SNPs) in *SLC25A12* was reported. This study aimed to test for association in *SLC25A12* in an independent data set of 327 families with autistic offspring.

Method: The authors analyzed two SNPs that were significant in the previous study group, as well as seven additional SNPs within the gene. Association analyses for individual SNPs as well as haplotypes were performed.

Results: There was no evidence of an association between *SLC25A12* and autism.

Conclusions: These results suggest that *SLC25A12* is not a major contributor to autism risk in these families.

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Autism is a neurodevelopmental disorder that has a strong, complex genetic component. Estimates suggest that multiple genes contribute to risk, but to our knowledge, no single gene of significant effect has yet been identified. Several genomic screens identified the 2q31–33 region (170–198 cM) as a consensus linkage region (1–4).

A number of potential candidate genes map within this region, and several have been evaluated for association with autism (3–5). These include *SLC25A12* (approximately 172 megabases [Mb]), which encodes a mitochondrial aspartate/glutamate carrier expressed in the brain. Ramoz et al. (5) reported significant association between autism and two single nucleotide polymorphisms (SNPs), rs2056202 in intron 16 (3′ end of the gene) and rs2292813 in intron 3 of *SLC25A12*, as well as the haplotype for the two SNPs (p=0.003, p=0.05, and p=0.002, respectively). To investigate these findings in our independent data set, we analyzed these two SNPs and seven other SNPs within this gene, covering the entire transcriptional unit.

Method

Association analyses were performed on 327 Caucasian families with autistic offspring, including trios (one affected child and two parents) and multiplex families (two or more affected chil-

TABLE 1. Results of Tests for Association Between Autism and Single Nucleotide Polymorphisms in the *SLC25A12* Gene for 327 Families With Autistic Offspring

SLC25A12 Marker	Position on Chromosome 2 (kilo base pairs)	Significance (p)	
		Pedigree Disequilibrium Test	Genotype Pedigree Disequilibrium Test
rs6433317	172,571	0.451	0.637
rs1878583[a]	172,551	1.000	0.760
rs2056202[b]	172,538	0.824	0.892
rs925881	172,529	0.456	0.645
rs3821095	172,510	0.310	0.443
rs970948	172,494	0.873	0.729
rs6758704	172,481	0.615	0.470
rs2292813[b]	172,470	0.566	0.624
rs11757[a]	172,467	0.745	0.366

[a] Located in translated region of the gene.
[b] Significant association reported by Ramoz et al. (5).

dren and parents). A complete description of the study was provided to the subjects, and written informed consent was obtained. The families included 230 from Duke University and 97 from Vanderbilt University. All of the affected offspring met the DSM-IV diagnostic criteria for autism. Detailed diagnostic evaluations of the family data have been previously described (5). Approval was obtained from both the Duke University and Vanderbilt University Medical Center institutional review boards.

Blood was obtained from the patients and other family members, and DNA was extracted from whole blood by using standard protocols. Nine SNPs (rs2292813, rs970948, rs1878583, rs11757, rs6758704, rs6433317, rs3821095, rs2056202, and rs925881) within *SLC25A12* were selected from the SNP database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/SNP/). All SNPs were ordered as TaqMan (Applied Biosystems, Foster City, Calif.) assays and genotyped according to the manufacturer's recommendation.

Hardy-Weinberg equilibrium was assessed, and pairwise linkage disequilibrium (D′ and r^2) between markers was calculated. The pedigree disequilibrium test and the genotype pedigree disequilibrium test were used to examine disease association. The family-based association test (6) was used for haplotype analyses. To run the haplotype analyses, we obtained global scores for a pairwise haplotype analysis that encompassed all SNPs within the gene. For each pair of SNPs, haplotypes observed in fewer than 10 families were not considered (6). TDT-GENEHUNTER (GENEHUNTER version 2.1; http://www.fhcrc.org/science/labs/kruglyak/Downloads/) was also used as a measure for linkage.

Results

Tests for Hardy-Weinberg equilibrium deviations were calculated for each marker in two groups of affected and unaffected individuals selected at random from each family. No polymorphism showed evidence of deviation from Hardy-Weinberg equilibrium in any data set.

The pedigree disequilibrium test and the genotype pedigree disequilibrium test showed no association with autism for any of the SNPs within *SLC25A12*, including the two SNPs for which Ramoz et al. found significant association (Table 1). In addition, there was no evidence of association ($p \leq 0.05$) with any specific pairwise haplotype according to haplotype analyses with the family-based association test.

To ensure that this lack of association was not due to a lack of informativity of the SNPs in our data set, the number of families informative for each SNP was calculated (a family was considered informative if it had at least one heterozygous parent). The number of informative families ranged between 124 (rs2292813 and rs1878583) and 249 (rs3821095). To capture linkage information, we analyzed the families that showed positive lod scores (logarithms of the odds ratios for linkage) (4) for the significant SNPs in the study by Ramoz et al. (5), rs2292813 and rs2056202. Analysis within these "linked" families also failed to detect significant association (pedigree disequilibrium test: p=0.09 and p=0.91 for 18 and 21 informative families, respectively). Additionally, a transmission disequilibrium test was run to assess linkage in the entire data set. The results were nonsignificant. A high amount of linkage disequilibrium ($D' \geq 0.93$, $r^2 \geq 0.76$) was found across the tested SNPs.

Discussion

We analyzed a panel of nine SNPs in *SLC25A12*, spanning the 110-kb transcriptional unit, in 327 autism families independent of those previously reported by Ramoz et al. (5). Markers were selected to span the gene with an average spacing of 10 kb. The selected markers included a silent mutation in the 5′ untranslated region (rs1878583) and an SNP in the 3′ untranslated region (rs11757), in addition to rs2056202 and rs2292813 (5). We found no evidence of association for any of the markers analyzed or for any specific pairwise haplotype in these data.

Ramoz et al. also reported positive linkage to the region and to the two significant SNPs in *SLC25A12* (approximately 172 Mb). We previously reported evidence for linkage to this same region within a subset of our families (4), with a peak lod score at D2S1776 (approximately 169 Mb), but in the current investigation we did not find evidence of linkage for any of the *SLC25A12* SNPs within the overall data set in this study. Moreover, we did not find evidence for significant association in the families with positive lod scores for *SLC25A12* SNPs.

There are several possibilities to consider when interpreting the lack of consistency between these two studies. The causative variation in *SLC25A12* could be an SNP different from the SNPs analyzed by Ramoz et al. but in strong linkage disequilibrium with them. However, given that we

analyzed SNPs covering the entire transcriptional unit, we would still expect to see an association. In addition, the association described by Ramoz et al. could be caused not by variation in *SLC25A12* but, instead, by variation in a nearby gene, which is in linkage disequilibrium with these two SNPs in their study group. It is also possible that the findings of Ramoz et al. could be due to a type I error, but this seems unlikely given the significance of their results.

Population differences could account for the different results. Owing to chance sampling, the Ramoz et al. (5) data set may include a subset of families that show the effect, or a real but unknown difference may exist between our two study groups. Undetected phenotypic differences between the two groups of families could exist, as it is well established that clinical symptoms vary widely within this diagnosis. Subtle differences in the expression of the disease in the various subgroups in the two studies could have significant genetic implications. Further investigation into the phenotype of families contributing to the association is needed in order to clarify these issues. In summary, we have failed to observe association findings similar to those reported by Ramoz et al. (5), but we cannot rule out the possibility that *SLC25A12* has a modest effect in a subset of families.

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