

## Confirmation of Association Between Autism and the Mitochondrial Aspartate/Glutamate Carrier *SLC25A12* Gene on Chromosome 2q31

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**Objective:** Autism is a neurodevelopmental disorder with childhood onset and a known major genetic component. A recent study identified a highly significant association between autism and a two-single-nucleotide-polymorphism haplotype in the *SLC25A12* gene, with a homozygote genotype relative risk between 2.4 and 4.8. The authors' goal was to investigate this

association with autism in Irish affected child-parent trios because replication in an independent sample is essential in the validation of such potentially important findings.

**Method:** Markers rs2056202 and rs2292813 were genotyped in a total of 158 trios (442 individuals). The Transmission Disequilibrium Test was used to examine these markers for association with autism.

**Results:** In agreement with the recent study, the authors found significant association between autism and the C alleles of both rs2056202 and rs2292813 as well as the two-marker haplotype.

**Conclusions:** These findings provide replication of the association between autism and *SLC25A12*.

(*Am J Psychiatry* 2005; 162:2182–2184)

**A**utism (number 209850 in the Online Mendelian Inheritance of Man, <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=300425>) is a neurodevelopmental disorder of childhood onset, with an estimated prevalence of 5.5–20.0 in 10,000 (1), characterized by impairments in social interaction, communication, and behavior. Greater monozygotic than dizygotic concordance in twins (60%–90% versus 0%) (2) and inflated sibling risk (3.0%–4.5%) indicate the importance of genetic factors in its etiology (3), with heritability estimates of 91%–93% (2), observations incompatible with simple Mendelian inheritance. Statistical modeling suggests that 3–15 loci may contribute to risk (4, 5) and that individual gene contributions are probably small.

Reports of linkage to 2q24–q33 (6–8) have implicated chromosome 2q as potentially harboring a susceptibility locus. A recent study (9) screened nine candidate genes across 2q31 for polymorphisms and explored genetic association in a sample of pedigrees including 671 autistic

children. An association was detected with two single nucleotide polymorphisms (SNPs) in the third and sixteenth introns of the *SLC25A12* gene: rs2056202 (I3–21A/G) and rs2292813 (I16+70A/G). In the present study we sought to confirm these findings in Irish affected parent-child trios.

### Method

One hundred sixty-seven affected trios (174 affected individuals, including six affected sibships) were recruited, and probands were diagnosed according to Autism Diagnostic Interview—Revised (10) and Autism Diagnostic Observation Schedule—Generic (11) criteria for autism/autism spectrum disorder. Exclusion criteria included known medical causes of autism, IQ <35 (or mental age <18 months), chromosomal abnormalities, or fragile X syndrome. The Eastern Regional Health Authority Child and Adolescent Psychiatry Ethics Committee granted ethical approval.

Genomic DNA was extracted from blood or buccal swabs by standard methods and quantified. The Amplifluor method (Chemicon Corp., Temecula, Calif.) was used to genotype SNPs rs2056202 and rs2292813. The risk allele for both SNPs is represented by C on the sense strand, termed G by Ramoz et al. (9), who examined the reverse strand.

Markers were examined for genotype errors and Mendelization inconsistencies with the PedCheck software program (12) in 158 affected trios. Allele and genotype frequencies from parents were calculated, tested for Hardy-Weinberg equilibrium, and examined for linkage disequilibrium ( $D'$ ,  $r^2$ ) by using the EMLD program (<https://epi.mdanderson.org/~quang/Software/pub.htm>). The markers and the two-marker haplotype were examined for association by using the Extended Transmission Disequilibrium Test program (13) and the TDTPHASE function of the UNPHASED package, which compensates for missing information and reconstructs haplotype phase where ambiguous, using an expectation-maximization algorithm (14).

## Results

Genotypes at both markers were shown to follow the expected Hardy-Weinberg distribution. The markers were verified to be in linkage disequilibrium ( $D'=1.0$ ,  $r^2=0.57$ ). For markers rs2056202 and rs2292813, 64 and 50 families, respectively, had at least one affected child and one heterozygous parent. Association was detected at each marker individually with the Transmission Disequilibrium Test and, when examined as a haplotype, with TDTPHASE (Table 1).

The associated alleles were identical to those reported by Ramoz et al. (9) as showing biased transmission to affected children. Sample size and minor allele frequencies were insufficient for useful estimation of genotype relative risks.

## Discussion

The association between autism and *SLC25A12* was originally reported by Ramoz et al. (9) in 411 families, 197 of which had at least one affected child and one parent heterozygous for the marker. If the reported relative risk estimates in this association are accurate, we expect our study to have good power to detect such an association, if disease allele frequency is approximately equal to that of the risk C alleles (0.8–0.9) (15). Our estimates of the effect size are of similar magnitude to those detected by Ramoz et al., suggesting that the risk factor may be the same in both studies.

The *SLC25A12* gene comprises a 2,037-base-pair open-reading frame, encoding the 678 amino acid mitochondrial aspartate/glutamate carrier, which resides in the mitochondrial inner membrane and is involved in the respiratory chain (16). It is difficult to hypothesize a model by which variation in this protein may contribute to the cause of autism because it is expressed predominantly in muscle and at lower levels in the brain (16). One could speculate that a defect in this gene could be compensated for by up-regulation or functional redundancy in some tissues, but that lack of compensatory mechanisms in tissues such as brain may lead to greater sensitivity to a slight deficiency in respiratory efficiency.

A functional role for the polymorphisms examined would be purely speculative because they are at opposite ends of the gene (approximately 68.3 kilobases [kb] apart), although they are in strong linkage disequilibrium with

**TABLE 1. Results of Analysis of *SLC25A12* Single Nucleotide Polymorphisms by Transmission Disequilibrium Test and Haplotype Analysis by TDTPHASE<sup>a</sup> in 158 Irish Affected Child-Parent Trios**

Item	Transmitted	Not Transmitted	Odds Ratio	p
Transmission				
Disequilibrium Test				
rs2056202-C	46	25	1.84	<0.02
rs2292813-C	34	18	1.89	<0.04
Haplotype analysis				
T-T <sup>b</sup>	21	39.2	1	<0.03 <sup>c</sup>
T-C	14.4	23.3	1.15	
C-T	0.0	0.0		
C-C	288.6	261.5	2.06	<0.004

<sup>a</sup> From the Extended Transmission Disequilibrium Test program (13); the TDTPHASE function of the UNPHASED package compensates for missing information and reconstructs haplotype phase where ambiguous. p values calculated by permutation (1,000 replicates).

<sup>b</sup> T-T was the reference haplotype.

<sup>c</sup> Global p value.

each other. The possibility remains that another functional variant exists elsewhere, in linkage disequilibrium with the SNPs examined, possibly in an adjacent gene or regulatory site. Examination of the HapMap (<http://www.hapmap.org>) data across the gene and adjacent areas shows that strong linkage disequilibrium relationships between markers extend across the entire gene as well as the adjacent *DNCI2*, *HAT1*, and *MAP1D* genes in a linkage disequilibrium block of approximately 370 kb, although these data are still relatively sparse. The exact location of any functional polymorphism responsible for susceptibility to autism, therefore, remains uncertain. However, given that we were testing a strong a priori hypothesis, we believe that the present study represents a confirmation of the association between autism and the C alleles at the rs2056202 and rs2292813 SNPs in the *SLC25A12* gene.

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Supported by grants HO1041, HO1071, and HO1142 from the Health Research Board, Ireland; National Alliance for Autism Research grant DO2071; and Wellcome Trust grant WO1014.

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