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Brief Research Communication

No Association Between the *APOE* Gene and Autism

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Autism is a neurodevelopmental disorder characterized by stereotypic and repetitive behavior and interests, together with social and communicative deficiencies. The results of several genomic screens suggest the presence of an autism susceptibility locus on chromosome 19p13.2-q13.4. The apolipoprotein E (*APOE*) gene on chromosome 19 encodes for a protein, apoE, whose different isoforms (E2, E3, E4) influence neuronal growth. APOE participates in lipid transport and metabolism, repair, growth, and maintenance of axons and myelin during neuronal development. The APOE protein competes with the Reelin protein for VLDL/APOER2 receptor binding. Several studies have reported evidence for an association between autism and the Reelin gene. Based on these data we tested for association between APOE and autism using family-based association methods in a data set of 322 autism families. Three promoter, one intronic, and one 3′ UTR single nucleotide polymorphisms (SNPs) in the *APOE* gene (−491a/t, −427c/t, −219g/t, 113c/g, and 5361c/t) as well as the APOE functional polymorphism (E2, E3, E4) were examined and failed to reveal significant evidence that autism is associated with APOE.

KEY WORDS: autism; association; APOE

Autism is a neurodevelopmental disorder characterized by stereotypic and repetitive behaviors and interests together with social and communicative deficiencies with an estimated prevalence of 34/10,000 [Yeargin-Allsopp et al., 2003]. The male to female ratio in autism is 4:1. Developmental abnormalities of this lifelong disorder manifest in the first 3 years of life [Lotspeich and Ciaranello, 1993; Folstein and Mankoski, 2000]. Both twin and epidemiologic studies provide evidence of a genetic component in the development of autism [Folstein and Rutter, 1977; Steffenburg et al., 1989; Bolton et al., 1994; Bailey et al., 1995; Pickles et al., 1995]. Siblings of an autistic proband have an estimated recurrence risk 50–150 times greater than the general population [Bolton et al., 1994; Pickles et al., 1995].

The neurobiological basis of autism is still an unknown. However, genetic evidence indicates that multiple susceptibility genes play a role in the genetic etiology of autism [Jorde et al., 1991]. Four genomic screens have suggested evidence for linkage to chromosome 19p13.2-q13.4 [International Molecular Genetic Study of Autism Consortium, 1998; Philippe et al., 1999; Liu et al., 2001; Shao et al., 2002b]. The apolipoprotein E (*APOE*) gene, which maps to chromosome 19q13.31 (68.29 cM; www.research.marshfieldclinic.org/genetics/), is both a locational and functional candidate gene for autism. *APOE* is involved in lipid transport and metabolism of the protein apoE [Mahley, 1988], and has been associated with repair, growth, and maintenance of axons and myelin in the nervous system during development and after injury [Ignatius et al., 1986]. The *APOE* gene encodes for a protein, apoE, whose different isoforms (E2, E3, E4) differentially influence neuronal growth [Nathan et al., 1994] and increase immune and neuroendocrine system dysregulation [Zhou et al., 1999]. ApoE competes with Reelin for VLDL/APOE receptor binding [D'Arcangelo et al., 1999]. Significant evidence for association has been reported with the Reelin gene and autism. Reelin maps to the

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chromosome 7 autism candidate region [International Molecular Genetic Study of Autism Consortium, 1998; Ashley-Koch et al., 1999; International Molecular Genetic Study of Autism Consortium, 2001; International Molecular Genetic Study of Autism Consortium (IMGSAC), 2001] and autism [Persico et al., 2001; Shao et al., 2002a; Zhang et al., 2002], although these results have not been consistent across data sets [Krebs et al., 2002]. Thus, we investigated our dataset of autism families for association of APOE functional polymorphism and five APOE single nucleotide polymorphisms (SNPs).

Families were ascertained by the Center for Human Genetics (CHG) of Duke University Medical Center (DUMC), the University of South Carolina School of Medicine (USCSM), and other collaborating institutions that are part of the Collaborative Autism Team (CAT), and the Autistic Genetic Resource Exchange Consortium (AGRE) through clinical referrals and recruitment of patients through organizations providing services to autism families. Samples and participation were given appropriate Institutional Review Board (IRB) approval. There were 322 autism families made up of 163 multiplex CAT [Shao et al., 2002b] and AGRE (www.agre.org) autism families (two or more sampled autism patients, their parents, and extended family) and 159 singleton autism families (one sampled autism patient and his or her parents) in the data set. Sporadic autism cases (one sampled autism patient and his parents with no other family history of autism; $N = 124$) were also included as singleton families. The AGRE data set included 86 multiplex families. Fifty-two of the CAT multiplex families were included in the CAT genomic screen that showed evidence of linkage with chromosome 19 [Shao et al., 2002b]. Our AGRE data set of 86 families overlapped with the data set of the AGRE genomic screen [Liu et al., 2001] which reported linkage to chromosome 19. The Autism Diagnostic Interview-Revised Third Edition (ADI-R) [Lord et al., 1994] interview was administered to the primary caregiver of the autistic individual to confirm the diagnosis. This diagnostic instrument is based on criteria from the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) and International Classification of Diseases 10 (ICD-10) [American Psychiatric Association, 1994; Volkmar et al., 1994]. Individuals with evidence of Angelman Syndrome, Prader–Willi Syndrome, Fragile X Syndrome, Tuberous Sclerosis Complex, or other rare neurological disorders were excluded from the dataset.

Genomic DNA was extracted from whole blood according to established protocols [Vance, 1998]. APOE genotyping was performed as previously described [Saunders et al., 1993; Shao et al., 2002b]. SNPs were genotyped that mapped to the promoter region and throughout the *APOE* gene using the NCBI SNP database (www.ncbi.nlm.nih.gov/SNP/) from the sequence found in GenBank (www.ncbi.nlm.nih.gov) (Fig. 1). Primers (Integrated DNA Technologies, Coralville, IA) for PCR were designed using MIT Primer3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) for −491a/t, 113c/g, −427c/t, and −219g/t. The primers for 5361ct and the probes (Integrated DNA Technologies) for all of the SNPs were designed using Primer Express® (Applied Biosystems, Foster City, CA). The reaction was performed in a 5 μl reaction volume consisting of 30 ng of genomic DNA, 2.5 μl TaqMan® reaction mix, 0.2 μM of each probe, and 2 μM of each PCR primer. Amplification was performed for 2 min at 50°C and 10 min at 95°C, followed by 40 cycles at 95°C for 15 sec and at 62°C for 1 min, in 384 Nunc PCR Plates (Nalge Nunc International) using Dual 384-Well GeneAmp® PCR System 9700 (Applied Biosystems). Sequence detection was done using the ABI PRISM® 7900HT sequence detection system (Applied Biosystems) and alleles were determined using the software Sequencing Detection System (Applied Biosystems). Quality control (QC) procedures were followed as described in Rimmler et al. [1999]. Genotype data were uploaded into the PEDIGENE® database and merged into the LAPIS management system for data analysis [Haynes et al., 1995].

Hardy–Weinberg equilibrium (HWE) was assessed using exact tests of linkage disequilibrium (LD) implemented in the genetic data analysis (GDA) program [Lewis and Zaykin, 2000]. A single proband was selected at random from each family to comprise the affected sample ($N = 309$). Both unaffected parents were selected from each family to comprise the unaffected sample ($N = 618$). Both the affected and unaffected samples were used to test for HWE for each marker. Each *P*-value was estimated using a permutation test with 3,200 permutations. A common pairwise measure of LD between markers, r^2 (sometimes also denoted by Δ^2) [Devlin and Risch, 1995] was calculated in both the affected and unaffected samples using software package GOLD [Abecasis and Cookson, 2000]. The value of r^2 is related to the amount of information provided by one locus about the other.

To test for association between autism and the APOE markers, the data were analyzed with the pedigree disequilibrium test (PDT) [Martin et al., 2000, 2001] and the likelihood-ratio test implemented in TRANSMIT

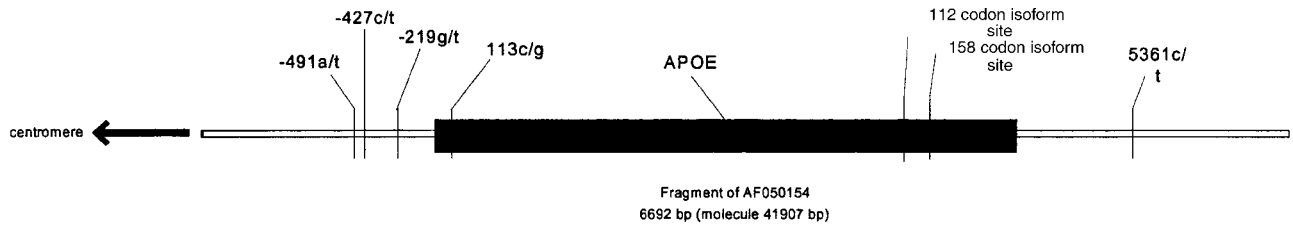

Fig. 1. Map of APOE functional polymorphisms and SNPs.

TABLE I. Marker Pairwise LD

	SNP491AT	SNP427CT	SNP219GT	SNP113CG	APOE	SNP5361CT
Unaffected sample (N = 618)						
SNP491AT		0.009	0.010	0.018	0	0.018
SNP427CT			0.001	0.001	0	0
SNP219GT				0.61	0	0.067
SNP113CG					0	0.043
APOE						0
Affected sample (N = 309)						
SNP491AT		0.021	0.004	0.01	0	0.024
SNP427CT			0.01	0.007	0	0.008
SNP219GT				0.60	0	0.084
SNP113CG					0	0.053
APOE						0

[Clayton, 1999]. In the likelihood ratio test in TRANSMIT, the robust variance estimator was used to test for correlations between multiple affected offspring. Haplotype analysis was performed using TRANSMIT, examining haplotypes for each successive set of adjacent SNPs.

There were no variants that showed deviations from HWE between the affected sample and the unaffected sample ($P \geq 0.06$). The pairwise LD measures r^2 in the unaffected sample are presented in Table I. The pairwise LD pattern in the affected sample is very similar to that in the unaffected sample (data not shown). Larger r^2 values indicate stronger LD, while $r^2 = 0$ indicates markers are in linkage equilibrium. There is little LD between SNPs except between SNP113CG and SNP219GT.

The results of the PDT analyses are shown in Table II. There is no evidence of significant ($P \geq 0.14$) association between autism and either the APOE functional polymorphism or the APOE SNPs in the overall data set. Subsetting by family source (Duke or AGRE) also failed to establish significance ($P \geq 0.05$, data not shown). Haplotype analysis also failed to show any significant association between haplotype frequency and autism.

APOE has proven to be a significant factor in the neurodegenerative diseases such as Alzheimer disease [Corder et al., 1993; Saunders et al., 1993], Parkinson disease [Li et al., 2002; Vance et al., 2002], and multiple sclerosis [Barcellos et al., 2003]. The extent if any of APOE's involvement in neurodevelopmental disorders such as autism is not yet established. In order to fully evaluate APOE as a candidate gene in addition to the APOE functional polymorphism, we genotyped several SNPs within the *APOE* gene. We found no evidence for linkage or association of APOE to autism although the possibility of an interaction effect with other candidate genes such as Reelin cannot be excluded. In summary, these data suggest that the involvement of APOE in autism risk, if any, is minor.

TABLE II. Marker-Specific Association Results

pdt	Two point results (pdt)
−491a/t	0.78
−427c/t	0.29
−219g/t	0.28
113c/g	0.31
5361c/t	0.14
APOE	0.93

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