

Identification of a Novel Gene on Chromosome 7q11.2 Interrupted by a Translocation Breakpoint in a Pair of Autistic Twins

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We report here the identification and characterization of a novel gene (*AUTS2*) that spans the 7q11.2 breakpoint in a monozygotic twin pair concordant for autism and a t(7;20) (q11.2; p11.2) translocation. *AUTS2* is 1.2 Mb and has 19 exons. The predicted protein is 1295 amino acids and does not correspond to any known protein. DNA sequence analysis of autism subjects and controls revealed 22 biallelic polymorphic sites. For all sites, both alleles were observed in both cases and controls. Thus no autism-specific mutation was observed. Association analysis with two exonic polymorphic sites and linkage analysis of four dinucleotide repeat markers, two within and two flanking *AUTS2*, was negative. Thus, although it is unlikely that *AUTS2* is an autism susceptibility gene for idiopathic autism, it may be the gene responsible for the disorder in the twins studied here.

Autism is one of a group of pervasive developmental disorders of brain function [1] characterized by abnormal or impaired development in social interaction and communication and a markedly restricted repertoire of activity and interests [2]. Both family and twin studies support a genetic etiology for autism [3–6]. Attempts to identify the location of autism susceptibility genes [7–13] provide suggestive evidence for linkage on chromosomes 1, 2, 5, 6, 7, 8, 10, 11, 12, 13, 15, 16, 18, 19, and X, although no single region is consistently positive in all studies. Several groups report evidence for linkage to chromosome 7q, with peaks occurring at *D7S477* (122 cM) [7], *D7S1813* (104 cM) and *D7S1824* (150 cM) [13], *D7S640* (139 cM) [14], *D7S2564* (42 cM) and *D7S1804*

(138 cM) [9], and between *D7S1824* and *D7S3058* (149–173 cM) [15].

A monozygotic twin pair concordant for autism that shares an identical balanced translocation involving 7q11.2 and 20p11.2 (t7;20) was previously reported [16]. The mother's karyotype was normal, whereas the father is deceased with no karyotype available. Because chromosome 7q is implicated in autism genetics by linkage studies, and the location of this translocation breakpoint overlaps with at least some of the positive chromosome 7 linkage peaks, we identified the chromosome 7 gene disrupted by this translocation as a potential autism candidate gene.

To identify the translocation breakpoint, we used 13 yeast artificial chromosome clones and 24 bacterial artificial chromosome (BAC) clones from 7q11.2 in fluorescence *in situ* hybridization (FISH) analysis of karyotypes from the translocation subjects. Two overlapping BACs, CITB-HSP-C 562K23 and djp232C07, hybridized to both the derivative 7 and derivative 20 chromosomes and the normal chromosome 7, but not to the normal chromosome 20, indicating that these clones span the translocation breakpoint (Fig. 1A). These two BACs were sample sequenced to 0.5× coverage and a BLAST search of GenBank identified a 214-nt exact match with an EST cluster KIAA0442 (AB007902) [17]. We screened a fetal brain cDNA library with a probe corresponding to the 214-nt sequence and identified two overlapping clones with 0.8-kb and 2-kb inserts. These clones and the KIAA0442 EST cluster were assembled into a 5973-nt sequence called autism-susceptibility gene-2 (*AUTS2*). We confirmed the accuracy and continuity of this sequence by RT-PCR amplifications of fetal brain RNA using three primer sets that amplified the 5' end (nt 550–2577), the middle (nt 2230–3976), and the 3' end

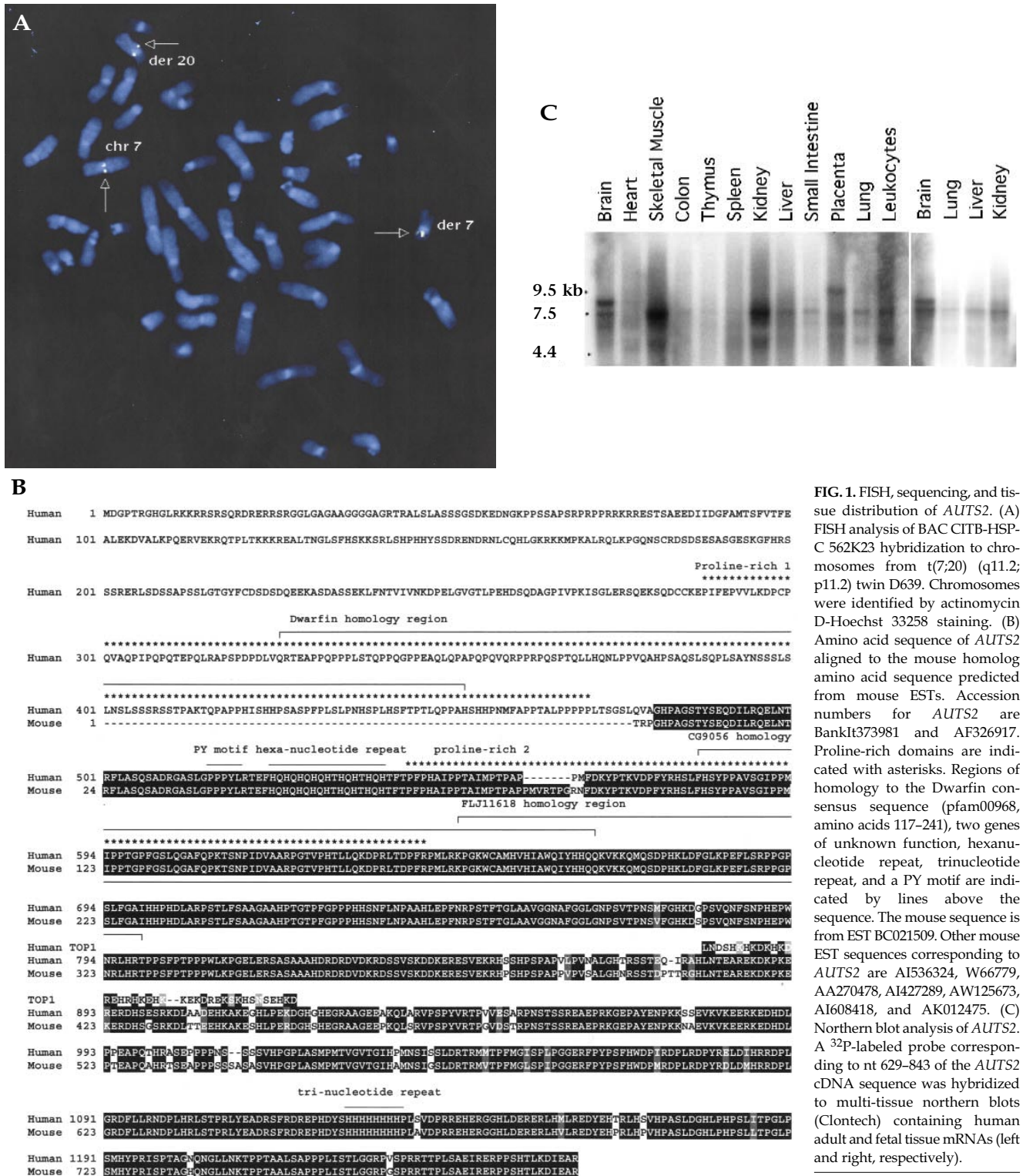


FIG. 1. FISH, sequencing, and tissue distribution of *AUTS2*. (A) FISH analysis of BAC CITB-HSP-C 562K23 hybridization to chromosomes from a (7;20) twin D639. Chromosomes were identified by actinomycin D-Hoechst 33258 staining. (B) Amino acid sequence of *AUTS2* aligned to the mouse homolog amino acid sequence predicted from mouse ESTs. Accession numbers for *AUTS2* are BankIt373981 and AF326917. Proline-rich domains are indicated with asterisks. Regions of homology to the Dwarfism consensus sequence (pfam00968, amino acids 117-241), two genes of unknown function, hexanucleotide repeat, trinucleotide repeat, and a PY motif are indicated by lines above the sequence. The mouse sequence is from EST BC021509. Other mouse EST sequences corresponding to *AUTS2* are AI536324, W66779, AA270478, AI427289, AW125673, AI608418, and AK012475. (C) Northern blot analysis of *AUTS2*. A ³²P-labeled probe corresponding to nt 629-843 of the *AUTS2* cDNA sequence was hybridized to multi-tissue northern blots (Clontech) containing human adult and fetal tissue mRNAs (left and right, respectively).

TABLE 1: Genomic organization and screening of *AUTS2*

<i>AUTS2</i> structure ^a						
Exon	Exon size (bp)	cDNA position	Intron size (bp)	Intron-exon junctions	Exon-intron junctions	Exon location in NT_007758.6 (first nucleotide)
1	≥ 630	1-630	299,324	NA	GCGCTGGAG-gtaaggggga	3276690
2	213	631-843	218,634	ttttctacag-AAAGATGTAG	ACTCAGACAG-gtgaggaag	2976737
3	102	844-945	16,303	tttgccttag-CTCAAGCC	GCTCAGTGAT-gtaagttaa	2757891
4	36	946-981	301,164	tattgcag-AGTTCAGC	GCTACTTC-gtaagtctatctc	2741487
5	30	982-1011	262,795	tcattttcag-TGTGACAG	AAGAGAAG-gtaagacccc	2440288
6	52	1012-1063	64,252	tgttttcag-GCATCAGA	AAACAAAG-gtaagaccca	2177462
7	472	1064-1535	1,411	tcccatgcag-ATCCGGAG	CAGTTTAAAG-gtgagtggc	2113159
8	254	1536-1789	1,109	gttccgatag-CAGCAGCA	TTACTCAG-gtaggacgga	2111277
9	221	1790-2010	1,690	tctctccag-AGCAAGAC	CTCCCATG-gtgcgtacc	2109915
10	45	2011-2055	3,481	ccctttacag-TTTGACAA	GGCACAGT-gtgagtttcat	2108005
11	95	2056-2151	2,384	tggccacag-CTCTTCCA	AGCCGAAG-gtaagaaacc	2104480
12	72	2152-2223	1,258	taattgtag-ACATCCAA	ACCCGAGG-gtacgtgcaa	2102001
13	30	2224-2253	1,717	ctttcacag-TTGACAGA	TGTTAAGG-gtaagaaagc	2100672
14	72	2254-2325	4,441	ctgttccag-AAACCAGG	AAGTCAAG-gtcagtccga	2098926
15	140	2326-2467	3,186	tgctgttag-AAACAGAT	TGCCGCTG-gtgagtgtgg	2094414
16	78	2468-2545	936	gacttaacag-GTGCTGCA	CCACCTAG-gtgagtccgc	2091087
17	84	2546-2629	1,171	ttgtttcag-AGCCTTTT	TTCCGTTA-gtgagtacct	2090074
18	223	2630-2852	2,316	gccatttcag-CACCCAAC	AAAGAAAG-gtacggaag	2088820
19	> 3122	2853-5972		tctccaccag-GGAAAGCG	NA	2086281

Table 1 continued on next page

(nt 3327-5938). The complete *AUTS2* sequence overlaps numerous other human ESTs (AW176610, T07024, AL044306, AA332687, AI971740, W47262, AA058448, N28293, AI290976, AW014542.1, AI625824, and AK025298). Recently, a genomic DNA sequence contig (NT_007758.6) became available that contains the entire *AUTS2* sequence. *AUTS2* has 19 exons with intron-exon junctions conforming to splice-site consensus sequences, and the gene spans 1.2 Mb (Table 1). The first 6 exons are separated by very large introns, whereas the last 13 exons are closely clustered. We amplified a smaller splice variant of *AUTS2* from fetal brain RNA that was missing 1623 nt (exon 2 to exon 13), with exon 1 joined to exon 14. This splice variant requires the removal of a 1.18-Mb segment of the initial transcript to join these two exons. Evidence for another splice variant comes from an EST (gi6442647) from a colon cDNA library that has exon 11 joined to exon 14.

We determined the position of the (t7;20) translocation breakpoint in *AUTS2* by generating somatic cell hybrids between a lymphoblastoid cell line from one of the twins and Chinese hamster ovary (CHO) cells. We used two of these hybrids, one with the derivative chromosome 7 as the only human chromosome 7 material (7pter-7q11.2), and the other with the derivative chromosome 20 as the only source of

human chromosome 7 material (7q11.2-7qter), to narrow the region of this breakpoint. Sequence-tagged site (STS) analysis of these two hybrids demonstrated that the breakpoint is between exons 2 and 3 of *AUTS2*. Although we did not identify the actual sequence of the breakpoint, we mapped it to a 2217-bp segment between nucleotides 2,909,577 and 2,911,794 of NT_007758.6. The 5' end of *AUTS2* is closest to the centromere.

The GenBank ORF finder predicted an ORF of 1259 amino acids from the cDNA sequence. The start codon, preceded by a Kozak motif, was at nt 326 and the stop codon was at nt 4105. The cDNA sequence contained 326 bp of 5'-UTR and 1867 bp of 3'-UTR, but no polyadenylation signal, suggesting that not all of the 3'-UTR sequence is present in the clones analyzed. We identified several mouse ESTs corresponding to human *AUTS2*. The longest mouse EST was 2518 nt (BC021509), with 85% nucleotide identity and 93% amino acid identity to *AUTS2* (Fig. 1B). The predicted human protein contained two proline-rich domains and regions of homology to the Dwarfism family consensus sequence (30% identity), human topoisomerase, and two genes of unknown function, CG0956 and FLJ11618 (Fig. 1B). *AUTS2* has no membrane-spanning domains. Predicted protein domains include a PY

TABLE 1: Continued

Primers for *AUTS2* exon amplification and mutation screening^b

Primer pairs	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Exon	PCR product (bp)	Polymorphism (position)
E1AS/E1AA	CACCAACAATAAGACCTCAGCC	GACCCCGGCGCAGCAGAACC	1	477	
E1BS/E1BA	GCGGAGTCCATGGCCCCGCG	CGGAGCCTCCCAGTCTCTTG	1	443	GTCT repeat (I+90)
E2S/E2A	CTCCCACTTAGTAGTAACACCC	CACACAGCTTTGTTGGACAACC	2	466	SNP C/T (I-16) SNP C/T (I-11)
E3S/E3A	TGTAATTCAGACTTGAACCAATGG	GGTGTTCACCTTTGACAAGG	3	287	
E4S/E4A	GTGATGAAAAGCCTGCAAAGG	TGCTCTTTAGGACTGAGAGAG	4	256	
E5S/E5A	ACTGGAGAAACTCTTTCTTTCC	ACATGAAGCTGGCATCCTACC	5	282	
E6S/E6A	GCACCACCTTATGGATGATTTGG	ATGAAACATCAACAGTAGCTTCAG	6	342	
E7S/E7A	CCCTCCTTATGCCACACTCGC	GAGAGGGCAAAGTCAGGCC	7	576	cSNP G/T (^A 303 ^S) cSNP A/G (^P 342 ^P)
E8S/E8A	CAGGGAGGATCTGTAAAGAGG	CAAATGCAACCTTGCAATCGG	8	489	SNP C/T (I-53) SNP T/C (I-23)
E9S/E9A	GTCCGATGTCCTTTTCTGAAGG	CCCATTTCGATCTCTGGTGG	9	387	SNP C/T (I-30) cSNP G/A (^I 544 ^T)
E10S/E10A	AATGACAGGGAAGCTTTGTAGG	CTAGTCATTGACTGATCTGTGG	10	228	
E11S/E11A	AAACTGAGATTACGTGGCTTGC	CAAGAGGACGCAGAACTTCC	11	298	SNP T/A (I-89)
E12S/E12A	GCAGAAGGGAAGGATCTTGC	AGCTAAGGAAGATGCGAGCAC	12	238	
E13S/E13A	TTAATTTCCCTCCTAATGAAGC	CCGTCATTCCAATGTCTTATTCC	13	248	
E14S/E14A	CTTTGGGGAAATTGAAGGTGG	GCCTCCTTCATCAGGTCTCC	14	265	
E15S/E15A	CGTTCACAGTCTCCAGCTCC	GGATGTGCTGCGATGTTGCC	15	380	SNP G/A (I+84)
E16S/E16A	CGTTGCATCTTCGAAAGTTGG	CACCAGCTTGCCCGGAGG	16	233	SNP C/T (I-20)
E17S/E17A	TAGGAAAAGTGGGACAGGCC	TCTCTAGAAAGGTCTTGAGCC	17	313	SNP A/G (I-67)
E18S/E18A	TGGTAAGTACCTTCATTACAGC	CCAGTTCCTACTGGTACTTGG	18	369	SNP G/A (I-10)
E19S/E19A	AGCCCTGTCCCGCCGACTCG	ATTGGTGAGACACTAACCAGTTGGG	19	3362	cSNP C/T (T+213) cSNP G/C (T+371) CTTA ins/del (T+530) cSNP G/C (T+583) ATG ins/del (T+1153) cSNP T/C (T+1393) cSNP A/G (T+1422) :cSNP T/G (T+1453)

^aIntron nucleotides are in lowercase letters, exon nucleotides are in capital letters. NA, not applicable.^bPrimer sequences are given as 5' to 3' and the sequences given are as synthesized. SNPs are listed as SNPs for those in introns and cSNP for those in the exons including the 3'-UTR. SNP locations for exons 1-18 are given as the number of nucleotides before the start of the exon (for example I-16) or after the end of the exon (for example I + 90). For exon 19, the locations of cSNPs in the 3'-UTR are given as the number of nucleotides after the termination codon (for example T+213). PCR reactions contained 20 pmol primers, 40 ng genomic DNA, and HotStarTaq DNA Polymerase master mixture (Qiagen) in 20 μ l. Except for exon 19, all amplifications were carried out as follows: 1 cycle at 95°C for 15 minutes, 33 cycles at 95°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. For exon 19, reactions were carried out as follows: 1 cycle at 95°C for 15 minutes, 40 cycles at 95°C for 30 seconds, 68°C for 6 minutes, and a final extension at 68°C for 10 minutes.

motif (PPPY) at amino acids 515-519 (Fig. 1B), several putative cAMP- and cGMP-dependent protein kinase phosphorylation sites, casein kinase phosphorylation sites, and *N*-myristoylation sites. Two putative *N*-glycosylation sites are at positions S⁴⁸⁶ and T⁴⁸⁷. Northern blot analysis of *AUTS2* revealed two strong bands at 7.5 and 8 kb in both fetal and adult brain (Fig. 1C). *AUTS2* was also strongly expressed in skeletal muscle and kidney with lower levels in placenta, lung, and leukocytes. Human fetal brain expresses *AUTS2* in frontal, parietal, and temporal lobes, but not in the occipital

lobe (data not shown). The size of the larger transcripts (7.5, 8, and 9.5 kb) suggests that the cDNA sequence obtained does not contain all of the 3'-UTR sequence and potential alternative polyadenylation signals may be used. Also, there may be additional splice variants of *AUTS2* that result in some of the smaller transcripts observed.

To determine whether genetic variation in *AUTS2* contributes to idiopathic autism, we carried out association and linkage studies for *AUTS2* using 65 nuclear multiplex autism families. These were 42 families in which two children had

TABLE 2: Association and linkage analysis of *AUTS2*

Association analysis ^a								
SNP	Allele	Affected subjects (autism families)		Parents	Normal controls			
A1228S	g	0.956 (195)		0.945 (363)	0.962 (177)			
	t	0.044 (9)		0.055 (21)	0.038 (7)			
P1347P	a	0.931 (190)		0.914 (351)	0.957 (176)			
	g	0.069 (14)		0.086 (33)	0.043 (8)			

Linkage analysis of <i>AUTS2</i> ^b								
Marker	Location	Estimated proportion of marker alleles shared IBD (for affected full sibs)			Simple linear regression analysis (effective)			
		Pairs	t-values	P values	D.F.	t-values	P values	
<i>D7S502</i>	78.7	64	-0.0428	0.5170	144	-0.7879	0.216033	
<i>D7S2500</i>	80.42	59	0.4454	0.3288	134	-0.5791	0.281758	
<i>D7S645</i>	80.42	63	-0.8851	0.8103	138	-0.2543	0.399805	
<i>D7S669</i>	90.42	64	0.2586	0.3984	142	-0.5176	0.302763	

^aAllele frequencies for cases are counts for one affected subject per family. Because families were ascertained on the basis of having two or more affected children, there is no proband in these families. Therefore, if both siblings had autism, one was randomly selected for the allele count. If only one child had autism and the second a less severe diagnosis (for example PDD), then the subject with autism was used. Likewise, if neither child had autism, but one had PDD and the second a less-severe diagnosis, the PDD subject was used. There are more families used to generate the allele frequencies than were used in the linkage analysis, because to be included in the linkage study, the diagnoses were restricted to autism and PDD, whereas for the association analysis, if one child had autism or PDD, and the second missed criteria for PDD, but was within ASD, then the autism or PDD subject was used. Comparison of affected with controls for A1228S or P1347P was not significant using the Fisher exact test ($P = 0.61$ and $P = 0.38$, respectively).

^bMarker positions are from Marshfield genetic maps. *D7S2500* is between *AUTS2* exons 2 and 3, and *D7S645* is between exons 1 and 2.

autism, 20 families in which one child had autism and one had pervasive developmental disorder (PPD), and 3 families in which two children had PPD. Both parents were sampled in 61 families, one parent in 3 families, and no parents in 1 family. We sequenced each *AUTS2* exon with 50–100 nt of flanking intronic sequences in 16 autism subjects from 16 different multiplex autism families and from 16 unrelated controls. We found 22 polymorphic sites, with 19 of these being single-nucleotide polymorphisms (SNPs), 2 being small (three and four nucleotide) insertion/deletion polymorphisms, and 1 being a two-allele polymorphism in a GTCT tetranucleotide repeat sequence (Table 1). The three SNPs in coding regions were an A1228S site and a P1347P site in exon 7, and a T1953T site in exon 9. At each site, each allele was observed in both affected and unaffected subjects. In addition, the cDNA sequence contains an interrupted hexanucleotide repeat (cagcac/cagcac/cagcac/cagcac/acc/cac/cagcac/cagcac/cagcac) at nt 1901–1949 (exon 9) and a trinucleotide repeat, (cac)₈, at nt 3701–3732 (exon 19). Neither site was polymorphic.

We analyzed exonic SNPs A1228S and P1347P for genetic association with autism in the 65 families and in unrelated

normal controls ($n = 96$). We selected A1228S because it is the only polymorphism that changes an amino acid. We assayed these polymorphisms by a primer extension assay (PRISM SnapShot ddNTP Primer Extension Kit, PE Biosystems, CA). The comparison of allele frequencies in parents and unrelated controls using chi-square tests indicates no evidence for association at the A1228S site ($\chi^2 = 1.01$, NS), but provides suggestive evidence at the P1347P site ($\chi^2 = 3.64$, $0.05 < P < 0.1$). No significant difference in allele frequency was obtained for either site when we compared affected subjects (one per family) with controls (Table 2).

We evaluated linkage of the *AUTS2* region using markers *D7S645* and *D7S2500*, which are within *AUTS2*, and *D7S502* and *D7S669*, which flank *AUTS2* (Table 2). We carried out linkage analysis with two different statistics. First, we computed the mean proportion of alleles shared identical-by-descent (IBD) for affected sibpairs. In the presence of linkage, an excess of IBD sharing is expected, and the associated t-statistic should be positive. Second, we regressed the squared difference in trait value (1 for discordant and 0 for concordant pairs) for all pairs of siblings onto the marker IBD-sharing estimate [18]. In the presence of linkage, the t-statistic for the slope of the regression should be negative. For this latter analysis, we estimated the effective number of degrees of freedom [19] because of the presence of non-independent pairs of siblings. We used SIBPAL to perform sibpair linkage analyses [20] and P values for both tests are based on one-tailed tests. We found no evidence for linkage to this region using

either approach (Table 2). The association and linkage studies indicate that *AUTS2* is probably not a susceptibility gene for a sizable proportion of autism cases. However, it is possible that disruption of *AUTS2* is the cause of autism in the twins studied here. We cannot exclude the possibility that mutations in *AUTS2* are the cause of autism in a small fraction of non-translocation subjects, either in coding regions or in the extensive intronic regions that were not screened in this study. Another possibility that cannot presently be excluded is that a gene disrupted on chromosome 20p is the cause of autism in these twins.

ACKNOWLEDGMENTS

We thank Richard C. Veith for support and John Wolff, Catherine Morgan, Hillary Massa, Tom Hinds, Stuart Yarfitz (all at University of Washington), and Manuel Villalon (Pontificia Universidad Catolica de Chile) for assistance. NIH grants PO1 HD 34565 (G.D.S. and G.D.), the Department of Veterans Affairs (G.D.S., C.E.Y., and W.H.R.), and NIH P50 HD33812 (G.D.S. and W.H.R.) supported this work. A U.S. Public Health Service Resource Grant (1 P41 RR03655) from the National Center for Research Resources supports S.A.G.E.

RECEIVED FOR PUBLICATION FEBRUARY 1; ACCEPTED JUNE 4, 2002.

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