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A Linkage Disequilibrium Map of the 1-Mb 15q12 GABA$_A$ Receptor Subunit Cluster and Association to Autism

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Autism is a complex genetic neuropsychiatric condition characterized by deficits in social interaction and language and patterns of repetitive or stereotyped behaviors and restricted interests. Chromosome 15q11.2-q13 is a candidate region for autism susceptibility based on observations of chromosomal duplications in a small percentage of affected individuals and findings of linkage and association. We performed linkage disequilibrium (LD) mapping across a 1-Mb interval containing a cluster of GABA$_A$ receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*) which are good positional and functional candidates. Intermarker LD was measured for 59 single nucleotide polymorphism (SNP) markers spanning this region, corresponding to an average marker spacing of 17.7 kb^{-1}. We identified haplotype blocks, and characterized these blocks for common (>5%) haplotypes present in the study population. At this marker resolution, haplotype blocks comprise <50% of the DNA in this region, consistent with a high local recombination rate. Identification of haplotype tag SNPs reduces the overall number of markers necessary to detect all common alleles by only 12%. Individual SNPs and multi-SNP haplotypes were examined for evidence of allelic association to autism, using a dataset of 123 multiplex autism families. Six markers individually, across *GABRB3* and *GABRA5*, and several haplotypes inclusive of those markers, demonstrated nominally significant association. These results are positively correlated with the position of observed linkage. These studies support the existence of one or more autism risk alleles in the GABA$_A$ receptor subunit cluster on 15q12 and have implications for analysis of LD and association in regions with high local recombination. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html. © 2004 Wiley-Liss, Inc.

KEY WORDS: linkage disequilibrium; association; autism; haplotype

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INTRODUCTION

Autism [MIM 209850] is a severe neurodevelopmental disorder defined by three major symptom domains including delayed or absent speech, deficits in social interaction, and repetitive behaviors and restricted interests. Autism typically presents before 3 years of age and results in life-long difficulties for affected individuals. Classic autism has an estimated prevalence of ~1/1,000 children with a preponderance of affected males [Fombonne, 1999]. Evidence from twin and family studies supports a substantial genetic component to this disorder [Folstein and Rutter, 1977; Steffenburg et al., 1989; Folstein and Piven, 1991; Bailey et al., 1995]. Autism exhibits clinical and genetic heterogeneity, has a complex genetic etiology, and is thought to result from oligogenic inheritance of alleles at an unknown number of genes. It is estimated that as many as 20 genes may contribute to overall risk, with locus heterogeneity resulting in different families possessing a different collection of susceptibility alleles [Pickles et al., 1995; Risch et al., 1999].

There are a number of findings that support a role for the GABA (γ-aminobutyric acid) neurotransmitter system in autism susceptibility. Reduced binding of radiolabeled GABA$_A$ receptor ligands was shown in autopsy brain specimens from individuals with autism [Blatt et al., 2001]. Chugani et al. [2001] used PET imaging in children with autism and also showed reduced GABA$_A$ receptors (NIH/ACC Meeting on Cellular and Molecular Mechanisms in Autism Genetics, 2001). The GABA$_A$ receptor agonist benzodiazepine is effective in treating seizure and anxiety disorders common in autism. Finally, elevated levels of circulating GABA and its essential precursor glutamate have been observed in children with autism [Moreno-Fuenmayor et al., 1996; Dhossche et al., 2002; Aldred et al., 2003].

A cluster of GABA$_A$ receptor subunit genes maps within the chromosome 15q11.2-q13 autism candidate region (Fig. 1). Interstitial duplications of this region are associated with a significant risk of autism, and risk is greater for duplications of maternal compared to paternal origin [Clayton-Smith et al., 1993; Browne et al., 1997; Cook et al., 1997; Mohandas et al., 1999; Roberts et al., 2002]. Maternal supernumerary pseudodicentric inverted duplicated marker chromosomes 15 (so-called idic(15) markers) carry two additional copies of a larger region and give rise to a more severe autistic phenotype [Robinson et al., 1993; Bundey et al., 1994; Cheng et al., 1994; Flejter et al., 1996]. Duplication-mediated autism

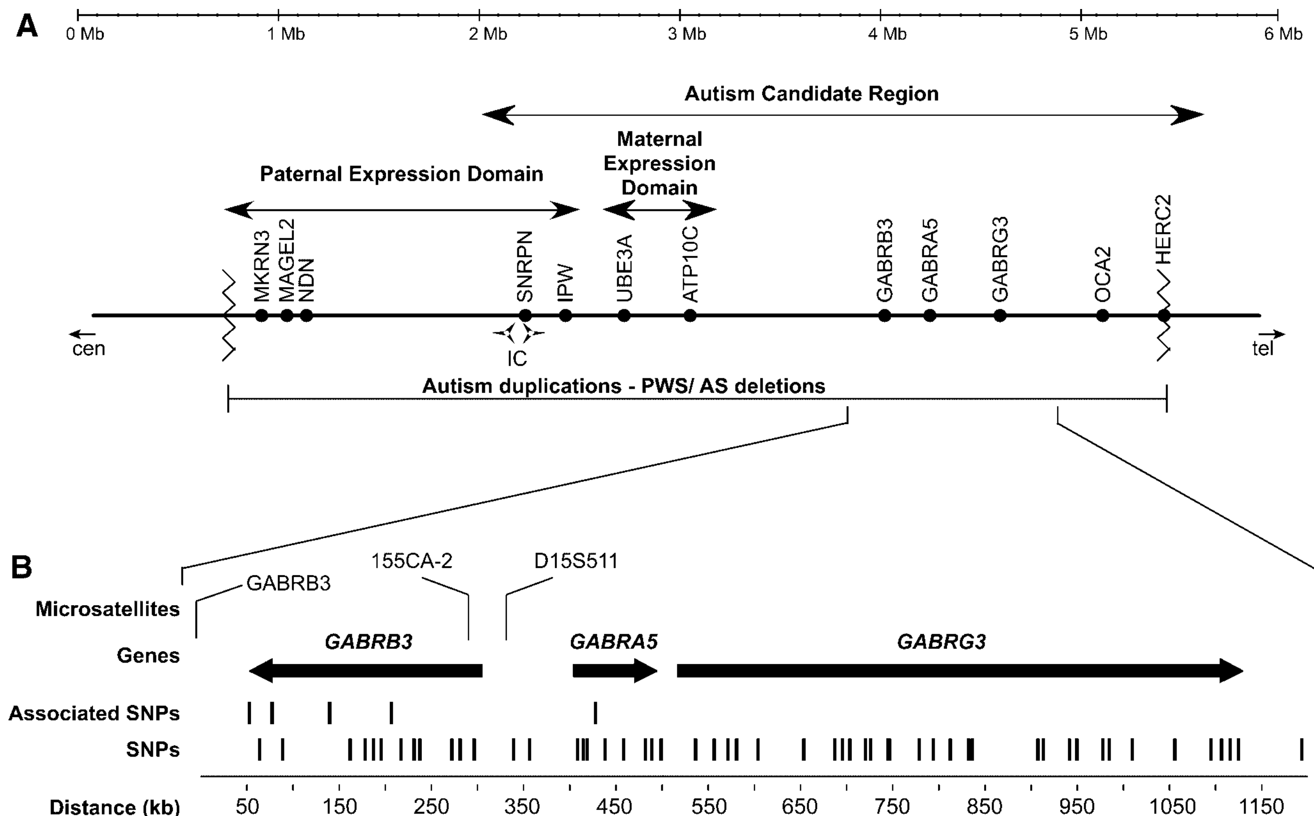

Fig. 1. Schematic map of the 15q11-q13 autism candidate region. **A**: Autism duplication and PWS/AS deletion interval. The region of chromosome 15q subject to interstitial duplication in cases of autism or deletion in PWS or AS is shown. Imprinted paternal expression (PWS) and maternal expression (AS) domains are indicated above the map relative to specific genes. The autism candidate region includes the 15q imprinting center, maternally-expressed genes and the cluster of $GABA_A$ receptor subunit genes. A scale in Mb for the interval is provided above the map. **B**: The $GABA_A$ receptor subunit gene cluster. The 1-Mb interval containing *GABRB3*, *GABRA5*, and *GABRG3* is expanded and shows relative position and transcriptional orientation (arrows) of each gene. Single nucleotide polymorphism (SNP) markers analyzed in this study are indicated by vertical hashes above the scale in kb; proximity of SNPs 5/6, 21/22, 40/41, and 45/46 in relation to the scale is such that these markers are not distinguished by separate hashes. Associated markers are shifted up from other SNPs. Reference microsatellite markers previously shown to be linked or associated are also indicated.

arguably stems from a dosage effect of genes in the duplicated intervals. Maternal or paternal deletions of the same region affected by interstitial duplications give rise to Angelman (AS [MIM 105830]) or Prader–Willi (PWS [MIM 176270]) syndrome, respectively, because of the loss of expression of imprinted genes in the interval (reviewed in [Jiang et al., 1998]; see Fig. 1). This is noteworthy since symptoms of autism can be associated with both AS and PWS. In addition to regions of paternal- and maternal-specific gene expression, there is an apparently non-imprinted region containing a cluster of $GABA_A$ receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*).

Accumulating genetic evidence also suggests the existence of genetic factor(s) in the GABA gene cluster region in idiopathic autism. Genomic linkage screens in autism have identified proximal 15q [CLSA et al., 1999; Philippe et al., 1999; Shao et al., 2002], but results are mixed as others have failed to detect significant linkage [IMGSAC, 1998; Risch et al., 1999; Auranen et al., 2000; Liu et al., 2001]. Two recent reports, including one from our group, demonstrated that subsetting autism families, based on variables from the Autism Diagnostic Interview-Revised (ADI-R), results in significantly increased evidence for linkage to the GABA region [Nurmi et al., 2003; Shao et al., 2003]. Several reports have documented findings of allelic association at microsatellite [Cook et al., 1998; Martin et al., 2000a; Nurmi et al., 2001; Buxbaum et al., 2002] and single nucleotide polymorphism (SNP) markers [Menold et al., 2001] in the GABA region. Two other groups, using microsatellite markers, did not identify association to autism in this region [Maestrini et al., 1999; Salmon et al., 1999]. Studies to date have involved analysis of only a small number of microsatellite or SNP markers and thus have not thoroughly surveyed this region for association. To test the hypothesis that common allele(s) in the GABA gene cluster confer risk for autism, we have undertaken a detailed analysis of linkage disequilibrium (LD) and allelic association across this 1-Mb region.

MATERIALS AND METHODS

Families

The sample for this study consisted of 123 multiplex families. Forty-eight multiplex families were recruited at the Tufts/New England Medical Center and 75 affected sib-pair families were obtained from the Autism Genetics Resource Exchange (AGRE; http://agre.org). The vast majority of families (~98%) are of Caucasian ethnicity. All affected individuals were at least 4 years of age and were clinically assessed with the ADI or ADI-R and most with the Autism Diagnostic Observation Schedule (ADOS). At least one sib had to meet ADI algorithm criteria for an autism diagnosis, while additional siblings may

be on the broader autism spectrum. Families were excluded from the study if probands had a known medical or neurological condition suspected to be associated with their autistic phenotype (e.g., fragile X syndrome). The procedures for clinical evaluation of affected individuals for the AGRE families have been previously described [Liu et al., 2001]. The study was undertaken after Institutional Review Board review and approval.

Molecular Analysis

SNPs from the dbSNP and Celera SNP databases were selected based on their map position, minor allele frequency, and, in one case, a finding of allelic association [Menold et al., 2001]. Database reference numbers and other details for the markers studied are cited in Table I (see the online Table I at http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html). Marker and exon locations and intermarker distances are based on the public (UCSC; July 2003 freeze; http://genome.cse.ucsc.edu/) and Celera (http://www.celera-discoverysystem.com/index.cfm) assemblies and published gene structure information for *GABRB3* and *GABRA5* [Kirkness and Fraser, 1993; Glatt et al., 1997]. Genetic (cM) distances are based on the deCODE genetic map [Kong et al., 2002]. Individual SNPs were genotyped by either fluorescent polarization template-directed dye terminator incorporation assay (FP-TDI) or TaqMan™. FP-TDI analysis was performed using Acycloprime™ kits according to the manufacturer's recommendations (Perkin-Elmer Life Sciences, Boston, MA). Products were analyzed using a VICTOR2™ multi-label plate reader instrument (Perkin-Elmer Life Sciences). TaqMan™ genotyping assays were performed according to manufacturer's recommendations (Applied Biosystems, Foster City, CA). Products were analyzed using an ABI 7900HT Sequence Detection System. PCR primers and probes for assays are provided in supplementary material online. This information is proprietary for the majority of markers, for which Assays-On-Demand™ were obtained from ABI.

Statistical Analyses

Initial analysis of SNP genotype data involved quality control checks consisting of verification of internal controls and assessment of Mendelian inconsistencies, followed by final haplotype consistency analyses using Simwalk2 [Sobel and Lange, 1996]. Conformity with anticipated Hardy–Weinberg equilibrium expectations was established, and SNPs were examined for intermarker LD using both the r^2 and D′ measures calculated by the Graphical Overview of Linkage Disequilibrium (GOLD; [Abecasis and Cookson, 2000]) software package. Similar to the definition described by Gabriel et al. [2002], SNP pairs were considered to be in strong LD if D′ values were >0.75. Neighboring SNPs were considered to be in a single LD block if all SNP pairs were in strong LD. Minimal block lengths were determined from intermarker spacing of SNPs defining the blocks. Transmission disequilibrium (TD) in autism families was determined using the pedigree disequilibrium test (PDT) statistic, developed for use with general pedigrees [Martin et al., 2000b]. Common haplotypes (≥5%) were identified using TRANSMIT [Clayton, 1999]; analysis of TD was performed using adjacent SNP pairs inclusive of loci significant in single marker analysis and haplotype tag SNPs for other multi-locus blocks. Results were considered significant at the nominal level for markers or haplotypes with $P < 0.05$.

RESULTS

We selected and genotyped 59 SNP markers that span the 1-Mb interval containing *GABRB3*, *GABRA5*, and *GABRG3* (Table I). Markers were primarily selected based on minor allele frequency and intermarker spacing to provide dense representation of regional LD. The average minor allele frequency was 0.37; six markers had a minor allele frequency less than 0.2; and one less than 0.1. This last marker (rs140674) was chosen based on a published report of nominal association in *GABRG3* [Menold et al., 2001]. The average intermarker spacing for the entire interval was 17.7 kb, while for individual genes it was 14.3, 10.3, and 18.7 kb, for *GABRB3*, *GABRA5*, and *GABRG3*, respectively. Genotyping for all SNPs was performed on DNA samples from 123 multiplex (48 New England and 75 AGRE) families. Genotypes at individual markers did not deviate from expectations of HWE (data not shown).

With the goal of detecting and characterizing potential allelic effects on autism risk, we initially analyzed single marker genotypes for evidence of TD. Data were analyzed using the PDT, and resulting *P* values are listed in Table I. Five of 19 markers, representing three distinct locations across the *GABRB3* gene, demonstrated significant association at the nominal level ($P < 0.05$). The first location corresponds to SNP 1 ($\chi^2 = 5.25$; $P = 0.02$) and SNP 3 ($\chi^2 = 6.98$; $P = 0.01$), which are located towards the 3′ end of the gene. The two remaining sites showing significant allelic effects are within intron 3. One involves adjacent SNPs 5 ($\chi^2 = 4.18$; $P = 0.04$) and 6 ($\chi^2 = 4.62$; $P = 0.03$). The final site corresponds to SNP 11 ($\chi^2 = 4.27$; $P = 0.04$). One marker within intron 5 of *GABRA5* (SNP 23) also showed evidence for association ($\chi^2 = 4.62$; $P = 0.03$). An initial examination of haplotypes, specifically at markers showing evidence for association, involved analysis of adjacent SNP-pairs using TRANSMIT. Table I details these results, and includes single marker allelic transmission data for significant SNPs. Consistent with results from individual markers, several two-SNP haplotypes demonstrated significant transmission distortion. None of the 30 SNPs located across the ~570-kb *GABRG3* gene showed evidence of association.

To characterize intermarker LD and haplotype structures across the entire 1-Mb region, D′ and r^2 measures were calculated from the genotype data using GOLD. D′ values for all intermarker combinations are represented in Figure 2, and 14 multi-SNP LD blocks (D′ > 0.75) are identified. Outside of these regions of relatively low haplotype diversity, LD between adjacent marker pairs is generally very low. While borders for individual blocks are not precisely defined, the multi-SNP blocks shown are represented by 38 SNPs and comprise a minimum of 263 kb of 1,040 kb, or 25%, of the entire interval. Average SNP coverage was higher for *GABRB3* (14.3 kb^{-1}) and *GABRA5* (10.3 kb^{-1}), in comparison to *GABRG3* (18.7 kb^{-1}) and intergenic regions (see Table I). Examination of minimal block lengths in proportion to total genomic DNA for genes individually reveals that multi-SNP blocks represent 40, 45, and 24% of the DNA encoding *GABRB3*, *GABRA5*, and *GABRG3*, respectively. Minimal block lengths ranged from 3.7 to 46.8 kb, and the average block size was estimated to be 18.8 kb for this Caucasian sample; this block size is consistent with previous reports [Gabriel et al., 2002]. Two sets of overlapping blocks were identified. Blocks 1-2-3 and 4-5-6 overlap based on strong LD between SNP 2 and 4-5-6, despite weak LD between other SNPs in the two blocks. SNP 22 was also found to bridge two overlapping blocks, and these findings are similar to those reported elsewhere [Daly et al., 2001; Dawson et al., 2002].

For all multi-SNP blocks, common haplotypes (>5%) were identified using TRANSMIT (Table II). This permitted identification of haplotype tags, or the subset of SNPs (htSNPs) that detect all common haplotypes for a given block. Of the 38 SNPs present within blocks, 7 were eliminated as redundant. At the current resolution, 82% of the SNPs in multi-locus LD blocks, or 88% of the total, were required to represent common alleles across this interval. Genotype data for the reduced set of SNPs

TABLE I. Chromosome 15q12 $GABA_A$ Receptor Subunit Cluster Single Nucleotide Polymorphism (SNP) Markers

Gene	SNP no.	Region	dbSNP rs#/celera hCV#	Alleles[a]	Minor allele frequency	Intermarker distance (kb)	Overall PDT (*P*)	Allele	T[b]	NT[b]	TRANSMIT (*P*)	
		Intergenic	GABRB3			52.9						
	1	Intergenic	hCV2911914	C/G	0.45	10.1	**0.02**	C	260	226		
								G	188	222	**0.02**	
GABRB3	2	Intron 8	rs2081648/hCV2911917	T/C	0.15	12.5	0.37					**0.02**
	3	Intron 7	rs1432007/hCV8866669	A/G	0.47	10.4	**0.01**	A	263	220		
								G	199	242	**0.04**	
	4	Intron 6	rs1426217/hCV2901088	G/A	0.38	46.3	0.21					
	5	Intron 3	hCV2901140	T/C	0.45	0.5	**0.04**	C	187	222		0.13
								T	279	244	0.07	
	6	Intron 3	rs4542636/hCV2901143	T/C	0.45	19.9	**0.03**	C	182	218		
								T	272	236		0.13
	7	Intron 3	rs754185/hCV2901163	T/C	0.34	14.7	0.82					
	8	Intron 3	hCV2901177	G/A	0.50	8.5	0.94					
	9	Intron 3	hCV2901182	A/G	0.28	7.6	0.82					
	10	Intron 3	rs1346149/hCV2061398	A/G	0.47	10.2	0.07				0.11	
	11	Intron 3	rs878960/hCV8865198	C/T	0.38	9.6	**0.04**	C	265	296		
								T	191	160		**0.04**
	12	Intron 3	rs1863464/hCV2901200	G/A	0.18	12.9	0.80					
	13	Intron 3	hCV245488	T/C	0.44	5.9	0.21					
	14	Intron 3	rs981778/hCV2901236	A/G	0.43	31.7	0.28					
	15	Intron 3	rs970408/hCV2901263	C/T	0.10	8.1	0.74					
	16	Intron 3	rs2059574/hCV2901280	T/A	0.49	11.7	0.61					
		Intron 3	155CA-2			2.4						
	17	Intron 3	rs3212337/hCV218360	C/T	0.36	36.7	0.78					
		Intergenic	D15S511			1.9						
	18	Intergenic	rs4632100	A/G	0.18	16.8	0.23					
	19	Intergenic	rs4506865	A/G	0.10	48.4	0.67					
GABRA5	20	Intron 3	rs2075716/hCV1843341	C/T	0.38	5.4	0.14					
	21	Intron 3	hCV474240	C/T	0.36	3.8	0.78					
	22	Intron 3	hCV11298361	G/A	0.41	8.6	0.37				0.07	
	23	Intron 6	hCV252720	T/C	0.43	9.8	**0.03**	C	176	211		
								T	288	253		**0.01**
	24	Intron 6	hCV27725	C/A	0.50	18.2	0.52					
	25	Intron 7	hCV42974	C/T	0.34	21.8	0.08					
	26	Exon 8	rs140682/hCV1028938	C/T	0.47	6.1	0.65					
	27	Exon 10	rs140685/hCV1028939	T/C	0.49	9.5	0.84					
	28	Intergenic	hCV2078419	T/A	0.39	34.4	0.89					
GABRG3	29	Intron 2	rs1432129/hCV8866584	C/A	0.47	18.4	0.18					
	30	Intron 2	hCV2078482	C/T	0.47	13.5	0.21					
	31	Intron 2	hCV2078497	A/G	0.45	8.8	0.25					
	32	Intron 3	hCV2078506	T/C	0.37	21.5	0.61					
	33	Intron 3	rs4078843/hCV2078548	G/A	0.31	45.2	0.89					
	34	Intron 3	rs4555125/hCV37817	A/G	0.49	32.4	0.18					
	35	Intron 3	rs1029937/hCV2665757	G/A	0.29	7.7	0.94					
	36	Intron 3	rs208174/hCV2665743	C/T	0.27	7.2	0.60					
	37	Intron 3	hCV2665737	C/T	0.17	15.6	0.60					

38	Intron 3	rs2286946/hCV2665715	A/G	0.48	5.2	0.66
39	Intron 3	rs741121/hCV2665706	G/T	0.46	17.1	0.85
40	Intron 3	rs208129/hCV2665692	A/T	0.42	1.6	0.66
41	Intron 3	rs208126/hCV2665687	G/T	0.43	29.4	0.16
42	Intron 3	hCV9408557	T/C	0.30	14.1	0.51
43	Intron 3	rs897173/hCV9408511	A/G	0.25	16.8	0.06
44	Intron 3	rs897177/hCV9408473	T/C	0.18	18.4	0.94
45	Intron 3	hCV9408434	C/T	0.44	3.7	0.48
46	Intron 3	hCV9408423	G/A	0.41	65.1	0.25
47	Exon 5	rs140674/hCV18418	T/C	0.04	5.1	0.71
48	Intron 5	hCV435176	A/G	0.44	26.0	1.00
49	Intron 5	hCV376685	G/A	0.27	7.5	0.38
50	Intron 5	hCV59714	A/G	0.27	26.0	0.28
51	Intron 5	hCV9399190	A/G	0.34	6.3	0.43
52	Intron 5	hCV458188	G/A	0.45	23.0	0.55
53	Intron 5	hCV473958	A/G	0.46	41.7	0.60
54	Intron 5	rs4550406/hCV374658	C/T	0.28	35.6	0.36
55	Intron 6	hCV34499	C/T	0.48	10.4	0.90
56	Intron 6	rs1871019/hCV11670850	A/G	0.40	8.4	0.33
57	Intron 6	hCV1846028	G/A	0.42	8.3	0.79
58	Exon 8	rs140679/hCV1845989	T/C	0.43	63.0	0.60
59	Intergenic	rs1382056/hCV8926104	G/A	0.48	—	0.75

Reference microsatellite markers are underlined, bold highlights $P \leq 0.05$.
[a]Major/minor allele.
[b]Transmitted (T), non-transmitted (N) allele counts.

was then analyzed using TRANSMIT to test for TD with autism; results of this analysis are presented in Table II. Transmissions at two multi-locus blocks, both containing SNPs showing association individually, were found to deviate significantly from that expected under the null hypothesis. The SNP 1-2-3 block ($\chi^2 = 8.02$; $P = 0.02$), located at the 3′ end of *GABRB3*, and the SNP 22-23-24 block ($\chi^2 = 15.5$; $P = 0.01$), located in *GABRA5*, both showed significant results.

To determine whether association at these sites was driven individually by either the New England or AGRE samples, significant results ($P < 0.05$) were examined in the two samples separately. Association within block 1-2-3 was derived from both sets of families. At other blocks or SNPs, there was either significance ($P < 0.05$) in one sample and a trending towards significance in the other or significance derived largely from one but not the other sample (data not shown).

DISCUSSION

Our results highlight the correlation between high local recombination rates, low LD and high haplotype diversity. The implication of this relationship is seen in the only modest reduction of genotyping using htSNPs and a requirement for dense SNP coverage for thorough representation of alleles in any association study. Application of this LD map to an autism dataset supports the existence of one or more risk alleles in the *GABRB3–GABRA5* region. Association was identified for a number of SNPs and haplotypes in *GABRB3*, as well as one SNP and corresponding haplotypes in *GABRA5*. These results correlate well with linkage previously reported in this region for autism [Shao et al., 2002] and phenotypic subsets of autism [Nurmi et al., 2003; Shao et al., 2003].

We have described a first-generation LD map and corresponding haplotype structures for this 1-Mb autism candidate region. This is the first report to detail LD and provide dense analysis for allelic association to autism for the $GABA_A$ subunit gene cluster. The average haplotype block size (18.8 kb) is consistent with previous reports utilizing samples of European ancestry [Gabriel et al., 2002]. Therefore, our data generally agree with the haplotype block structure proposed for the human genome, and add to the literature of detailed LD analyses across large physical regions [Daly et al., 2001; Jeffreys et al., 2001; Patil et al., 2001; Dawson et al., 2002; Gabriel et al., 2002; Clark et al., 2003].

This study reinforces the correlation between high local recombination, low LD and high haplotype diversity, noted previously by others [Dawson et al., 2002; Cardon and Abecasis, 2003]. The sex-averaged genetic map estimates that the rate for this interval is 4 cM/Mb, compared to a genome-wide average of 1.3 cM/Mb. Thus, our findings are not entirely unexpected. Dense SNP coverage and genotyping a large fraction of markers become prerequisites for conducting a thorough disease association study for regions of low LD. A higher resolution of SNPs, particularly in *GABRG3* and intergenic regions, will undoubtedly identify additional blocks and reveal a lower average block length, although a high proportion of SNPs will still be required to detect all common alleles across the region. Thus, a limitation in the current study is a marker density that does not identify all blocks. While our study did not utilize a very dense SNP map (e.g., 5 kb^{-1}), the average minor allele frequency (0.37) was high, and markers were analyzed in a number of families sufficient to permit effective establishment of haplotype phase and structure. Some genome-wide estimates of the ability to eliminate redundant SNPs using haplotype tags [Gabriel et al., 2002] do not reflect the complexity of analyzing such regions of great haplotype diversity and low LD.

The findings of suggestive association in *GABRB3* occur in multiple locations within the gene. Those which cluster

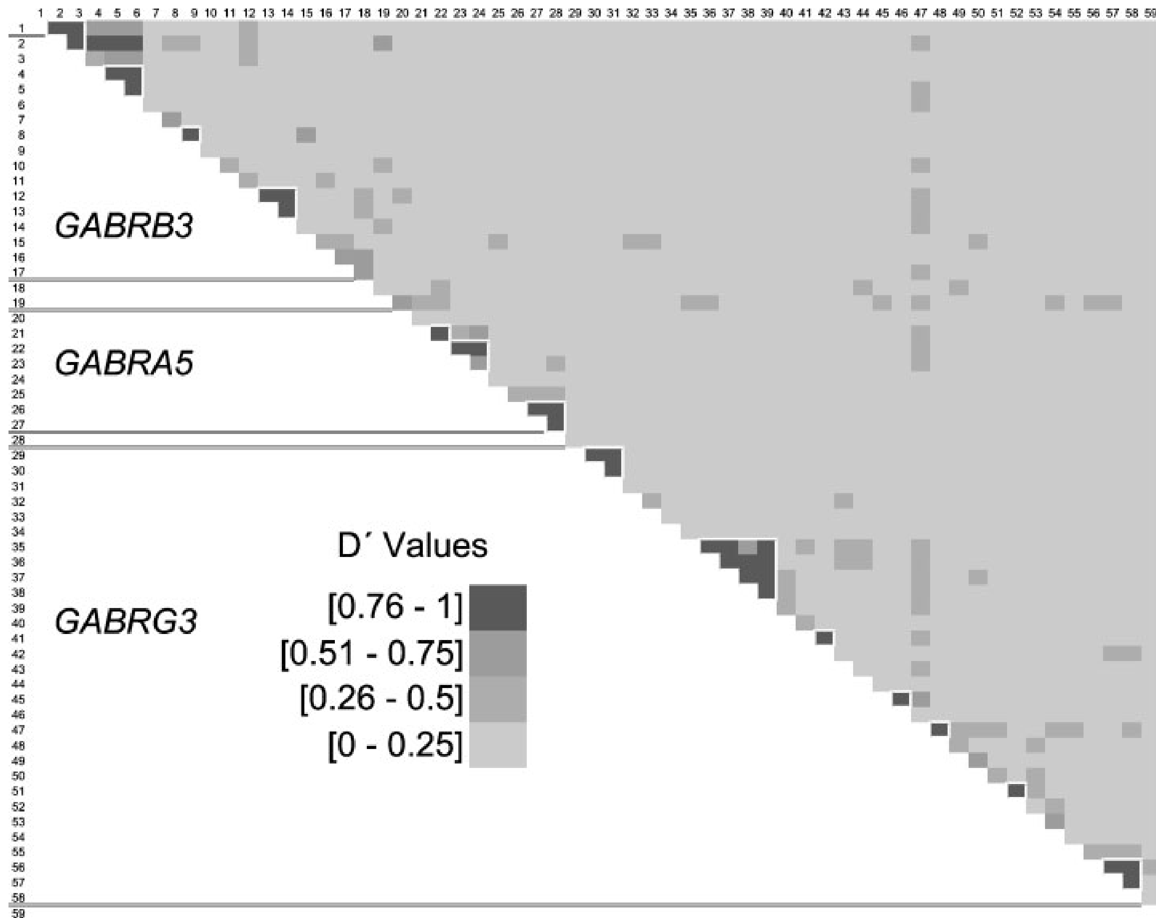

Fig. 2. Intermarker linkage disequilibrium (LD) and haplotype blocks in the 15q12 $GABA_A$ receptor subunit gene region. D′ values corresponding to each pair-wise SNP combination are plotted, and LD/haplotype blocks are revealed as regions of high LD (black squares) along the diagonal and are outlined with white borders.

towards the 3′ end of the gene lie ~50 kb from the GABRB3 microsatellite marker shown to be linked [Shao et al., 2002, 2003] and associated [Martin et al., 2000a] by one group. It is unclear whether the GABRB3 microsatellite marker, centromeric to our first SNP, lies within the same LD block containing SNPs 1-2-3. Two sites within the ~150-kb intron 3 showed association. SNPs 5 and 6 are in a single block located at the centromeric end of this intron. The more telomeric site, corresponding to SNP 11, lies ~80 kb centromeric to microsatellite marker 155CA-2 (see Fig. 1), found associated to autism by two groups [Cook et al., 1998; Buxbaum et al., 2002]. Our own linkage studies in autism subsets have pointed to this region, and peak linkage occurs at the 5′ end of *GABRB3* at D15S511 [Nurmi et al., 2003], ~40 kb from 155CA-2.

Comparison of our data to that in a single published report of association analysis of SNPs in this region in autism shows only two markers in *GABRA5* and two in *GABRG3* common to both studies [Menold et al., 2001]. None of these markers demonstrated association in the current study, although the *GABRG3* exon 5 SNP showed nominal association in the [Menold et al., 2001] report. Unfortunately, a very low minor allele frequency (0.04 in our sample) for the *GABRG3* exon 5 marker substantially hampers power to detect association, and this could explain the difference. Additionally, the [Menold et al., 2001] report described analysis of both multiplex (91) and trio (135) families, whereas this study involves only multiplex families. In contrast to this report, the [Menold et al., 2001] study failed to detect association in *GABRB3* or *GABRA5*, although a much smaller number of markers (9) was examined for these genes.

The observation of association at alleles at three distinct locations within *GABRB3* as well as *GABRA5* could be explained by the existence of multiple autism risk alleles for these two genes. Such a scenario is consistent with the published data and a hypothesis that dup(15)-mediated autism is a contiguous gene duplication effect requiring the $GABA_A$ subunit genes in addition to the imprinted, maternally-expressed genes [Sutcliffe et al., 2003]. Relative strength of genetic effects, power to detect those effects and potential phenotypic specificity of given genes or alleles bear consideration. Larger datasets will be required to provide power for detection of heterogeneous alleles and for analysis of phenotypic subsets. Increased evidence for linkage in families when data are covaried for "insistence on sameness" [Shao et al., 2003] or in which affected individuals have savant skills [Nurmi et al., 2003] suggests a possible phenotypic specificity or bias of allelic effects in this region. While confounding clinical, locus, and allelic heterogeneity can explain difficulties in detecting significant association, the absence of strong association does not allow us to exclude the possibility that one or more of the associated alleles represents a false-positive result.

While suggestive association was detected in *GABRB3* and *GABRA5*, the current study involves multiple analyses on genotype data for a large number of SNP markers, and none of

TABLE II. TRANSMIT Haplotype Analysis for Linkage Disequilibrium (LD) Blocks

Haplotypes	Transmissions				TRANSMIT	
	Frequency	Observed	Expected	χ^2	Global χ^2	*P*
1-2-3						
C - C - A	0.17	93.8	87.8	1.24		
C - T - A	0.37	205.2	189.4	4.23		
G - T - T	0.45	201.0	222.8	8.01	8.02	**0.02**
4-5-6						
G - C - C	0.44	200.0	218.4	4.57		
A - T - T	0.36	195.0	182.9	2.30		
G - T - T	0.19	105.0	98.2	1.45	5.68	0.13
8-9						
A - T	0.21	104.1	106.4	0.10		
G - T	0.48	240.9	235.7	0.51		
A - G	0.30	151.9	152.3	0.01	3.39	0.34
12-13-14						
G - T - A	0.62	318.0	308.8	1.53		
A - C - G	0.16	77.0	79.5	0.18		
G - C - G	0.22	103.0	110.6	1.47	2.87	0.41
21-22						
C - A	0.41	209.9	200.8	1.45		
C - G	0.24	115.5	122.1	1.06		
T - G	0.35	171.6	173.0	0.04	2.56	0.46
22-23-24						
G - C - A	0.05	28.4	25.2	0.93		
A - T - A	0.39	199.9	194.1	0.52		
G - T - A	0.06	27.2	30.5	0.67		
G - C - C	0.37	163.6	184.9	6.93		
G - T - C	0.11	67.8	56.0	4.89	15.50	**0.01**
26-27-28						
C - C - A	0.36	171.2	179.8	1.36		
C - C - T	0.12	60.8	58.7	0.18		
C - T - T	0.06	30.4	28.2	0.39		
T - T - T	0.44	221.5	222.1	0.01	6.76	0.34
29-30-31						
A - C - G	0.44	209.8	219.8	1.77		
C - C - A	0.08	39.6	39.4	0.00		
C - T - A	0.47	244.5	234.7	1.82	1.95	0.58
35-36-37-38-39						
G - C - C - A - G	0.35	173.5	172.4	0.02		
G - C - T - A - G	0.17	78.0	81.2	0.42		
G - C - C - G - T	0.22	117.5	111.1	0.93		
A - T - C - G - T	0.25	124.0	128.7	0.50	2.38	0.79
41-42						
G - C	0.28	136.9	133.3	0.23		
G - T	0.29	156.7	151.0	0.64		
T - T	0.42	198.4	208.6	1.73	2.79	0.43
45-46						
T - A	0.33	171.9	164.2	1.00		
C - G	0.57	270.9	278.1	1.10		
T - G	0.07	36.2	37.5	0.12	1.43	0.70
47-48						
T - A	0.54	276.0	274.2	0.08		
T - G	0.46	224.0	225.8	0.08	0.80	0.78
51-52						
A - A	0.48	244.0	237.5	0.69		
A - G	0.21	104.0	103.8	0.01		
G - G	0.31	150.0	156.6	0.88	1.25	0.74
56-57-58						
G - A - C	0.38	180.0	188.3	1.17		
A - G - T	0.62	314.0	305.7	1.17	1.17	0.28

Gray denotes htSNPs, bold highlights $P \leq 0.05$.

these data are corrected for multiple comparisons. Therefore, given a concern over potential type I error, these association results must be interpreted cautiously. We analyzed 59 SNPs, although not all tests were independent, since a number of these markers are in LD. If we assumed 35 independent tests (one test per block and per SNP between blocks), then an adjusted significance threshold could be estimated to be $0.05/35 = 0.0014$. Using this criterion, none of these results are statistically significant. On the other hand, using this model, we would expect only $35 \times 0.05 = 1.75$ (i.e., ~2) significant

($P \leq 0.05$) results, and there is only a 10% chance of observing four or more independent nominally significant results in this dataset. We see six individually significant SNPs representing four sites not in LD. There is also a clustering of positive results in *GABRB3* in proximity to markers previously shown to exhibit linkage and association. These considerations argue against type I error in this case. Ultimately, replication of these findings in independent samples will be key to determination of significance for these data.

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