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# Evaluation of the Chromosome 2q37.3 Gene *CENTG2* as an Autism Susceptibility Gene

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**Autism is a highly heritable neurodevelopmental syndrome with a complex genetic etiology for which no disease genes have yet been definitively identified. We ascertained three subjects with autism spectrum disorders and chromosome 2q37.3 terminal deletions, and refined the deletion breakpoint regions using polymorphism mapping and fluorescence in situ hybridization (FISH) probes. We then genotyped polymorphic markers downstream from the breakpoint region in a sample of autism affected sibling pair families. Both the chromosomal breakpoints and linkage analyses focused our attention on the gene centaurin gamma-2 (*CENTG2*), an attractive candidate gene based also on its function and pattern of expression. We therefore assessed *CENTG2* for its involvement in autism by (1) screening its exons for variants in 199 autistic and 160 non-autistic individuals, and (2) genotyping and assessing intra-genic polymorphisms for linkage and linkage disequilibrium (LD). The exon screen revealed a Ser → Gly substitution in one proband, an Arg → Gly substitution in another, and a number of additional variants unique to the autism families. No unique variants were found in the control subjects. The genotyping produced strong evidence for linkage from two intronic polymorphisms, with a maximum two-point HLOD value of 3.96 and a posterior probability of linkage (PPL) of 51%. These results were contradicted, however, by substantially weaker evidence for linkage from multi-point analyses and by no evidence of LD. We conclude, therefore, that 2q37.3 continues to be a region of interest for autism susceptibility, and that *CENTG2* is an intriguing candidate gene that merits further scrutiny for its role in autism.** **© 2005 Wiley-Liss, Inc.**

**KEY WORDS: linkage; linkage disequilibrium; autistic disorder; chromosomal abnormalities**

## INTRODUCTION

Autism (MIM 209850) is a neurodevelopmental syndrome consisting of deficits in social interaction and communication, specific ritualistic-repetitive behaviors, and a characteristic course [Rutter et al., 1993]. Onset is typically first noted at the age when relevant behaviors such as speech and complex social interactions become observable, and symptoms generally continue throughout life [Gillberg, 1993]. Family and twin studies have demonstrated that the predisposition to develop autism is largely genetically determined, with a sibling relative risk between 50 and 100 and a heritability estimate of at least 90% [Szatmari et al., 1998]. These same studies also show that the mode of inheritance for autism is likely to be complex, characterized by multiple genetic variants that confer susceptibility by interacting in as yet unknown patterns [Pickles et al., 1995; Risch et al., 1999; Szatmari, 1999]. While the identification of these variants has thus far largely been thwarted by such complexity, an array of increasingly sophisticated approaches are being brought to bear on the search.

The investigations presented here integrate cytogenetic, statistical, molecular, and clinical methodologies in a focused examination of chromosome 2q37.3. Our attention was drawn to this region for two reasons. First, we identified terminal 2q37 deletions in three autistic individuals, a finding consonant with a growing number of similar cases [Burd et al., 1988; Gorski et al., 1989; Stein et al., 1992; Fisher et al., 1994; Conrad et al., 1995; Phelan et al., 1995; Friedman et al., 1997; Viot-Szoboszlai et al., 1998; Ghaziuddin and Burmeister, 1999; Smith et al., 2001; Wolff et al., 2002; Daniel et al., 2003; Casas et al., 2004; Lukusa et al., 2004]. Second, in follow-up analyses of our initial genome-wide screen [CLSA, 1999], we identified a modest linkage signal close to the 2q telomere in a subgroup of families with severe language impairment (maximum multipoint heterogeneity LOD score of 1.13 at 252 cM; unpublished data).

We therefore, as described in this report, proceeded to investigate these initial findings more intensively. Since few of the reported 2q37.3 chromosomal abnormalities have been delineated with precision, we began by demarcating the deletion breakpoints in our cases using polymorphism and fluorescence in situ hybridization (FISH) mapping. We also genotyped polymorphic markers along 2q37 in a large sample

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of affected sibling pair families. Results from both of these approaches focused our efforts on the gene centaurin gamma-2 (*CENTG2*), which codes for a cytoskeletal protein involved in multiple neuronal processes in the developing and mature brain [Jackson et al., 2000]. We screened the coding sequence of *CENTG2* for variants and genotyped numerous intragenic polymorphisms that were tested for linkage and linkage disequilibrium (LD). While the chromosomal deletion cases support the growing interest in 2q37.3, the results of the *CENTG2* screen were mixed with both supportive and confuting evidence regarding its potential contribution to autism susceptibility.

## SUBJECTS AND METHODS

### Recruitment and Assessment of Chromosome Deletion Cases

Deletion probands 1 and 2 were recruited through an ongoing study of chromosomal abnormalities in autism. Subjects are recruited from throughout the United States at a clinical and data collection center at the University of North Carolina (UNC), Chapel Hill. Subjects must have a DSM-IV diagnosis of autism, Asperger's syndrome, or pervasive developmental disorder not otherwise specified (PDD NOS), and are excluded if they have associated medical conditions (e.g., tuberous sclerosis or fragile X syndrome), evidence of gross central nervous system injury, or have suffered severe perinatal events. All accessible medical records are acquired, and blood is drawn that is used for cytogenetic tests and DNA extraction. If a chromosomal abnormality is detected, we attempt to perform the more detailed assessment described in the affected sibling pair ascertainment below. Deletion proband 3 was referred to us by collaborators at the University of California, Irvine (UCI). The ascertainment and evaluation of this subject has been described in detail in a previous report [Smith et al., 2001].

### Chromosome Deletion Breakpoint Delineation

For all three deletion probands, the deletions had been identified clinically by karyotyping, and additional molecular delineation had been performed for proband 3. The focus of the cytogenetic work for this study, therefore, was to refine the delineation of the breakpoint intervals using polymorphic markers and FISH probes. We first selected markers within 2q37 from the Marshfield [Broman et al., 1998] and NCBI UniSTS genetic maps. These markers were genotyped in the probands and their parents, and the results examined for evidence of loss of heterozygosity (LOH). Parental DNA was not available for proband 2, so polymorphisms from this individual were simply examined for the presence of two alleles. When known markers were exhausted, we used the utility tandem repeats finder [Benson, 1999] to identify additional tandem repeats for PCR. Repeats found to be polymorphic were used to assess the deletions, with this process continuing until the breakpoint gaps were maximally narrowed.

For the FISH probe analysis, bacterial artificial chromosome (BAC) clones across the region of interest were identified through NCBI physical map. BAC clones thus identified were ordered from Research Genetics, Huntsville, AL. Individual clones were cultured, and DNA was isolated and used for polymerase chain reaction (PCR) to confirm that each clone was positive for the corresponding gene sequences. Purified BAC clone DNA was labeled with spectrum green or spectrum orange using the Vysis labeling system. Labeled BAC clone DNA was hybridized to human Cot 1 DNA to block repetitive sequences. Thereafter, it was hybridized to metaphase and interphase nuclei on prepared slides. Following the washing of slides according to standard protocols, slides were examined using direct fluorescence microscopy at appropriate wavelengths.

### Patient Ascertainment and Sample Used to Screen *CENTG2* and 2q37.3

Examination of 2q37.3 markers for linkage and LD was performed in a sample of affected sibling pair families ascertained under a previously described protocol [CLSA, 1999]. Briefly, families were recruited from the Midwest and Mid-Atlantic United States through data collection sites at the University of Iowa (UIA) and the University of North Carolina (UNC). All affected individuals were at least 3 years old and were required to meet the ADI-R [Lord et al., 1994] and the ADOS [Lord et al., 1989] or ADOS-G [Lord et al., 2000] criteria for autism. Affecteds were excluded if they had fragile X syndrome (based on fragile X DNA testing) or any neurological or medical condition thought to be associated with autism. DNA was extracted from whole blood using standard techniques, and this sample provided 117 families with at least two affected siblings. These families, as per the CLSA protocol, were split into two sets. The 58 families in Set 1 have been analyzed previously in a genome-wide linkage screen [CLSA, 1999]. That study reported on a total of 75 families, 15 of which are no longer available. Genotyping in the 59 Set 2 families has not been previously reported.

Exon screening was performed using DNA from 199 independent autistic individuals from the Iowa sample and a comparison group of 160 unrelated Iowans of similar ethnicity who did not have autism. DNA for 169 of the autistic individuals came from the UIA and UNC sites, while DNA for an additional 30 affecteds was graciously provided by Dr. Susan Folstein from Tufts University-New England Medical Center, who had ascertained these individuals using the CLSA protocol described above. All individuals (or, when appropriate, their guardians) provided written, informed consent for participation in this study.

### *CENTG2* Coding Sequence Screening

The 4112 bp *CENTG2* cDNA contains 2415 bp of coding sequence across 17 exons, with two alternatively spliced exons (11B and 12B in Fig. 2) present in shorter cDNA. Exons, including flanking splice-junction sequences, were screened with a total of 27 amplicons (primers available upon request), none of which exceeded 250 bp in length, using single strand conformational polymorphism (SSCP) analysis. All amplicons were screened in the entire autism and comparison groups.

PCR was performed with 20 ng of genomic DNA amplified in a reaction mixture containing 1.0 L of PCR buffer (100 μM Tris-HCl (pH8.8), 500 μM KCl, 15 μM $MgCl_2$, 0.01% gelatin (w/v)), 200 μM each of dATP, dCTP, dGTP, and dTTP, 2.5 pM of each primer, and 0.05 U of Taq DNA polymerase, increased to a final volume of 10.0 μL with water. Samples were initially denatured at 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 50–62°C for 30 sec (see Table I), and 72°C for 30 sec. PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels at 20 W for approximately 3 hr at room temperature while being cooled by a fan. The gels were then silver stained using standard protocols [Bassam et al., 1991]. Amplicons containing SSCP shifts were forward and reverse sequenced on an Applied Biosystems (ABI, Foster City, CA) model 377 automated sequencer using dye terminator chemistry. Sequence data were analyzed using Sequencher 4.0 (Gene Codes, Ann Arbor, MI).

The only exception to this SSCP-based screening was for exon 1. This section of *CENTG2* was highly homologous to *CENTG2* pseudogenes, hindering the development of unique amplicons small enough for sensitive SSCP assays. We there-

TABLE I. Deletion Breakpoint Delineation

| BAC FISH | | | Deletion proband 1 | | Deletion proband 2 | | Deletion proband 3 | |
|---|---|---|---|---|---|---|---|---|
| Marker | Probes[a] | MB[b] | Marker | FISH | Marker | FISH | Marker | FISH |
| D2S1363 | | 226.86 | ■ ■ | — | ■ ■ | — | ■ ■ | — |
| D2S1349 | | 228.01 | — | — | hmz | — | — | — |
| ATA20H03 | | 228.29 | — | — | ■ ■ | — | — | — |
| D2S427 | | 232.03 | ■ ■ | — | ■ ■ | — | ■ ■ | — |
| D2S1279 | | 233.78 | ■ ■ | — | ■ ■ | — | — | — |
| D2S1357 | | 235.93 | — | — | ■ ■ | — | — | — |
| AC010148.AAT | RP11-367B19 | 236.02 | — | — | — | ■ | ■ ■ | ■ ■ |
| D2S336 | | 236.06 | ■ ■ | | — | | hmz | |
| D2S407 | | 236.31 | — | — | hmz | — | hmz | — |
| D2S2973 | | 236.32 | — | | hmz | | hmz | |
| AC092576.AAAT | RP11-7N16 | 236.53 | — | — | — | ■ | hmz | ■ |
| AC093785.AT | RP11-212M1 | 236.57 | — | — | — | — | ■ | ■ |
| AC093785.AT2 | | 236.60 | — | | — | | ■ | |
| AC012305.ATT | | 236.61 | ■ ■ | — | — | ■ | — | ■ |
| AC012305.ATTT | | 236.63 | ■ ■ | | — | | ■ | |
| AC012305.CTTT | RP11-84G18 | 236.68 | ■ ■ | | — | | hmz | |
| AC012305.GT1 | | 236.72 | ■ ■ | | — | | hmz | |
| AC012305.GT2 | | 236.74 | ■ ■ | | — | | hmz | |
| AC012305.CA2 | | 236.77 | ■ | | — | | ■ | |
| AC073989.CA | RP11-473L20 | 236.88 | ■ | — | — | ■ | — | ■ |
| AC073989.GT | | 236.90 | ■ | | — | | hmz | |
| D2S2202 | | 236.92 | ■ | — | — | — | ■ | — |
| D2S1397 | RP11-485M18 | 237.08 | ■ | — | — | — | ■ | ■ |
| D2S1359 | | 238.16 | ■ | — | hmz | — | hmz | — |

■ ■, heterozygous; ■, deletion; hmz, homozygous but uninformative;—, not typed.
[a]Shading shows approximate extent of region covered by BAC clones to which FISH probes were hybridized.
[b]Physical map locations based on UCSC July, 2003 Assembly. Shading shows proximal portion of *CENTG2* covered by deletion screening.

fore forward and reverse sequenced a unique 324 bp fragment spanning this exon in 48 affected individuals and 48 control subjects.

### Identification and Genotyping of Intra-Genic and Telomeric Polymorphisms

Polymorphisms within and downstream from *CENTG2* were identified by searching SNP, STS, and other polymorphism databases, and by using TRF. PCR was performed as described above, and PCR products were electrophoresed on 6% denaturing polyacrylamide gels at 60 W for ~2 hr. Gels were visualized using silver staining and read blindly with respect to sample status by two independent raters with discrepancies resolved by re-genotyping. SNPs were genotyped using TaqMan® Pre-Designed SNP Genotyping Assays and Custom TaqMan SNP Genotyping Assays, run on a StrataGene Mx3000P qPCR thermocycler.

### Analysis of Polymorphisms for Linkage and LD

Two-point and multi-point heterogeneity LOD (HLOD) scores were calculated in the same manner as in our genome-wide linkage screen, using the MLINK and HOMOG subroutines of the LINKAGE package [Lathrop et al., 1985] under one simple recessive model (disease allele frequency = 0.10, penetrance = 50%) and one simple dominant model (disease allele frequency of 0.04, penetrance = 40%). Multi-point analyses were calculated using all available markers. Allele frequencies and marker heterozygosities were determined from parental genotypes. Normal allele penetrance was set to zero (no phenocopies). We also performed two-point and multi-point analysis of the markers using the posterior probability of linkage (PPL) statistic, which integrates out parameters of the trait model, thereby providing a largely model-free analysis [Vieland, 1998; Logue et al., 2003]. The PPL is calculated on the straightforward probability scale (0,1), with 1 indicating certain linkage and 0 indicating no linkage. The prior probability of linkage is set to 0.02, so that a PPL > 0.02 indicates some degree of evidence in favor of linkage. Of import to the language-based subgroup analyses described below, the PPL enables results from multiple samples or subgroups of families to be integrated en bloc sequentially. This protects homogeneity in those groups from the potentially diluting effect of simply merging all families into one large sample [Vieland et al., 2001]. Lastly, Genehunter 2.1 [Kruglyak et al., 1996] was used to calculate the TDT statistic for all polymorphisms using one randomly selected affected sibling from each family.

### Subgroup Analyses

Subsequent to our genome-wide screen, we attempted to address the apparent heterogeneity of autism by splitting the ASP families into two groups based on their language characteristics. Families were classified as *language impaired* if neither autistic sibling had developed phrase speech by 36 months, and otherwise as *language normal* (the average normal onset of phrase speech is 18 months). Analyses of the two resultant subgroups at our two strongest loci (7q and 13q) revealed that the predominance of the linkage signals arose from the *language impaired* families [CLSA, 2001], a pattern that has since been replicated [Buxbaum et al., 2001; Alarcon et al., 2002; Shao et al., 2002]. In addition, we had assessed, by direct questioning, whether the ASP parents had a history of developmental language difficulties, including delayed onset of speech, trouble learning to read, or persistent trouble with spelling. Parents who experienced any of these difficulties were classified as *language impaired* (affected) and otherwise as *language normal* (unaffected). Incorporation of this data into the analyses further strengthened the evidence for linkage at

both loci. We thus provided support for the hypotheses that (1) language-based classification defines more homogeneous subgroups of autistic families, and (2) parental language phenotype is in some way genetically related to autism. In light of this, we analyzed the markers genotyped for this study in a similar manner, examining the evidence for linkage and LD in the pooled Set 1 and Set 2 samples and in the two language based subgroups from each set (for a total of four groups), and incorporating parental language phenotypes.

## RESULTS

### Clinical Assessments of Deletion Probands

Detailed clinical assessments of the deletion probands are provided in online supplementary material. Briefly, probands 1 and 3 met ADI-R and ADOS-G criteria for autistic disorder; proband 2 has not been assessed with these instruments, but has been given a DSM-IV diagnosis of PDT, NOS. Probands 1 and 2 have mild mental retardation, while proband 3 has a normal IQ.

### Deletion Breakpoint Delineation in the Deletion Probands

For proband 1, previous karyotyping of this child had revealed a single terminal deletion, 46,XY,del(2)(q37.1) which was not found in karyotypes of the parents. Subsequent genotyping in this study of a total of 35 polymorphisms revealed that the maternal chromosome was deleted and led to delineation of the breakpoint to a 33 kb region within intron 1 of *CENTG2* (Table I and Fig. 2).

Karyotyping of proband 2 had revealed a de novo 2q37 telomeric deletion: 46,XY,del(2q)(q37.1q37.3). While parental DNA was not available for this individual, combined FISH and marker data narrowed the deletion breakpoint to a 100–150 kb interval ~500 kb upstream from *CENTG2* (Table I and Fig. 2). The deletion also appeared to be terminal, as no markers or FISH probes demonstrated two chromosomes telomeric to the breakpoint.

For proband 3, previous karyotypes of the patient and parents had detected a de novo 2q37 deletion with the breakpoint interval narrowed to a span of ~5 MB using BAC FISH probes and polymorphic markers (Smith ref). The interval was further narrowed in this study using 45 polymorphic markers and additional FISH probes. The markers reduced the breakpoint interval to a 600 kb region approximately 200 kb upstream from the proband 1 breakpoint interval. The additional FISH probes confirmed the marker results, narrowing the interval to ~500 kb and placing the breakpoint ~200–500 kb upstream from *CENTG2* (Table I and Fig. 2).

### *CENTG2* Coding Sequence Screen

Results of the coding sequence screen are shown in Table II. Sixteen variants were identified: four non-synonymous and six synonymous coding sequence variants, and six intronic variants. Of the non-synonymous variants, S82G, R745G, and P801T (see Fig. 1) were found in one autism family each. The probands containing S82G and R745G were from trios, while P801T was found in both the affected and unaffected sibs from a discordant pair. S82 and R745 are both highly conserved amino acids within, respectively, ras and ankyrin functional domains; P801, conversely, is not in a known functional domain. The fourth non-synonymous variant, I618V (rs2034648), was common in both the autism and control populations and, while itself not conserved, is within the ArfGap functional domain.

Of the synonymous coding sequence variants, four were common in both the autism and control samples, while two—T1222C and C2686T— were found in one autism family each; no variants were found that were unique to the control subjects. T1222C was found in a trio proband, while the T allele of C2686T was transmitted from a mother to both of her affected children. Similarly, the intron 12 AG deletion was found in one ASP family in which it was transmitted from the father to both

TABLE II. Results of *CENTG2* Coding Sequence Screen

Coding sequence variants

| Exon | cDNA variant | Amino acid effect | Functional domain | dbSNP | Comment[a] |
|---|---|---|---|---|---|
| 1 | C703T | None, N43N | — | rs8178993 | Common |
| 2 | A781G | None, P210P | — | ss9807226 | Common |
| 3 | A818G | S83G | Ras | — | One trio proband |
| 6 | T1222C | None, N217N | — | — | One trio proband |
| 8 | C1451A | None, R294R | — | — | ASP father and one sib |
| 8 | C1528T | None, T319T | — | rs2292708 | Common |
| 15 | A2423G | I618V | ArfGap | rs2034648 | Common |
| 16 | C2686T | None, D705D | — | — | One ASP family (mother and both affecteds) |
| 17 | A2805G | R745G | Ankyrin | — | One trio proband |
| 17 | C2923T | None, P784P | — | ss9807225 | Common |
| 17 | C2972A | P801T | — | — | One trio proband and unaffected sib |

Intronic variants

| Intron | BAC | Nucleotide within BAC | Variant | dbSNP | Comment[a] |
|---|---|---|---|---|---|
| 1 | AC012305 | 81238 | A/C | rs3738998 | Common |
| 9 | AC074874 | 107882 | T/A | ss9807227 | Common |
| 10 | AC079400 | 50563 | T indel | ss9807228 | common |
| 12 | AC079400 | 110517 | AG deln | — | One ASP family (father and both affecteds) |
| 12 | AC019047 | 45468 | T indel | rs2123511 | Common |
| 16 | AC019047 | 151211 | C/T | rs2278884 | Common |

[a]"Common" indicates variants with minor allele frequencies > 10%.

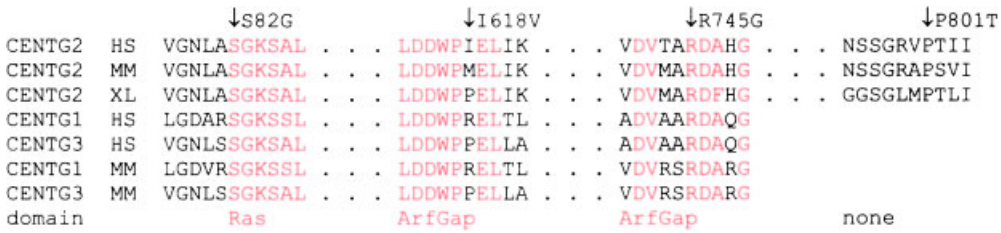


Fig. 1. Non-synonymous *CENTG2* coding sequence variants. S82G and R745G were found in one autism trio proband each. I618V was common in both autistic individuals and psychiatrically normal controls. P801T was found in both of a discordant sib pair. Red letters indicate conserved amino acids in known functional domains of *CENTG2*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

affected children. Phenotypic information available on this father revealed multiple characteristics similar to the core features of autism, including a rigid personality style and deficits in social interactions and friendships, while the mother was phenotypically normal.

### Analysis of Polymorphisms for Linkage and LD

Sixteen polymorphisms were identified, genotyped, and analyzed for evidence of linkage and LD (Table III and Fig. 2; primers available upon request). Two of these, D2S2968 and D2S427, had been genotyped in half the families for our initial linkage screen, and were thus genotyped in the remainder of the sample for this study.

Two-point linkage analyses revealed, under the recessive model, strong evidence in favor of linkage at two markers: D2S1397 and CENTG2.32112 (Table IV and Fig 3). For both markers, the evidence for linkage arose predominantly from the language impaired subsets of families. For D2S1397, the evidence for linkage arose primarily from the Set 1 families, while for CENTG2.32112 the evidence arose primarily from the Set 2 families. Six of the remaining markers within *CENTG2* produced LOD scores $>1.0$ and/or PPLs greater than 0.02, suggesting some evidence in favor of linkage.

In contrast to the two-point results, however, multi-point analyses provided little support for the presence of linkage. Three-point, four-point, and all-marker analyses were universally low across the entire region of interest (Fig. 3). In addition, the single-sibling TDT analyses failed to identify evidence for LD at any of these markers, and no evidence of allelic instability was detected at either of the CAG trinucleotide repeats.

TABLE III. 2q37.3 Polymorphisms That Were Genotyped

| Polymorphism | Type | Heterozygosity[a] | MB |
|---|---|---|---|
| D2S427 | Micro | 0.79 | 232.03 |
| CAGL234 | Micro | 0.65 | 236.71 |
| CENTG2.32112 | Mini | 0.30 | 236.72 |
| CENTG2.71457 | Mini | 0.50 | 236.76 |
| CENTG2.TAAA | Micro | 0.64 | 236.83 |
| CENTG2.214374 | Mini | 0.47 | 236.90 |
| CENTG.GTTG | Micro | 0.74 | 236.92 |
| hcv1543812 | SNP | 0.34 | 236.95 |
| D2S1397 | Micro | 0.68 | 237.08 |
| rs4663627 | SNP | 0.45 | 237.08 |
| CENTG2.GGAT | Micro | 0.88 | 237.09 |
| rs765880 | SNP | 0.48 | 237.23 |
| D2S2968 | Micro | 0.65 | 237.86 |
| 2Q112721.GTTT | Micro | 0.66 | 238.59 |
| 2Q16776.AAGG | Micro | 0.88 | 239.14 |
| 2Q17028.GATA | Micro | 0.76 | 240.18 |

Primers available in supplementary online material shading indicates markers within *CENTG2*.
[a]Heterozygosity was determined using all parental genotypes.

## DISCUSSION

The genetic and phenotypic complexity that typifies psychiatric disorders necessitates integration of multiple approaches in the attempt to identify susceptibility genes. In the current study, the delineation of breakpoint intervals for three terminal deletions of chromosome 2q37.3 in individuals with autism or autism spectrum disorders provides support for this as a region that is likely to harbor an autism susceptibility gene. The chromosomal studies were then complemented by subsequent linkage analyses of 2q37 that took into account phenotypic heterogeneity. These analyses focused our attention on the gene *CENTG2*, which we therefore examined by screening its coding sequence for genetic variants and by examining intra-genic polymorphic markers for linkage and LD. These studies combined to produce a mixed picture of the relationship between *CENTG2* and autism susceptibility.

The chromosome 2q37.3 terminal deletions that we describe join a growing body of similar reports in the literature (for a recent review of 2q terminal deletions, see [Casas et al., 2004]). While the most common syndrome associated with such abnormalities is albright's hereditary osteodystrophy (AHO) [Phelan et al., 1995; Bijlsma et al., 1999; Casas et al., 2004], the presence of autism or autistic traits is also common [Burd et al., 1988; Gorski et al., 1989; Stein et al., 1992; Fisher et al., 1994; Conrad et al., 1995; Phelan et al., 1995; Friedman et al., 1997; Viot-Szoboszlai et al., 1998; Ghaziuddin and Burmeister, 1999; Smith et al., 2001; Wolff et al., 2002; Daniel et al., 2003; Casas et al., 2004; Lukusa et al., 2004]. The specific chromosomal sub-band locations of the breakpoints do not appear to be predictably associated with autistic features [Casas et al., 2004], though only two such cases pursued breakpoint delineation beyond karyotyping [Giardino et al., 2001; Daniel et al., 2003], and both demonstrated breakpoint boundaries in close proximity to those presented in this study.

The first described a child with multiple medical problems, abnormal facies, no AHO phenotype, moderate developmental delay, and multiple autistic features [Daniel et al., 2003]. Genotyping of polymorphic markers in the proband and parents showed that the first deleted marker was D2S2202, which is in intron 3 of *CENTG2* (Fig. 2). A second report of interest described a family with multiple still births and a child with dysmorphic facial features, multiple physical anomalies, and mild MR [Giardino et al., 2001]. Karyotyping of this child revealed a subtelomeric translocation t(2;16)(q37;q24), which FISH studies refined to 46,XY,der(2)t(2;16)(q37.3;q24.3), producing partial monosomy for distal 2q37. The mother, maternal grandfather, and a subsequent conceptus were found to have balanced t(2:16) translocations. The affected child was quite young at the time of the report and appears not to have

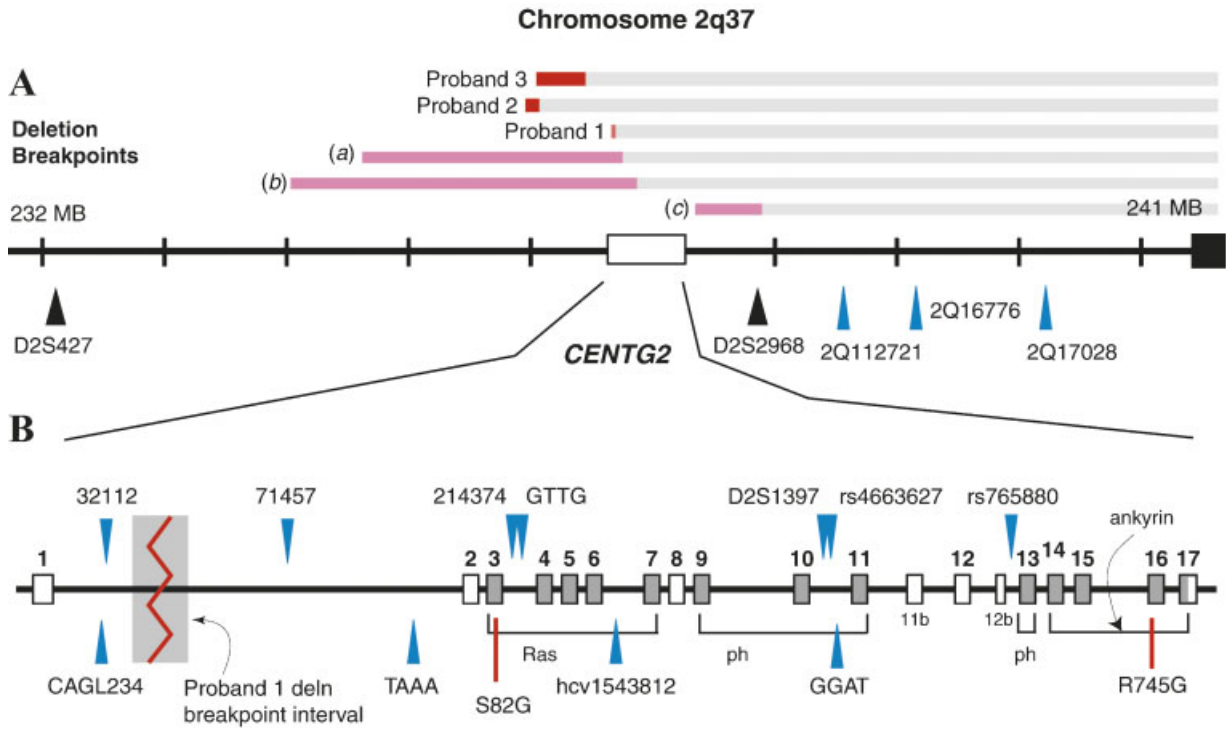


Fig. 2. Chromosome 2q37 and *CENTG2* findings. **A**: Deletion breakpoint intervals for deletion probands 1, 2, and 3. Red bars indicate breakpoint intervals, and gray bars indicate extent of the deletions. Pink bars show deletion breakpoints for three cases from the literature with autistic features, 2q37.3 deletions, and some degree of molecular delineation of the breakpoint intervals; (**a**) is from [Giardino et al., 2001], (**b**) is from [Daniel et al., 2003], and (**c**) is from [Giardino et al., 2003]. Black arrows indicate markers previously genotyped in part; blue arrows indicate markers developed and genotyped for the current study. **B**: *CENTG2* structure and analyses. Numbered boxes indicate exons; shading in exons indicates functional domains (named underneath). Red straight lines show locations of two non-synonymous coding sequence variants. Blue arrows indicate markers within *CENTG2* that were genotyped for the current study. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

been assessed for autism, and no phenotypic information was provided for the mother or grandfather. Additional FISH studies were performed using YAC clones, and the first deleted clone (746H11) contained exon 1 of *CENTG2* (Fig. 2). Thus these two anomalies appear to interrupt 2q37 near the deletions that we describe.

The deletions described in this and these previous reports cause monosomy for 7–10 MB of genomic material that con-

TABLE IV. Two-Point Linkage Results

| | | Maximum HLODs in family subgroups[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Family Set 1 | | | Family Set 2 | | | |
| MARKER | MB Pos | Language impaired n = 42 | Language normal n = 16 | Pooled n = 58 | Language impaired n = 37 | Language normal n = 22 | Pooled n = 59 | PPL |
| D2S427 | 232.03 | 1.83 | 0.00 | 1.02 | 0.99 | 0.02 | 0.77 | 0.04 |
| CAGL234 | 236.71 | 0.97 | 0.14 | 0.84 | 0.28 | 0.62 | 0.71 | 0.02 |
| CENTG2.32112 | 236.72 | 1.48 | 0.08 | 0.66 | 3.51 | 1.15 | 2.41 | 0.37 |
| Centg271457 | 236.76 | 0.09 | 0.01 | 0.06 | 0.61 | 0.67 | 0.69 | 0.02 |
| CENTG2.TAAA | 236.83 | 0.42 | 0.00 | 0.15 | 0.43 | 1.14 | 0.96 | 0.02 |
| Centg21437 | 236.90 | 0.25 | 0.09 | 0.20 | 1.18 | 0.81 | 1.87 | 0.02 |
| CENTG2.GTTG | 236.92 | 1.33 | 0.01 | 0.37 | 0.92 | 1.66 | 0.86 | 0.03 |
| hcv1543812 | 236.95 | 1.89 | 0.02 | 1.20 | 0.75 | 0.08 | 0.56 | 0.05 |
| D2S1397 | 237.08 | 3.96 | 0.58 | 4.03 | 0.85 | 0.36 | 0.18 | 0.51 |
| rs4663627 | 237.08 | 1.69 | 0.19 | 1.68 | 0.58 | 0.63 | 0.57 | 0.04 |
| CENTG2.GGAT | 237.09 | 1.30 | 0.60 | 1.79 | 0.48 | 0.52 | 0.86 | 0.03 |
| rs765880 | 237.23 | 1.13 | 0.05 | 0.91 | 0.70 | 0.08 | 0.50 | 0.03 |
| D2S2968 | 238.01 | 0.81 | 0.00 | 0.15 | 0.62 | 0.15 | 0.35 | 0.02 |
| 2Q112721.GTTT | 238.59 | 0.50 | 0.39 | 0.79 | 0.65 | 0.54 | 0.75 | 0.03 |
| 2Q16776.AAGG | 239.14 | 0.50 | 0.11 | 0.61 | 0.72 | 0.52 | 0.88 | 0.02 |
| 2Q17028.GATA | 240.18 | 0.96 | 0.14 | 0.68 | 0.46 | 0.63 | 1.08 | 0.02 |

Shading indicates markers within *CENTG2*.
[a]HLODs are reported for a simple recessive model (disease allele frequency = 0.10, penetrance = 50%).

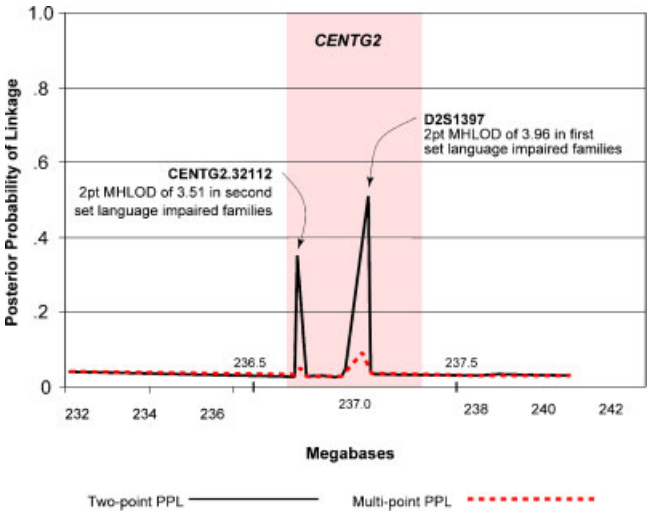


Fig. 3. Linkage analyses of 2q37 and *CENTG2* markers. The black line shows two-point PPLs and the dashed red line shows multi-point PPLs using all markers. The pink shaded area demarcates the boundaries of *CENTG2*. Note that the scale for the area containing *CENTG2* is expanded compared to the rest of the graph. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tains over 100 genes and RNA transcripts. We therefore genotyped markers across the interval to test for linkage and/or LD. In this initial pass, we found minimal evidence for linkage downstream from *CENTG2* that contrasted with strong evidence for linkage within the gene. In addition, *CENTG2* was suggested as a candidate gene because of its function and pattern of expression. Centaurin proteins (at least 10 have been identified in humans) are involved in phosphatidylinositol 3,4,5-trisphosphate (PI 3-phosphate) and ADP ribosylation factor signaling pathways [Jackson et al., 2000]. Both of these pathways influence neuronal processes such as cell growth and survival, differentiation, metabolism, transcription, vesicular trafficking, and cytoskeletal organization [Fruman et al., 1998; Chavrier and Goud, 1999; Leevers et al., 1999; Rameh and Cantley, 1999; Roth, 1999; Donaldson and Jackson, 2000; Rodgers and Theibert, 2002]. In line with this, the PI 3-phosphate pathway is activated by neurotrophic, differentiation, and growth factors [Rodgers and Theibert, 2002]. *CENTG2* is expressed in multiple regions of fetal and adult brain, and centaurin proteins are found in neuronal growth cone vesicles and in synapses of developing and mature neurons [Kreutz et al., 1997; Sedehizade et al., 2002; Aggensteiner and Reiser, 2003]. Lastly, the gene coding for *centaurin alpha-1*, a relative of *CENTG2*, is located within the neurofibromatosis (NF) micro-deletion region on chromosome 17q11.2, and NF is associated with an elevated rate of autism.

We therefore genotyped additional markers within *CENTG2* and screened the coding sequence for variations. The genotyping identified two markers that produced strong evidence for linkage based on two-point heterogeneity LOD scores and PPLs. The signal at D2S1397 arose primarily from the language impaired subgroup of the Set 1 families, while the signal at CENTG2.32112 arose primarily from the language impaired subgroup of the Set 2 families. The majority of the remaining *CENTG2* markers provided at least a modest degree of supportive evidence for linkage. Multi-point analyses, however, produced results that stood in stark contrast to the two-point results with LOD scores and PPLs near zero across the entire interval. These negative results were consistent for three-point, four-point, and all marker analyses.

The discrepancy between the two- and multi-point results is striking and, while we do not know with certainty its cause, a number of possibilities exist. The first is genotyping error; we rigorously examined our genotypes, however, using multiple methods for detecting and removing errors, and do not believe that errors in our genotyping could account for the degree of disparity between the results. A second possibility is that the two-point scores were false positives that were revealed as such by the greater informativeness of the multi-points. Conversely, however, the two-point linkage signals may be detecting a real linkage that is lost in the multi-points. Multi-point analyses are generally assumed to more accurately assess linkage due to their use of all available genetic information. While true for "perfect" datasets, imperfections that characterize real data such as missing parents, map errors, missing genotypes, and genotyping errors—even when these all occur at low frequencies—have been shown to inordinately degrade multi-point, as compared to two-point, linkage signals [Sullivan et al., 2003]. Thus it may be that the two-point analyses more accurately represent the evidence for linkage in our CLSA sample. The finding of strong linkage in distinct sets of families at two different markers in close proximity to each other seems an unlikely chance occurrence, and subsequent marker-to-marker analyses found no evidence for disequilibrium between D2S1397 and CENTG2.32112.

The coding sequence screen of *CENTG2* produced similarly mixed results. We found, in two independent autistic subjects, non-synonymous variants that altered single amino acids—glycine for serine in one case (S82G) and glycine for arginine (R745G) in another—that were both in conserved regions of functional domains (Fig. 1). We also found one non-synonymous variant in both sibs of a discordant pair, though the affected amino acid was not in a known functional domain. Lastly, we found numerous synonymous and intronic variants, a number of which were unique to the autism sample and segregated with the disorder within the sib-pair families; no unique variants were found in the non-autistic control subjects.

These variants could be interpreted to implicate *CENTG2*. S82G and R745G substitute significantly different amino acids in presumably sensitive regions of the protein, and it has become clear that synonymous and intronic variants can alter multiple aspects of gene expression and processing. Conversely, non-synonymous variants, while more likely to be deleterious, often occur without any associated known effect on phenotype. Furthermore, we are limited in our ability to assess the effect of these variants by our typically small pedigrees and by the daunting task of assessing their functional significance.

In conclusion, we present a constellation of evidence derived from investigations assessing the contribution of chromosome 2q37 and the gene *CENTG2* to autism susceptibility. The terminal deletions that we report provide further support for 2q37.3 as a region of interest in autism genetics, and the molecular breakpoint delineations refine the proximal interval boundary. Results from the examination of *CENTG2*, however, are mixed, with some supporting and others refuting the gene's involvement in autism heritability. Thus, while *CENTG2* merits further scrutiny as an autism disease gene, the numerous other plausible candidate genes in the region must be considered for examination as well.

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