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Cytogenetic and Genome Research

Molecular genetic delineation of a deletion of chromosome 13q12→q13 in a patient with autism and auditory processing deficits

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Abstract. In a sporadic case of autism and language deficit due to auditory processing defects, molecular genetic studies revealed that a chromosomal deletion occurred in the 13q12→q13 region. No chromosome abnormalities were detected in the parents. We determined that the deletion occurred on the paternally derived chromosome 13. There are two previous reports of chromosome 13 abnormalities in patients with autism. The deletion in the subject described in this paper maps between the two chromosome 13 linkage peaks described by Bradford et al. (2001) in studies of subjects with autism and language deficits. The 9-Mb region deleted in the patient described here contains at least four genes that are expressed in brain and that play a role in brain development. They are NBEA, MAB21L1, DCAMKL1 and MADH9. These genes therefore represent candidate genes for autism and specific language deficits.

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Through molecular genetic delineation of chromosome deletions in patients with autism spectrum disorders, candidate genes for these disorders may be identified. Here we report the fine structure mapping of a de novo chromosomal deletion in 13q in a patient with autism and language deficits due to auditory processing defects. The deletion includes the distal third of band 13q12 and the proximal two thirds of band 13q13. Results of our molecular cytogenetic studies and polymorphic marker analysis indicate that in this patient there is a deletion of approximately 9 Mb that extends from 28.52 Mb to 38.06 Mb. Results of polymorphic marker analysis revealed that the deletion arose on a paternally derived copy of chromosome 13. Given the advanced state of human genome sequencing it is now possible to determine which genes are deleted in our patient and to identify brain expressed genes that represent candidate genes for autism and language processing.

There are two other published reports of the co-occurrence of autism and deletions of the proximal region of chromosome 13. Ritvo et al. (1988) reported on the simultaneous occurrence of autism and retinoblastoma in a patient with a deletion that extended from 13q12 to 13q14. Steele et al. (2001) described a case of autism with a de novo deletion of 13q14→q22.

Specific evidence for linkage of chromosome 13 markers and autism was reported by Bradford et al. (2001). These investigators analyzed the effect of incorporating language information and parental structural language phenotypes into the genome screening for autism. Their results revealed that two distinct peaks of linkage occurred on chromosome 13q. The deletion in our patient maps in a position between the map locations of the two linkage peaks described by Bradford et al. (2001).

Materials and methods

Clinical evaluation

Our patient was born by normal vaginal delivery. Birthweight was 6 lb 1 oz, length was 21 inches. He had no feeding problems. Developmental milestones include smiling at one month, sitting at 9 months, walking alone at

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Table 1. Chromosome 13 polymorphic markers examined: map position, PCR primers and PCR conditions. Fifteen microsatellite repeat markers were studied. HTR2A displays a single nucleotide polymorphism that we examined by DNA sequencing.

Primer		Sequence	Size (bp)	Position	Het.	Temp.	Conditions
D13S217	For	ATG CTG GGA TCA CAG GC	160–174	23,352 kb	0.67	60	Q sol'n
	Rev	AAC CTG GTG GAC TTT TGC T					
D13S1229	For	GGT CAT TCA GGG AGC CAT TC	137–151	25,471 kb	0.74	60	Q sol'n
	Rev	CCA TTA TAT TTC ACC AAG AGG CTG C					
D13S260	For	AGA TAT TGT CTC CGT TCC ATG A	158–173	26,424 kb	0.77	57	$MgCl_2$
	Rev	CCC AGA TAT AAG GAC CTG GCT A					
D13S1493	For	ACC TGT TGT ATG GCA GCA GT	223–248	27,996 kb	0.80	57	$MgCl_2$
	Rev	GGT TGA CTC TTT CCC CAA CT					
D13S1293	For	TGC AGG TGG GAG TCA A	119–139	28,725 kb	0.77	57	$MgCl_2$
	Rev	AAA TAA CAA GAA GTG ACC TTC CTA					
D13S218	For	GAT TTG AAA ATG AGC AGT CC	187–195	33,019 kb	0.65	57	$MgCl_2$
	Rev	GTC GGG CAC TAC GTT TAT CT					
D13S325	For	TCC TTT AAG TGT CTA GAG AGG AGG	195–231	37,160 kb	0.78	57	$MgCl_2$
	Rev	TCT CTC TCA GAA GTT TGG AAG C					
D13S1227	For	AAG CCA TCA CTG TGT TCC C	124–156	37,530 kb	0.80	57	Q + $MgCl_2$
	Rev	TGC TTG GGT GGA ATG C					
D13S291	For	ATG GCC AGA CTT CCC ACT	240–253	38,806 kb	0.71	57	$MgCl_2$
	Rev	CCA GGC TCA CAT GCT AAC A					
D13S1272	For	TTT ATC GCT AAC ATC TGG CA	246–254	39,072 kb	0.71	57	Q sol'n
	Rev	AGC ATT CAT TGG TGG CA					
D13S1312	For	TCT TCC CAG AAT ATA TGG GA	106–144	39,919 kb	0.81	57	$MgCl_2$
	Rev	AGC TGT AAA AGT GTT TGT TTG ATG T					
HTR2A	For	AGA TGC CAA GAC AAC AGA TAA TGA C	105	41,396 kb		60	Q sol'n
	Rev	CTC ACC TTT TCA TTC ACT CCG					
D13S161	For	CAC ACT GCA CAC AAT TCT AAA	88–100	41,771 kb	0.57	57	$MgCl_2$
	Rev	CTT AAA TTA ACG TAT TTG GGG TTT T					
D13S287	For	CTG AAT TGC TTT GAG ACA TT	139–153	41,876 kb	0.59	57	$MgCl_2$
	Rev	CCA GTG TAG GGC TGA TAT AGA					
D13S1317	For	CTT GGA AAC CAA CAA GTC AC	221–241	61,060 kb	0.86	57	Q sol'n
	Rev	ATT TTG CCA CCT AGA ACG G					
D13S800	For	AGG GAT CTT CAG AGA AAC AGG	295–319	67,870 kb	0.75	60	Q sol'n
	Rev	TGA CAC TAT CAG CTC TCT GGC					

15 months. He had his first words at two years of age. When evaluated at 3 years and 8 months he had many single words. He could not easily put two words together. Clinical examination was normal and evaluation by a dysmorphologist revealed no abnormalities. MRI of the brain was carried out on a 1.5 Tesla Picker superconducting magnet. The sequences obtained included Sagittal T1-weighted, axial proton density and T2-weighted and coronal 3D gradient echo volume sequences. No mass lesions were seen; ventricles and sulci were within normal limits and no structural abnormalities were identified.

Language testing

Extensive language assessment was carried out when the patient was 6 years old. At that time auditory processing skills were judged to be the subject's greatest weakness and were thought to affect his higher-level auditory comprehension and expressive language skills. Further detailed language evaluation was carried out at the age of 8 years and 9 months. The Token Test for children (TTTFC) (DiSimoni, 1978) assesses the ability to follow directions of increasing length and complexity. The subject's scores indicated continued difficulties with auditory processing skills of memory and sequencing. In addition as the complexity of the task increased his abilities to remember the direction and sequence correctly, decreased. The subject's overall score for the TTTFC was 476. The age mean for this test is 500 with a standard deviation of 5. The revised test of auditory perceptual skills (TAPS-R) (Gardner, 1996) assesses various areas of the individual's auditory-perceptual skills. The subject exhibited severe auditory difficulties in the areas of following directions and reasoning. Auditory word discrimination was an area of strength. The auditory perceptual quotient in this patient was 67. The age mean is 100 with a standard deviation of 15.

The CELF-3 test (Semel et al., 1995) assesses basic foundations of content and form that characterize mature language: word meanings (semantics) word and sentence structure (morphology and syntax) and recall and retrieval of spoken language (memory). Due to short attention span the entire CELF 3 test was not administered. The sub-tests administered revealed weakness in morphology and semantics. The Goldman-Fristoe-Woodcock test of auditory discrimination can be used as an index of an individual's ability to listen to and understand a message in the presence of competing sound (Brown et al., 1987). The subject was able to perform adequately when there was an absence of background noise, however when noise was introduced his ability to discriminate was significantly reduced.

Theory of mind assessment

This was examined using five tasks designed to assess a child's understanding of the appearance-reality distinction (Flavell et al., 1983; Sapp et al., 2000), of another's false beliefs and emotions (Baron-Cohen, 1989; adapted from Baron-Cohen et al., 1985; Harris et al., 1989; Wimmer and Hartl, 1991; Hughes et al., 2000), and of one's own false beliefs (Wimmer and Hartl, 1991). These tasks have been frequently used to assess theory of mind in typically developing children and in children with autism, and recent research into their psychometric properties indicate good test-retest reliability especially among aggregated scores of multiple measures (Hughes et al., 2000).

Theory of mind tests were administered twice over a two-week interval. The child's performance on the first order belief systems was inconsistent across tasks and over time. The child failed a second order false belief task at both testing sessions and exhibited some difficulty in responding verbally to questions tapping an understanding of the appearance of reality distinction. Although he showed some understanding of emotional states, he does not appear to appreciate the beliefs underlying these states. In sum the child appears to have a tentative understanding of the mental state of belief but only in response to very limited straightforward circumstances.

Evaluation for autism

There was no family history of autism. This child was administered the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1989) (Module 3) at age 6 years, 2 months. His score measuring impairments in Communication quality was 2 (scores of 2 or higher indicate Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS); scores of 3 or higher indicate Autistic Disorder). His score measuring Qualitative Impairments in Reciprocal Social Interaction was 5 (scores of 4 or higher indicate PDD-NOS; scores of 6 or higher indicate Autistic Disorder). His combined Communication (2) and Social (5) scores was 7 (scores of 7 or higher indicate

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PDD-NOS; scores of 10 or higher indicate Autistic Disorder). The ADOS diagnosis for this child was PDD-NOS. This diagnosis was confirmed by clinical examination by pediatric neurologist, Pauline A. Filipek, M.D.

The Autism Diagnostic Interview (ADI) (Lord et al., 1994) was also administered to the mother of this boy when he was 6 years, 2 months of age. His score on the scale measuring qualitative impairments in reciprocal social interaction was 15 (scores of 10 or higher indicate Autistic Disorder). His score on the communication impairments scale was 12 (scores of 8 or higher indicate Autistic Disorder). On the scale measuring repetitive behaviors and stereotyped patterns, his score was 6 (scores of 3 or higher indicate Autistic Disorder). His profile also contained five indications of abnormal development before 36 months (scores of 1 or more indicate Autistic Disorder).

IQ testing

The Stanford-Binet test 4th Edition (Thorndike et al., 1986) was administered to this child when he was 8 years, 7 months of age. His Test Composite (IQ) was 78 at the 8th percentile and in the Slow Learner (IQ's of 68–78) Range relative to age-mates (mean = 100, standard deviation = 16). In a subscale pattern typical of autistic children, his Verbal Reasoning IQ was 70 (3rd percentile) and comprised his lowest area score, while his Abstract/Visual Reasoning IQ was significantly higher at 90 (27th percentile). The Quantitative Reasoning IQ was 88 (23rd percentile) and the Short-term Memory IQ was 80 (11th percentile). The Memory for Beads visual memory subtest scaled score (mean = 50, SD = 8) was 45 at the 27th percentile and the Memory for Sentences scaled subtest score was 38 at the 7th percentile relative to age-mates.

Routine cytogenetic studies

Analysis of metaphases from peripheral blood revealed that the patient had an unbalanced chromosome complement with an interstitial deletion on chromosome 13. The breakpoints of the deletion were estimated to be 13q13.2 and 13q14.1. Chromosome analyses on peripheral blood from the parents revealed normal karyotypes.

Molecular cytogenetic studies

Cultured white blood cells (lymphoblastoid cell lines) from the patient and his parents were used to produce slides with spreads of metaphase chromosomes and interphase nuclei. We utilized information from the Human Genome projects as archived on the NCBI website (http://www.ncbi.nlm.nih.gov/), to identify a series of linearly ordered BAC clones on chromosome 13q. BAC clones were ordered from Research Genetics/In Vitrogen. BAC clone preparations were plated out on agar and single colonies of each specific BAC were isolated and grown overnight in liquid culture medium. BAC clone DNA was extracted by alkaline lysis according to the procedure recommended by Research Genetics. DNA from BAC clones was labeled using Spectrum Green dUTP (Vysis) or Spectrum Red dUTP (Vysis) and nick translation. Labeled BAC clone DNA was ethanol precipitated along with Cot1 human DNA to block repetitive sequences. These precipitates were then dried, and dissolved and used for FISH studies. Hybridization of probes to chromosome preparations and pre- and posthybridization washes were carried out according to procedures recommended by Vysis.

Analysis of polymorphic markers and genotyping

The purpose of these studies was to further define extent of the deletion and to determine the parental origin of the deletion chromosome. The polymorphic markers used, their map position, PCR amplification primers, and conditions are summarized in Table 1.

The markers were amplified using PCR. The reaction mix of 25 µl contained 0.50 µg of DNA, 2.5 µl of Qiagen 10× buffer (100 mM Tris, 500 mM KCl), 1.3 µM forward primer, 1.3 µM reverse primer, 0.30 mM dNTPs, and 1.2 U Taq. We also added 5.0 µl of Qiagen Q solution and/or 1.5 mM of $MgCl_2$. The thermocycling was carried out in an MJ Research Inc PTC-100 and MJ Research Inc Minicyclers. The amplification program consisted of an initial incubation at 94 °C for 1 min, followed by 30 cycles of denaturing at 92 °C for 40 s, annealing at either 57 °C or 60 °C for 40 s, and extension at 75 °C for 90 s, with a final extension at 75 °C for 5 min. For individual marker conditions, see Table 1.

Once the fragment was amplified, the PCR product was run on a polyacrylamide gel to confirm that the correct fragment was amplified. The 25-ml gel consisted of 6 % acrylamide:bis (19:1), 10 % glycerol, 1× TBE, 2.1 mM ammonium persulfate, and 0.24 % N,N,N′,N′-Tetramethylethlyenediamine.

Table 2. Delineation of region on chromosome 13 deleted in the patient reported here. The deletion region was determined on the basis of FISH analysis with BAC clones that map to chromosome 13 and on the basis of analysis of polymorphic markers. The map positions of the BAC clones and the polymorphic markers are based on data in NCBI databases.

BAC (RP11-)	Region	
144H23	21,623 kb–21,730 kb	+/+
90M5	24,392 kb–24,554 kb	+/+
D13S1229	25,470,884–25,471,018 bp	+/+
173P16	25,660 kb–25,774 kb	+/+
D13S260	26,424 kb	+/+
270H22	27,439 kb–27,661 kb	+/+
D13S1493	27,995,410–27,995,639 bp	+/+
37L2	28,112 kb–28,215 kb	+/+
218I21	***28,521 kb–28,689 kb***	***+/–***
307O13	***29,346 kb–29,533 kb***	***+/–***
98D3	***29,998 kb–30,169 kb***	***+/–***
157B21	***30,602 kb–30,678 kb***	***+/–***
D13S218	***33,018,815–33,019,003 bp***	***+/–***
186J16	***33,832 kb–33,917 kb***	***+/–***
D13S325	***37,159,835–37,160,043 bp***	***+/–***
160G19	***37,915 kb–38,058 kb***	***+/–***
D13S291	38,805,645–38,805,891 bp	+/+
71C5	38,908 kb–39,073 kb	+/+
D13S1312	39,919,241–39,919,346 bp	+/+
480G1	40,316 kb–40,427 kb	+/+
106H11	40,575 kb–40,633 kb	+/+
147L20	41,306 kb–41,412 kb	+/+
HTR2A Ex3	41,396,423–41,396,527 bp	+/+
83N23	41,412 kb–41,616 kb	+/+
D13S287	41,876,131–41,876,279 bp	+/+
103K9	47,389 kb–47,576 kb	+/+
109K20	61,007 kb–61,139 kb	+/+
309J13	67,784 kb–67,966 kb	+/+
D13S800	67,869,608–67,869,903 bp	+/+

+/+ Indicates that the BAC clone mapped to both members of the chromosome 13 pair; +/-indicates that the BAC clones hybridized to only one member of the chromosome 13 pair (bold italies). In the case of polymorphic markers +/+ indicates that the child inherited a different allele from each parent; +/- indicates that the child inherited an allele from only one parent (bold italics).

After the amplification was verified, the PCR product was diluted. The dilutions ranged from 1:1 to 1:30, depending on the intensity of the fragment as determined by examining the gel. Approximately 0.1–0.2 ng of diluted PCR product was then added to 2 fM of Applied Biosystems Genescan-500 Tamra internal land standard and 2.0 µl deionized formamide. The mixture was then denatured for 5 min at 94 °C in the thermocyclers mentioned above. Following the denaturing, the samples were immediately placed on ice to prevent renaturation. The samples were then run on the Applied Biosystems 377 sequencer. The gel used for the Sequencer was the Long Ranger Singel from Biowittaker Molecular Applications. The gel was analyzed using Applied Biosystems Genescan and Genotyper programs. We compared the marker fragment sizes of the proband with those of the parents for discrepancies.

Results

Results of molecular cytogenetic studies and analysis of polymorphic DNA markers undertaken in blood samples and cell lines from a child with autism and a deficit in higher level auditory comprehension and expressive language are presented in Tables 2–4 and in Figs. 1, 2.

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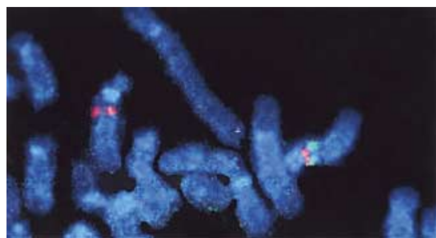

Fig. 1. Results of FISH analysis of metaphase chromosomes. Note that the probe labeled red (BAC RP11-90M5) hybridizes to both chromosomes. The probe labeled green (RP11-307O13) hybridizes to one chromosome only.

FISH studies revealed that six BAC clones assigned to chromosome 13q12→q13, in the region between 28,521 kb and 38,058 kb, hybridized to only one member of the chromosome 13 pair in the patient's cells. Results of these studies are summarized in Table 2. An example of FISH studies on metaphase chromosomes is shown in Fig. 1. This figure illustrates that clone RP11-90M5 (labeled red) hybridizes to both members of the chromosome 13 pair. Clone RP11-307O13 (labeled green) hybridizes to only one member of the chromosome 13 pair.

The presence of a deletion on one member of the chromosome 13 pair was confirmed by results of polymorphic marker analysis. Results of analysis of polymorphic markers are summarized in Table 3. ABI tracings of a subset of the markers are illustrated in Fig. 2. The paternal DNA sample is designated 50.101, the maternal sample is 50.102 and their child's sample is 50.201. The child is heterozygous for markers D13S1493 and D13S291. He is hemizygous for the marker D13S218 which maps at approximately 33,019 kb and he was also hemizygous for D13S325 which maps at approximately 37,160 kb. For both markers the child failed to inherit a paternal allele. As may be seen from Table 3 the child inherited both maternal and paternal alleles for markers at other locations on chromosome 13.

We utilized information in NCBI databases to determine a list of genes that map within the region we showed to be deleted. These genes are listed in Table 4. We considered four of the genes in this region to be of particular interest since they are expressed in brain and play a role in brain development. These genes are: NBEA, MAB21L1 (which maps within NBEA), DCAMKL1 (which contains a doublecortin domain) and MADH9.

Table 3. Results of chromosome 13 polymorphic marker analysis, the allele size of the markers in the father (50.101) mother (50.102) and child (50.201) are provided

	50.101	50.102	50.201	Conclusion	Position
D13S217	160.7 168.8	160.5 168.3	168.6	Inconclusive	23,352 kb
D13S1229	?	?	134.8 144.3	Heterozygous	25,471 kb
D13S260	167.0 172.6	159.3 172.6	159.3 172.6	Heterozygous	26,424 kb
D13S1493	230.6 238.6	230.5 234.6	234.6 238.5	Heterozygous	27,996 kb
D13S1293	123.6 127.6	123.6 131.5	123.6	Inconclusive	28,725 kb
D13S218	190.3	190.3 194.2	194.2	Hemizygous	33,019 kb
D13S325	212.1 224.7	217.0 221.2	217.2	Hemizygous	37,160 kb
D13S1227	174.2	174.2	173.9	Inconclusive	37,530 kb
D13S291	244.9 248.8	241.1 244.9	244.9 248.8	Heterozygous	38,806 kb
D13S1272	250.6	252.4 254.2	250.6 254.3	Heterozygous	39,072 kb
D13S1312	129.8	123.8	123.6 129.6	Heterozygous	39,919 kb
HTR2A	A/A	G/G	A/G	Heterozygous	41,396 kb
D13S161	92.6	87.4 92.6	92.5	Inconclusive	41,771 kb
D13S287	148.0 149.8	147.9 151.6	147.5 151.3	Heterozygous	41,876 kb
D13S1317	234.5 236.5	222.6 234.6	234.6 236.8	Heterozygous	61,060 kb
D13S800	298.7 302.9	293.7 297.8	298.6 302.7	Heterozygous	67,870 kb

Table 4. Genes that map within the deletion region on chromosome 13q that is deleted in the subject with autism and auditory processing deficits reported here

Gene locus [a]	Gene function	Map location in kb
NBEA	Neuron specific protein kinase A anchor protein	29,500–30,230
MAB21L	*C.elegans* neural development protein homolog	within NBEA
DCAMKL1	Doublecortin and Calmodulin kinase domains	30,330–30,690
SPG20	Mutant in spastic paraplegia	30,860–30,905
CCNA1	Role in cell cycle	30,995–31,000
RFXAP	Transcription factor	31,380 –31,385
MADH9	Member of SMAD family of proteins	31,410–31,480
ALG5	Dolichol phosphate glucosyl transferase	31,510–31,560
OIP2	Transcription factor	31,560–31,570
P38IP	Transcription factor	31,570–31,620
OSF2	Osteoblast specific factor	32,120–32,155
TRPC4	Transient receptor potential channel	32,200–32,430
STOML3	Stomatin- like membrane protein	33,530–33,550
LHFP	Lipoma HMGIC fusion partner	33,905–34,160
COG6	Component of oligomeric golgi complex	34,220–34,250
FOXO1A	Forkhead transcription factor	35120–35,220
MRPS31	Mitochondrial ribosomal protein	35,290–35,332
SLC25A15	Solute carrier, ornithine transporter	35,350–35,220
ELF1	ETS domain transcription factor	35,490–35,620
WBP4	WW domain forming binding protein	35,622–35,644
MTRF1	Mitochondrial translation release protein	35,775–35,824
RGC32	Cell cycle regulator	36,018–36,032
KIAA0564	Protein with unknown function	36,127–36,522
AKAP11	Protein kinase A anchor protein	36,832–36,884
TNFSF11	Tumor necrosis factor ligand 11	37,132–37,168
EPSTI1	Epithelial stromal interaction protein	37,448–37,553
MCJ	Contains DNAJ domain	37,584–37,668

[a] Gene symbols are from HUGO nomenclature database and NCBI Locus Link database.

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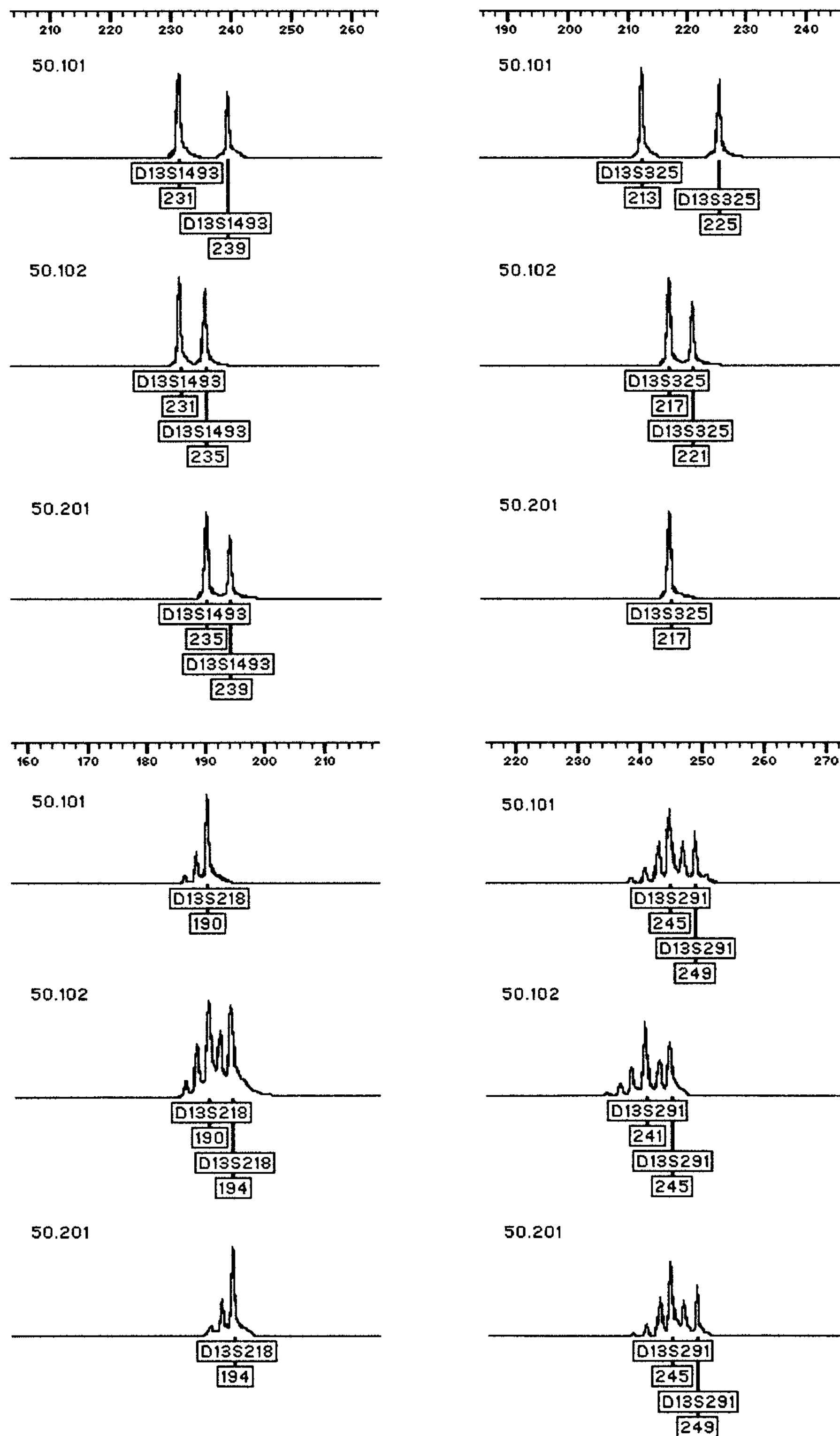

Fig. 2. Genotype profiles of polymorphic markers. Note that for markers D13S1493 (top left) and D13S291 (bottom right) the proband (50.201) inherited an allele from both the father (50.101) and the mother (50.102). For markers D13S218 and D13S325 the proband (50.201) failed to inherit a marker from father (50.101).

Discussion

We report the presence of a chromosomal deletion in 13q that includes the distal third of band 13q12 and the proximal two thirds of band 13q13, a region corresponding to the region extending from 28.5 Mb to 38.06 Mb. The question arises whether hemizygosity for specific genes on chromosome 13 contributes to or is sufficient to lead to autism and language deficits. In reviewing consequences of haplo-insufficiency Fisher and Scambler (1994) proposed that some developmental pathways are particularly susceptible to dosage effects because of exquisite sensitivity to levels of critical proteins. They also

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postulated that proteins involved in the assembly of intermolecular multi-subunit complexes in which subunit stoichiometry is important, would be deleteriously affected by hemizygosity. They proposed further that the level of expression of the non-deleted gene may influence the phenotype.

Hemizygous deletions are particularly deleterious when they occur in chromosomal regions that are imprinted, e.g. the Angelman region on chromosome 15q (Clayton-Smith and Laan, 2003). If, for example, maternal genes in the 13q12→q13 region are silenced through imprinting, the deletion of the paternally derived 13q12→q13 region would be particularly harmful. Evidence for imprinting in the proximal region of 13q was reported by Demenais et al. (2001) and by Velagaleti et al. (2001).

The 9-Mb region deleted in the patient described here contains at least four genes that are expressed in brain and that play a role in brain development. They are NBEA, MAB21L1 and DCAMKL1 and MADH9. These genes therefore represent candidate genes for autism and specific language deficits.

Neurobeachin (NBEA) is a neuron-specific multi-domain protein with a high-affinity binding site for a regulatory subunit of protein kinase A, (Wang et al., 2000). It contains a BEACH WD 40 sequence domain in its C terminal region. At least ten distinct mammalian proteins with BEACH WD40 domains have been identified. Neurobeachin is found throughout the cytoplasm of the neuronal cell body and neuron processes. It also occurs in a sub-population of synapses specifically at the post-synaptic plasma membrane. Neurobeachin functions as a protein kinase anchor protein AKAP. The anchoring of protein kinase to specific sites is associated with many transfer processes including hormone secretion and modulation of neurotransmitter receptors and ion channels. Wang et al. (2000) postulate that neurobeachin plays a role in post-Golgi neuronal membrane trafficking. Recent studies on the domain structure of neurobeachin crystals have led to the identification of plekstrin type domains. Neurobeachin EST's in Unigene HS3821 are from neuronal and endocrine tissues. At the protein level Wang et al. (2000) detected neurobeachin only in brain lysates, including forebrain, cerebellum and brainstem. They postulated that the low level neurobeachin mRNA in endocrine tissue is not translated into protein. Developmental changes in neurobeachin expression have been documented in mouse brain. Expression is highest in neonatal brain and in adult brain levels are 50% of those in neonatal brain.

A gene designated MAB21L1 is contained within the neurobeachin gene. This gene is homologous to a *C. elegans* gene *Mab21* that plays a role in neural development. The MAB21L1 gene was reported with the designation CAGR by Margolis et al. (1999). Within MAB21L1 there is a CAG repeat that is unstable and undergoes repeat expansion. There are no definitive reports of neurological diseases associated with expansion of this gene. Wong et al. (1999) analyzed *Mab21* gene expression in mouse embryos. They reported that both *Mab21* and *Mab21L1* genes are expressed in overlapping domains at all stages of embryogenesis. *Mab21* gene expression was first detected in the neuroepithelium of the cephalic neural folds in E8 embryos. In E10–E14.4 embryos both *Mab21* genes were expressed in the encephalic vesicle and in the neural tube. Expression also occurred in the optic tissue, mid-brain branchial arches and limb buds. *Mab21L1* was also found in the otic pits.

DCAMKL1 encodes a brain-specific transmembrane kinase. The N-terminal 345-amino-acid region shows 78% homology to doublecortin. The C-terminal 427-amino-acid region contains two transmembrane domains and is 98% homologous to a calmodulin-dependent kinase-like domain. DCAMKL1 is thought to play a role in cortical development. Several different splice variants of DCAMKL1 occur. Burgess and Reiner (2002) reported that DCAMKL1 splice alternatives are differentially expressed in embryonic and adult brain. One splice variant of this gene is designated CPG16 (candidate plasticity gene 16). Burgess and Reiner (2000) demonstrated that DCAMKL1 (designated DCKLl1 by them) is microtubule associated and that it is expressed in the growth cones of post-mitotic neurons. Vreugdenhil et al. (2001) reported that in hippocampus of adult rats alternate splice products of the *Dcamkl1* (*Dckl1*) gene are expressed. They postulated that these different transcripts play a role in controlling neuronal plasticity.

The doublecortin-encoding gene, DCX, maps to the X chromosome Xq22.3→q23 and encodes doublecortin, a brain-specific putative signaling protein that is mutated in several neuronal migration defects including X-linked lissencephaly and subcortical band heterotopia, (Sossey-Alaoui and Srivastava, 1999, Srivastava et al., 1999). Developmental cortical migration defects have been reported in autism on the basis of neuroradiologic and neuropathologic studies (Piven et al., 1990). Corbo et al. (2002) induced targeted mutations in the DCX gene in mice. They reported that in females heterozygous for DCX mutations and in hemizygous males there was disruption of lamination in the hippocampus. This was most severe in the CA3 region. Behavioral studies indicated that the abnormal cytoarchitecture correlated with defects in context and cued conditioned fear tests.

MADH9 is a member of the SMAD family of proteins that mediate the TGF beta signaling pathway. TGF beta signaling plays a role in proliferation and differentiation of many different cell types. TGF beta signaling pathways have been shown to play a role in differentiation at synaptic junctions (Packard et al., 2003). MADH9 is expressed in brain and in many other tissues.

Chromosome 13 and autism

Ritvo et al. (1988) reported the simultaneous occurrence of autism, sporadic retinoblastoma and reduced esterase D activity and a deletion on chromosome 13. In their patient who met DSMIII and National Society for Autistic children criteria for autism, the red cell esterase D levels were 50% of normal. Chromosomes were analyzed using trypsin-Giemsa banding and a deletion that included band 13q13 and portions of bands 13q12 and 13q14. Esterase D and the retinoblastoma gene (RB) map telomeric to the deletion in our patient. It is of interest to note that the patient with autism that Ritvo described and our patient have deletions of 13q13 and a portion of 13q12.

Steele et al. (2001) described a case of autism with a de novo deletion of 13q13→13q22. They noted that the RB gene was not deleted. It seems likely that the deletion in our patient is

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more centromeric than the deletion described in the autism case reported by Steele et al. (2001).

Bradford et al. (2001) reported the effect of incorporating linkage information and parental structural language phenotypes into analyses in two chromosomal regions where the highest MMLS/het LOD scores were found in their genome screening for autism, namely chromosome 13q and 7q. The results of their updated linkage analyses revealed that two distinct peaks occurred on chromosome 13q. One peak was obtained with marker D13S217, which maps at 23.351 Mb and another peak was obtained with marker D13S800 which maps at 71.843 Mb. D13S217 and D13S800 are the only chromosome 13 markers for which Bradford et al. (2001) reported linkage results. The deletion in the subject we describe here maps between the two chromosome 13-linkage peaks described by Bradford et al. (2001).

In summary, results of cytogenetic and linkage studies reported in the literature and results of studies in our patient, indicate that there may be two autism and language deficit determining loci on chromosome 13q, one at 13q12→13q13 and another at 13q22. Molecular genetic studies of the patient that we report revealed a deletion in the 13q12→q13 region. Four of the loci that map in this region play a role in brain development: these are NBEA, MAB21L1 (which maps within NBEA), DCAMKL1 and MADH9. Further studies in other autistic subjects are required to confirm if one or more of these genes play a role in the etiology of autism.

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