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# Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new markers for deletion screening by PCR

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**Abstract.** We recently studied a patient who meets criteria for autistic disorder and has a 2q37 deletion. Molecular cytogenetic studies were carried out using DNA isolated from 22 different 2q37 mapped BACs to more precisely define the extent of the chromosome deletion. We also analyzed 2q37 mapped polymorphic markers. In addition DNA sequences of BACs in the deletion region were scanned to identify microsatellite repeats. We describe four new polymorphic microsatellite repeat markers in the 2q37.3 region. These markers enabled

us to determine the parental origin of the deletion in our patient. DNA from 8–13 unrelated individuals was used to determine heterozygosity estimates for these markers. We review four genes deleted in our patient – genes whose known functions and sites of expression in the brain and/or bone make them candidates for involvement in autism and/or the osteodystrophy observed in patients with 2q37.3 deletions.

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During the past decade, the increasing study of autism has clarified the spectrum of autistic abnormalities and emphasized the importance of early recognition and therapeutic intervention (Filipek et al., 2000). These advances have been accompanied by the development of standardized testing. Current information indicates that autism spectrum disorders occur with a frequency of 1 in 150 individuals. The most consistent brain abnormalities involve the structures of the limbic system, especially the amygdala (Aylward et al., 1999; Baron-Cohen et al., 2000; Howard et al., 2000) and the cerebellum (Saitoh and Courchesne, 1998). Twin studies provide significant evidence that genetic factors play a role in autism (Ritvo et al., 1985; Bailey et al., 1995). Available evidence from cytogen-

etic studies and linkage analysis indicates that autism is genetically heterogeneous. Genetic heterogeneity in autism complicates mapping of underlying loci by linkage analysis.

Patients with chromosome abnormalities, particularly abnormalities that result in gene loss or gene disruption, provide an important resource for identification of regions of the genome containing genes involved in autism. A number of different cytogenetic abnormalities have been reported in autistic individuals. These include defects in chromosomes 15 and 7 (Cook et al., 1997; Bass et al., 2000; Smith et al., 2000; Ashley-Koch et al., 2000; Warburton et al., 2000). Autistic features may also occur in patients with fragile X mental retardation (Gurling et al., 1997). There are nine published cases of autistic disorder or autistic type behaviors in patients with cytogenetic abnormalities involving chromosome 2q37 (Burd et al., 1988; Stein et al., 1992; Conrad et al., 1995; Ghaziuddin and Burmeister, 1999; Borg et al., 2000; Wolff et al., 2000). In these cases the size of the deletion was not determined by molecular methods.

We recently studied a patient who meets criteria for autism disorder and has a deletion in the 2q37 region on one member of the chromosome 2 pair, detected in routine cytogenetic studies. Based on chromosome banding studies it was not possible

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to determine which of the three sub-bands in the chromosome 2q37 region were deleted. Given the current availability of mapping and sequence information resulting from the Human Genome Project, we undertook molecular cytogenetic studies and analysis of genetic markers in this patient to more precisely define the extent of the chromosome deletion. Here we report clinical findings, results of psychometric evaluation and molecular genetic studies. We obtained 22 BAC clones that map to chromosome 2q37. These BACs were used in fluorescence in situ hybridization (FISH) analysis of chromosomes. DNA sequences of BACs in the deletion region were scanned to identify microsatellite repeats. We describe four new polymorphic microsatellite repeat markers in the 2q37.3 region. These markers enabled us to determine the parental origin of the deletion in our patient. Heterozygosity estimates for these markers are given, based on analysis of DNA from between 8 and 13 unrelated individuals.

We combine this information with a review of previous accounts of deletions on 2q37.3. We review four genes deleted in our patient – genes whose known functions and sites of expression in the brain and/or bone make them candidates for involvement in autism and/or the osteodystrophy observed in patients with 2q37.3 chromosome deletions.

## Materials and methods

### Case report

Clinical features included prominent forehead, deep set eyes, low nasal bridge, narrow palpebral fissures, bulbous nasal tip, hypoplastic alae nasae, prominent columella, high arched palate, bifid uvula. The patient's hands revealed brachymetaphalangism. The fourth metacarpal bones were shortened, the index finger was the longest finger, and thumbnails were short and broad. The feet were small, with brachymetaphalangism of the third, fourth and fifth metatarsals. Weight was at the 10th percentile for age. Height was well below the 3rd percentile and head circumference was at the 10th percentile for age.

*Developmental history and psychometric analysis:* The parents noticed a lack of eye contact during infancy. The patient walked at 39 months and single words were heard at 48 months of age. Acquisition of bowel and bladder control occurred at 60 months. Imitative and imaginative play, instrumental gestures and pointing were absent before age 5. There was little response to social or verbal gestures from others. Fine motor skills were severely impaired. School testing indicated mental retardation. However, she began to make rapid catch-up cognitive progress after learning to type her responses with one finger at age 13. Although she presently has a limited spoken vocabulary consisting of single words and largely ungrammatical phrases, she can type full sentences that are complex and grammatically correct. She graduated from regular high school classes and currently, is a sophomore at a four-year college. She seldom spontaneously offers social or emotional information, she can however respond by typing short responses to most questions about her feelings. Fine motor skills remain severely impaired for writing and most daily living tasks. The diagnostic criteria (DSM-IV) for Autistic Disorder were met in an evaluation using the Autism Diagnostic Observation Schedule-Generic (Lord et al., 1989) and the Autism Diagnostic Interview (Lord et al., 1994) (Tables 1 and 2).

*Cognitive function:* Because of fine motor limitations, only verbal tasks and multiple choice questions could be administered to test current cognitive functioning. She obtained a Verbal Reasoning Standard Age Score of 107 (Average Range) by typing answers to questions from the Stanford-Binet: Fourth Edition instrument (Thorndike and Hagan, 1986). She obtained a Standard Score of 98 (Average Range) at the 45th percentile relative to the normative sample of age-mates on the Peabody Picture Vocabulary Test Third edition instrument (Dunn, 1997).

**Table 1.** ADOS-G (Module 4) scores

Categories	Patient score	Autism cutoff <sup>a</sup>
Qualitative impairments in social interaction	5	3
Impairment of communication	9	6
Social + communication impairments	14	10

<sup>a</sup> Note that scores at or above cutoff indicate autism.

**Table 2.** ADI scores

Categories	Patient score	Autism cutoff <sup>a</sup>
Qualitative impairment of reciprocal social interaction	30	10
Impairment of communication	23	8
Repetitive behaviors	7	3
Abnormal development evident before 36 months	5	1

<sup>a</sup> Note that scores at or above cutoff indicate autism.

### Molecular cytogenetic studies

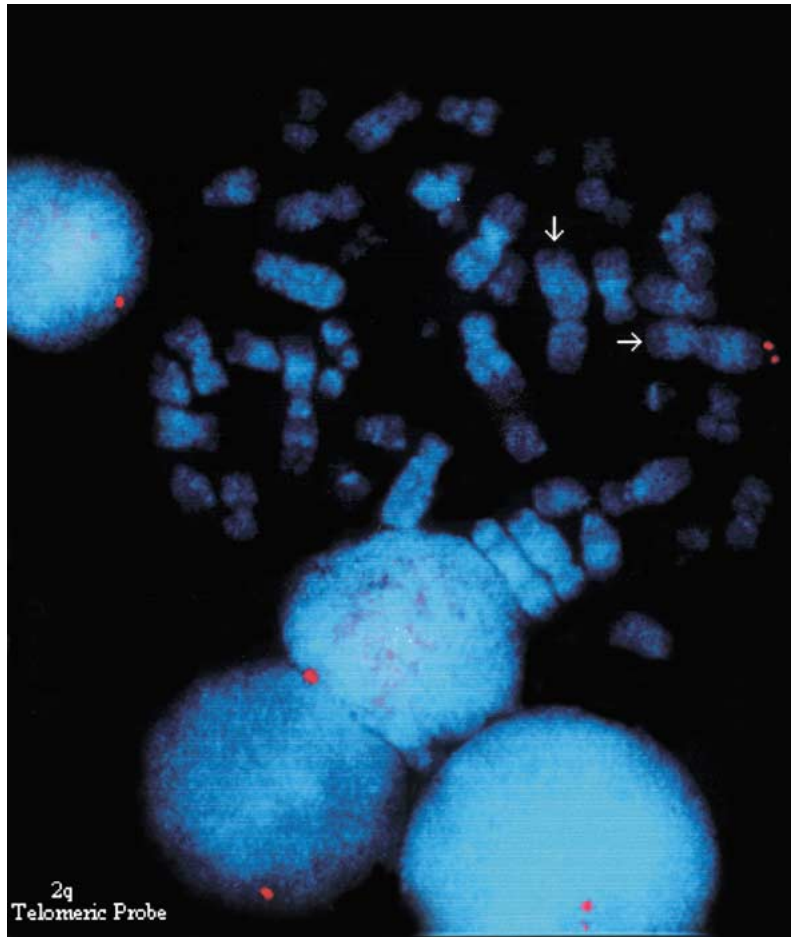
White blood cells and cultured lymphoblastoid cell lines from the patient, her parents and her brother, were used to produce slides with spreads of metaphase chromosomes and interphase nuclei. These were then reacted with a Spectrum orange dUTP labeled 2q telomeric probe (Vysis). Slides were examined using fluorescence microscopy (Fig. 1). Chromosome preparations from the patient and her parents were examined using FISH with a Spectrum orange dUTP labeled chromosome 2 painting probe (Vysis).

We utilized information from the Human Genome Project as archived on the NCBI website (<http://www.ncbi.nlm.nih.gov>), to identify a series of linearly ordered BAC clones on chromosome 2q37. BAC clones were ordered from Research Genetics. BAC clone preparations were plated out on agar plates and single colonies of each specific BAC were isolated and grown overnight in liquid culture medium. DNA was extracted from cultured BAC clones using alkaline lysis and the procedure recommended by Research Genetics. DNA from individual BAC clones was labeled using Spectrum Green dUTP (Vysis™). Labeled BAC clone DNA was ethanol precipitated along with Cot 1 human DNA to block repetitive sequences. This was then used in FISH studies on metaphase chromosomes and interphase nuclei from the patient's peripheral blood lymphocytes and cultured cells (Fig. 2). Hybridization and post-hybridization washing of slides was carried out according to the dUTP spectrum green manufacturer's protocols (Vysis).

### Analysis of polymorphic markers

We analyzed marker D2S140, defined as the most telomeric polymorphic marker on chromosome 2q37 using primer sequences defined in the Marshfield database (<http://research.marshfieldclinic.org/genetics>). We scanned the DNA sequence of contigs mapped to chromosome 2q37 to identify dinucleotide repeats that could serve as polymorphic markers in this region. Sequences flanking the repeats were used to design primers for amplifying the repeat containing segment. Primer sequences were entered into BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) to determine that they were unique and, based on current sequence information, the polymorphism detected with each primer set would be limited to one locus in the genome. Table 4 contains information on the chromosome 2q37.3 mapped BAC clones containing dinucleotide repeats that proved to be polymorphic. The D2S140 polymorphism maps within the BAC AC011298. We have identified a polymorphism that maps to a different region of BAC AC011298. We also examined a dinucleotide repeat within the most telomeric 2q37.3 BAC clone AC084227. This repeat showed a very low level of polymorphism and was not included in further analyses. In Table 4 we indicate the sequence of

**Fig. 1.** FISH analysis of metaphase chromosomes using a 2q telomeric probe (Vysis). The two members of the chromosome 2 pair are indicated with arrows. Note that the probe hybridizes to only one member of this pair. In interphase nuclei there is only one probe signal.



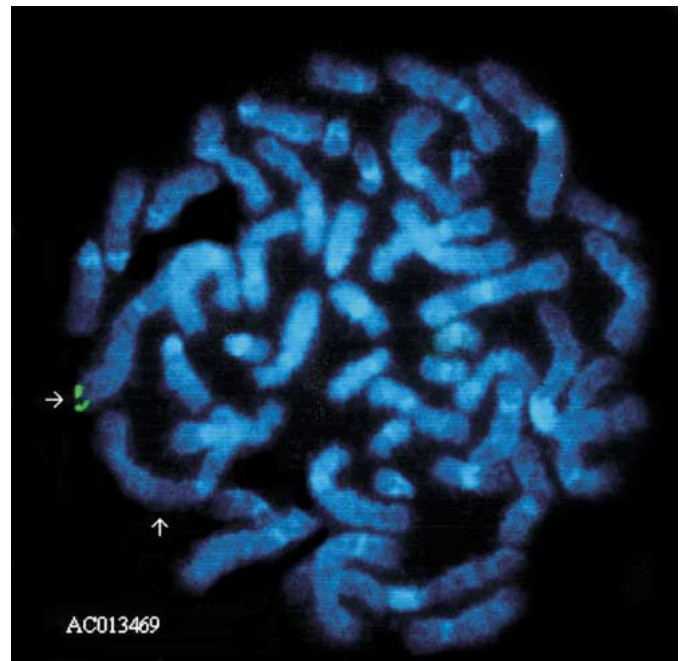
the primers used to examine polymorphisms. The 5' primer in each primer set was fluorescein labeled and PCR products were generated from genomic DNA. These PCR products were then electrophoresed on an ALF Pharmacia electrophoresis system with laser detection. The gel peaks representing different alleles were compared to labeled size standards to determine the allele sizes.

## Results

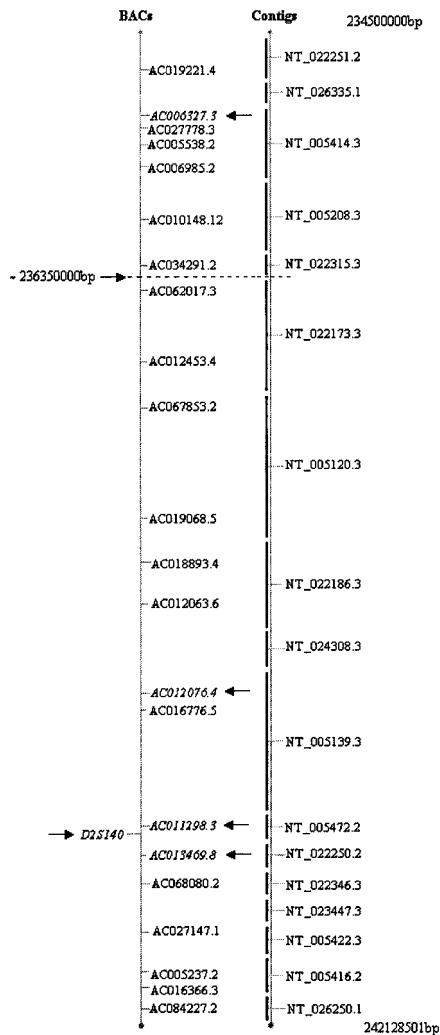
### *Cytogenetic studies*

In slides from the patient a 2q telomeric probe signal was observed on only one member of the chromosome 2 pair (Fig. 1). There was no evidence that the 2q telomeric region was translocated to another chromosome. The parents and the patient's sibling showed no evidence of chromosome 2q37.3 deletion. The chromosome 2 painting probe (Vysis) revealed no evidence for a chromosome 2 translocation in the patient or in her parents.

Results of our studies using 22 BAC clones in FISH experiments are summarized in Fig. 3 and Table 3. DNA of each of the BAC clones used yielded unique signal in the 2q37.3 region after blocking with Cot 1 human DNA. Based on our analyses using 22 BAC clones and the current human genome map, the proband is deleted for approximately 5 MB on chromosome 2q37.3 (see Fig. 3, Table 3).



**Fig. 2.** FISH analysis of metaphase chromosomes using DNA from BAC clone AC013469 labeled with Spectrum green dUTP (Vysis). The two members of the chromosome 2 pair are indicated with arrows. Note that the probe hybridizes to only one member of this pair.



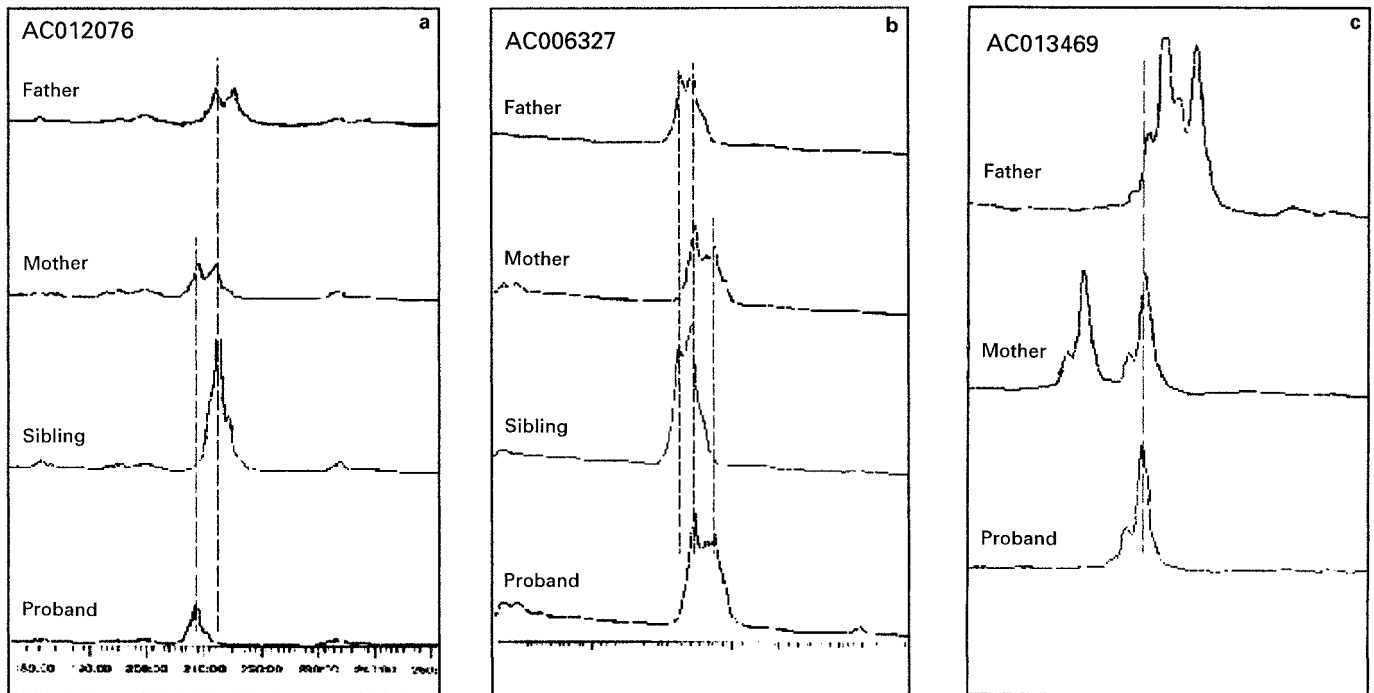
### Studies with newly identified polymorphic probes

These results are summarized in Table 4. The range of allele sizes found in analysis of DNA from 8–13 unrelated individuals is indicated in Table 4. Figures 4a, b and c illustrate laser scans obtained from electrophoresis of the AC012076, AC006327 and the AC013469 dinucleotide repeat PCR products, respectively. The family described here proved to be informative for three of the new dinucleotide repeat markers in the 2q37.3 region. For two of these markers, one corresponding to sequence in BAC AC012076 and another in BAC AC013469, the patient did not inherit a paternal allele. These BAC clones were also shown to be deleted from one member of the chromosome 2 pair by FISH. Taken together these results indicate that the deletion in our patient arose on the paternally derived chromosome. The polymorphism in marker AC006327 was informative in this family. The patient inherited a different sized allele from each parent and this indicated that the 2q37.3 deletion occurred below this marker. The map positions of the four new polymorphic markers are illustrated in Fig. 3. To determine the informativeness of the newly identified polymorphisms, DNA samples from between 8 and 13 unrelated individuals were typed and allele frequencies calculated. In Table 4 we list the expected heterozygosity for each marker (assuming

**Fig. 3.** Linear order in the 2q37.3 region of contigs and the BAC clones analyzed to determine extent of deletion in our patient. BAC clones and contigs shown above the dotted line at 236.35 MB were present on both members of the chromosome 2 pair. BAC clones shown below the dotted line (between 236.35 MB and the end of the chromosome 242.128 MB) were present on only one member of the chromosome 2 pair in our patient. In the DNA sequence of BAC clones marked with arrows we identified dinucleotide repeats which exhibited polymorphisms.

**Table 3.** Sequenced contigs and genes in the 2q37.3 region deleted in our patient with autistic disorder

Contig accession #	BACS analyzed within contig	Genes within contig	Description
NT 022713	AC062017, AC012453	HDAC4	HDAC4 - histone deacetylase 4,
NT 005120	AC067853, AC019065	KIAA1099, FLJ22527	KIAA1099 - KIAA1099 protein, FLJ22527 - hypothetical protein FLJ22527
NT 022186	AC018893, AC012063	COP9	COP9 - COP9 homolog
NT 024308		MGC2771, FLJ12538, LOC51052, LOC65621, LOC82737	MGC2771 - hypothetical protein MGC2771, FLJ12538 - hypothetical protein FLJ12538 similar to ras-related protein RAB17, LOC51052 - preproprolactin-releasing peptide, LOC65621 - similar to collagen, type VI, alpha 3 ( <i>H. sapiens</i> ), LOC82737 - hypothetical gene supported by NM_004369
NT 005139	AC012076, AC016776	LRRFIP1, SCLY, RAMP1, LOC82431, HES6, PER2, ASB1	LRRFIP1 - leucine rich repeat (in FLII) interacting protein 1, SCLY- putative selenocysteine lyase, RAMP1 - receptor (calcitonin) activity modifying protein 1, LOC82431 - similar to TAR DNA binding protein; TAR DNA-binding protein-43 ( <i>H. sapiens</i> ), HES6 - hypothetical protein HES6, PER2 - period ( <i>Drosophila</i> ) homolog 2, ASB1 - ASB-1 protein
NT 005472	AC011298	ATSV	ATSV - axonal transport of synaptic vesicles
NT 022250	AC013469	NDUFA10	NDUFA10 - NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10 (42 kDa)
NT 022346	AC068080	GCP1	GCP1 - glypican 1
NT_023447		none	
NT 005422	AC027147	STK25, KIAA0793, NEDD5	STK25 - serine/threonine kinase 25 (Ste20, yeast homolog), KIAA0793 - KIAA0793 gene product, NEDD5 - neural precursor cell expressed, developmentally down-regulated 5
NT 005416	AC005237, AC016366	KIAA0135, PPP1R7, PRO2900, HDLBP	KIAA0135 - KIAA0135 protein, PPP1R7 - protein phosphatase 1, regulatory subunit 7, PRO2900 - hypothetical protein PRO2900, HDLBP - high-density lipoprotein binding protein (vigilin)
NT 026250	AC084227	none	



**Fig. 4.** (a) Electrophoresis profile of PCR product of microsatellite repeat polymorphisms. Profile of polymorphism in BAC AC012076. Note that the proband failed to inherit a paternal allele. This locus therefore maps within the 2q37.3 deletion region. (b) Electrophoresis profile of microsatellite polymorphism in BAC AC006327. Note that the proband inherited a different sized allele from each parent. This locus therefore maps outside the 2q37.3 deletion region. (c) Electrophoresis profile of microsatellite polymorphism in BAC AC013469. Note that the proband failed to inherit a paternal allele.

**Table 4.** New microsatellite repeat polymorphisms identified in the 2q37.3 region

Marker <sup>a</sup>	Primer sequence	Result in proband	Range of allele sizes	Expected heterozygosity	Frequency of most common allele	Size of most common allele
AC006327	F-TTAACTCTCTCCCCGACAA R-TAAGCAGGCAAAGGGAGAAA	Not deleted	205–215 bp	0.73	0.42	209 bp
AC012076	F-AACCACGGTACCACTCACA R-GTTGCAGTGAGCCAAGATCC	Deleted	212–223 bp	0.73	0.41	218 bp
AC011298	F-TGAATGAGGTCACCCTCTCC R-TTTCCTCAAGCACCAACCTAA	Uninformative	200–212 bp	0.77	0.38	201 bp
AC013469	F-GCCATTCTAGTCCTCTGTCTCA R-AAAAATAGCTGGGCGTGGT	Deleted	320–335 bp	0.88	0.17	327, 329 bp

<sup>a</sup> Note that markers are given the designation of the BAC clone in which they occurred.

Hardy-Weinberg equilibrium) and the frequency and size of the most commonly observed allele.

#### *Genes in the deletion region*

Examination of the Genome resources Website at NCBI reveals that the 5-Mb region between the chromosome 2 telomere and BAC AC062017 the most centromeric BAC that is deleted in our patient, is covered in ten sequenced contigs. Within these contigs there are 15 genes, sequences corresponding to three ESTs (expressed sequence clones) and eight hypothetical proteins. It is important to note that currently there are gaps of unknown length between contigs of sequence in this

region. We cannot rule out the possibility that there are additional genes. Among the 15 known genes, three are expressed in regions of the brain affected in autism and, based on what is currently known about their functions, can be considered as candidate genes for autism: Glypican 1, Vigilin, a gene designated as axonal transporter of synaptic vesicles (ATSV). In addition there is approximately 1182 bp of sequence that is homologous to the homeobox gene, GBX2, Genbank Locus link ID 2637. The GBX2 gene is expressed in brain. Glypican 1 and Vigilin are also abundantly expressed in skeletal tissue including bone and can be considered as candidate genes for the osteodystrophy observed in patients with 2q37.3 deletions.

## Discussion

Nine patients with autism and chromosome 2q37 deletions have been described in the literature. Combining the clinical features in these patients and in our patient, autistic features include difficulties in communication and social interactions, repetitive stereotypic behaviors, and developmental delay. Physical abnormalities include prominent brow in eight of the ten patients, and deep set eyes and low nasal bridge in six patients. Six of the ten patients were shorter than average and their weight was disproportionately high compared to height. In six of the ten patients, head circumference was larger than expected based on height and weight. Five of the ten patients were described as hypotonic and two of the ten had brachymetaphalangism with shortening of the fourth and fifth fingers and fourth and fifth toes and abnormal spacing between fingers and toes.

The exact extent of the chromosome deletions was not determined in the nine patients with autism and 2q37 deletions previously described in the literature. It is possible that the extent of the deletion varies among the different patients. All of the patients have autism. It would therefore be important to determine the shortest region of overlap of the different deletions and to use sequence information to identify genes within that region.

Four patients with 2q37.3 deletion were reported to have a phenotype described as McCune Albright osteodystrophy-like syndrome and developmental delay (Wilson et al., 1995). It is not clear from the published report whether or not the patients had autistic behaviors. These patients were found to be normal for the Gs-alpha protein which is defective in McCune Albright osteodystrophy and which is encoded by a gene on chromosome 20 (Wilson et al., 1995). Four additional patients with a small deletion in 2q37.3 and clinical phenotype similar to that of McCune Albright osteodystrophy have been described (Phelan et al., 1995). It is of particular interest that the Glypican 1 gene and the Vigilin gene that map within the terminal 2q37.3 region are known to be abundantly expressed in skeletal tissues.

On the basis of the findings in our patient and in patients reported in the literature, we hypothesize that deletions of human chromosome 2q37.3 lead to a contiguous gene syndrome. The largest deletions are associated with autism and a number of other developmental abnormalities. These include mild facial dysmorphology and skull abnormalities (frontal bossing), linear growth retardation and hypotonia. Osteodystrophy represents part of the phenotype of the contiguous gene syndrome associated with deletions on chromosome 2q37.3. Smaller deletions within this region can be expected to show a more limited number of abnormalities.

There is a growing body of evidence indicating that cryptic telomeric rearrangements play an important role in the etiology of mental retardation. Subtle subtelomeric abnormalities in mentally retarded subjects have been demonstrated by fluorescence in situ hybridization (Knight et al., 1999) and through use of subtelomeric polymorphic markers (Slavotinek et al., 1999). In a pilot study of ten autistic patients one patient with a telomeric deletion (2q37.3 deletion) was found (Wolff et al., 2000). It is clear that a more comprehensive analysis of patients with autism should be undertaken to determine if sub-telomeric

deletions occur with higher frequency in this population than in normal individuals. Such screening could be carried out using telomeric BAC clones described here. The polymorphic markers that we identified will also be useful for screening for deletions by PCR.

In considering the potential candidacy for involvement in autism of genes that map in 2q37.3, we have concentrated on genes that are known to be abundantly expressed in regions of the brain that are reported to be abnormal in autism, particularly genes that appear to play a role in brain development. Aylward et al. (1999) concluded on the basis of MRI findings in conjunction with neuro-histopathology that in their autistic patients there was underdevelopment of the neural connections between limbic structures, including amygdala and hippocampus, and other parts of the brain, particularly the cerebral cortex. Developmental malformations of the amygdala are postulated to underlie the social-cognitive impairments characteristic of high functioning autism. The amygdala malformation may reflect incomplete neuronal pruning in early development (Howard et al., 2000). Functional MRI studies in patients with autism have revealed deficits in the amygdala response (Baron-Cohen et al., 2000). Cerebellar changes in autism have also been described (Saitoh and Courchesne, 1998).

In evaluating the four genes that map in the 2q37.3 region and are abundantly expressed in brain, glypican 1 is perhaps the most likely candidate gene for autism. Glypican 1 (Gpc1) is a 558-amino acid protein that is encoded by nine exons; it is one of six homologous cell surface heparan sulfate proteoglycans (HSPGs) in mammals (Lander et al., 1996). Glypicans are thought to act as co-receptors for growth factors and other cell-cell signaling molecules (Lander et al., 2000). Glypican 1 is a major proteoglycan of the developing brain, a number of studies indicate that although it is expressed by most neurons during early development, with time it becomes particularly prominent in structures of the limbic system (amygdala, hippocampus, parts of the cortex), thalamus, and cerebellum (Litwack et al., 1994, 1998; Karthikeyan et al., 1994). It is interesting to note that in autism neuroanatomical and functional studies have correlated abnormalities in the human limbic system (especially amygdala) and cerebellum with autism. In the rodent glypican 1 is expressed throughout development; after the first postnatal week or so the expression of a potentially compensatory homolog (glypican 2) disappears (Stipp et al., 1994, Ivans et al., 1997). Thus, it might be expected that the neuropathology associated with a loss of glypican 1 function may not have onset at birth.

It is interesting to note that glypican 1 is abundantly expressed in skeletal tissues. Deletion or disruption of this gene may play a role in the osteodystrophy that occurred in our patients and which has also been described in other patients with 2q37.3 deletions.

We consider Vigilin as a candidate gene for autism based on its map position in the terminal portion of 2q37 and based on its structure and potential function (Plenz et al., 1994). Both Vigilin and FMR1 protein contain KH domains that appear to be involved in RNA binding and transport from the nucleus to the cytoplasm (Kanamori et al., 1998). Vigilin is expressed in many tissues including brain. Molecular genetic studies have

shown that mutations in the KH domains of the FMR1 lead to fragile X mental retardation (Siomi et al., 1994; Musco et al., 1996). Intact function of the KH domain is therefore key to the prevention of fragile X mental retardation, a condition that is associated with autism. Most commonly FMR1 product is deficient in cases of fragile X mental retardation because triplet repeat expansion in the 5' gene region interferes with gene transcription (Bardoni et al., 2000). It is not clear why mental retardation with or without autism results from deficiency of FMR1 protein. Vigilin is abundantly expressed in cartilage and bone (Plenz et al., 1993) and it is therefore a candidate gene for the osteodystrophy observed in patients with 2q37.3 deletions.

The gene encoding axonal transporter of synaptic vesicles protein (ATSV), maps in the terminal 2q37.3 region. Sequence corresponding to this gene is present in BAC AC011298 (contig NT\_005472), which is deleted from one member of the chromosome 2 pair in our patient. The protein encoded by the ATSV gene is an axonal motor protein, a member of the kinesin family, and has close homology with the murine Kif1a protein (Locus link NCBI NLM.) Studies in mice who are deficient for the Kif1a protein revealed that Kif1a mediated axonal transport plays a critical role in the viability, maintenance and function of neurons, particularly mature neurons (Yonekawa et al., 1998).

A fourth gene that may be considered as an autism candidate gene is the gastrulation homeobox gene, GBX2. Sequence corresponding to the distal half of this gene is present in contig NT\_005120 that lies within the region deleted in our patient. We have not yet been able to identify sequenced BACs in GenBank that contain the proximal (5') 943 base pairs of this gene. It is possible that this gene region is encoded by sequence that lies some distance from the region that encodes the 3' half of GBX2. Alternately it is possible that the sequence in contig NT\_005120 does not represent the true GBX2 gene. Homeobox genes are important to consider as candidate genes for autism based on their role in brain development (Rodier, 2000).

The question arises: by what mechanisms could the hemizygous deletion predispose to autism? It is possible that haploinsufficiency for one or more genes within the region is sufficient. Or, that deletion of a key gene on one chromosome uncovers the presence of mutant allele in the homologous gene on the other member of the chromosome pair. In this scenario the patient has no active gene product. The 2q37.3 deletion may only cause autism if it occurs in a specific patient along with a mutation at one or more autism predisposing gene loci elsewhere in the genome. If the deletion is not sufficient to cause autism we could expect to encounter individuals who do not manifest the autistic phenotype but have deletions that are identical in extent to the 2q37.3 deletions in autistic individuals.

In considering these questions it is important to take into account recent theories about autism. One currently held theory states that in each case of autism several genes are mutated or altered and the autistic phenotype is due to the cumulative effect of changes at several gene loci (Pickles et al., 1995; Risch et al., 1999). On the basis of autism recurrence risks in families, a model with three interacting loci was proposed (Pickles et al., 1995). Analysis of allele sharing of markers in affected members of multiplex families with autistic disorder led to the pro-

posal that there is multigenic inheritance of autism with allele sharing at 15 susceptibility loci (Risch et al., 1999).

Given the results of linkage studies and the finding of autism in patients with different deletions of different chromosome regions, it seems likely that autism is due to defects in a number of different genes in different regions of the genome. The question then arises as to whether or not these different genes share homology or are functionally related in a common pathway. It is also possible that different autism predisposing genes are only functionally related in so far that they affect development of the same specific region of the brain e.g. the amygdala, the hippocampus and their projections to the cortex. Perhaps certain genetic changes e.g. deletions of specific chromosome regions, loss of function mutations in critical genes, lead to severe disruption of development and autistic disorder without the presence of additional mutations of autism predisposing genes elsewhere in the genome.

In a recent paper, evidence for location of an autism susceptibility gene on chromosome 2q was presented based on linkage analysis in multiplex families (Buxbaum et al., 2000). Maximum LOD scores were observed with markers D2S364 and D2S335. These markers map on chromosome 2q at 188 and 175 cM respectively. The 2q37.3 deletion region that we identified in our patient corresponds to a region of the genetic map between 240 and 269 cM.

The genetic heterogeneity in autism and the scarcity of large families with multiple members affected with autism complicates fine mapping of autism based on linkage analysis alone. Patients with chromosome abnormalities, especially those abnormalities that result in gene deletion through loss or through interruption of a gene region by translocation, may provide a unique resource for identification of regions of the genome that are important in the etiology of autism. Depending on which particular gene region is involved and which genes are deleted or interrupted, autism may be only one of the manifestations of the phenotype. Fine mapping of the deletion regions in patients with autism is important for identifying candidate genes for autism. In subsequent studies it will be important to search for mutations in those candidate genes in patients with autism who do not have chromosome abnormalities.

We identified four new polymorphic markers in the 2q37.3 deletion region. These markers may serve as a screening tool to detect deletions. Also it is possible that in patients without 2q37.3 deletions, examination of polymorphic markers may be informative since specific alleles of these markers may be in linkage disequilibrium with the autism phenotype. The polymorphisms that we have characterized in the 2q37.3 region are also useful in defining the parent of origin of the deletion. Defining the parent of origin of a deletion may be particularly important if there is evidence for imprinting in a specific chromosome region.

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