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Research Letter

Description of a Case of Distal 2p Trisomy by Array-Based Comparative Genomic Hybridization: A High Resolution Genome-Wide Investigation for Chromosomal Aneuploidy in a Single Assay

To the Editor:

We report a 13-month-old boy who has a chromosomal abnormality of trisomy 2p that was successfully delineated by array-based comparative genomic hybridization (CGH). A cervical cystic lymphatic anomaly detected by first trimester ultrasonography prompted chorionic villus sampling, which revealed a karyotype of 46,XY,add(9)(p24) without further identification of the additional material on chromosome 9. Both parents had normal karyotypes. At birth, the neonate was noted to have metopic and unilateral coronal synostosis, thinning of the corpus callosum, atrial septal defect (ASD), moderate left ventricular hypertrophy (LVH), ocular hypopigmentation, and poor foveal reflex. He developed apnea associated with seizures, failure to thrive, and developmental delay. The origin of the extra material was not identifiable until sequential M-FISH and Rx-FISH studies were completed. These techniques demonstrated that the extra material originated from the distal short arm of chromosome 2 [Lee et al., 2001]. The final karyotype was 46,XY,add(9)(p24)de novo.ish der(9)t(2;9)(p21;p24)(wcp2+,wcp9+): a translocation consistent with trisomy of 2pter-2p21 material.

At 13 months of age, the child's weight, height, and head circumference were less than the 5th centile. He had minor frontal asymmetry following cranial repair, low posterior hairline; facial asymmetry and midfacial hypoplasia; upslanting eyes, bilateral epicanthal folds, and minor left blepharoptosis; fine, blond, and medially flared eyebrows; orbital hypertelorism; short nose with anteverted nares, bulbous tip, and depressed nasal root; low-set and posteriorly rotated ears with thick helices; wide philtrum, thin upper lip, small oral aperture, and a bifid uvula; short and wide neck; pectus excavatum; shallow sacral dimple; hypoplastic scrotum with partially descended testes; small hands with widened fingertips and spooned nails; and small feet (Figs. 1 and 2). On neurologic evaluation, he had horizontal nystagmus, no tracking, and diffuse hypotonia. He was able to roll from back to front, turn to sound, and sit with support. He babbled and said "Mama." He reached for objects and was able to bring his pacifier to his mouth.

Improvements in molecular cytogenetic-based techniques now provide alternate and complimentary means for detecting and identifying chromosomal rearrangements and imbalances, which are otherwise ambiguous after routine GTG-banded analysis. Techniques such as spectral karyotyping [Schrock et al., 1996], M-FISH [Speicher et al., 1996], and Rx-FISH [Muller et al., 1998] combine the sensitivity of fluorescence in situ hybridization (FISH) with a whole genome perspective. The limitation of each technology can be partially overcome by the combined usage of different techniques for a given clinical case.

CGH is another whole genome assay, but is more specifically designed to detect and quantify changes in DNA copy number in a single assay. Traditionally, test DNA was hybridized to normal metaphase chromosomes and the relative fluorescence of the two dyes measured along the length of each chromosome to assess deviations from the expected 1:1 ratio. Many of the limitations of chromosomal-based CGH have been overcome by the use of microarrays with carefully chosen genomic DNA samples from Bacterial or P1 Artificial Chromosome clones (BACs or PACs, respectively). An increase or decrease in DNA copy number can be detected when the ratio of the two fluorescent colors (test vs. reference DNA) for any spot on the array is significantly greater or less than one. We performed array CGH using this patient's DNA and, in a single assay, have obtained unbiased, confirmatory, and more precise delineation of the chromosome rearrangement compared to that previously obtained by the combined use of conventional chromosome banding, M-FISH, and Rx-FISH.

Patient and male reference genomic DNAs were fluorescently labeled with Cy3 and Cy5 dyes according to standard random priming protocols. The DNAs were combined, denatured, and applied to 2–4 Mb resolution human DNA microarrays, according to the manufacturer's recommendations (Spectral Genomics, Inc., Houston, TX). Slides were hybridized for 48 hr, washed in 50% formamide, 2 × SSC solutions at 45°C, and scanned on a GenePix 4000B Axon scanner. Images were analyzed with SpectralWare software provided by Spectral Genomics, Inc.

A gain of chromosomal material was noted in the patient, corresponding to the distal short arm of chromosome 2, confirming our previous results. The additional chromosome 2 material extended from the end of the short arm to BAC RP11-89F19 (Fig. 3). Using publicly available maps of the human genome, this particular BAC was localized to band 2p22.1 with its proximal end designated as nucleotide 38776387. This suggested a gain of approximately 38.8 million bases of DNA—information that could not reliably be extracted from classical cytogenetic or even multicolor FISH testing. The derivative 9 chromosome presumably has a terminal break allowing for the attachment of chromosome 2 material at its end. The chromosome 9 ratio plot did not show evidence of the loss of material at the distal end (data not shown). This is likely due to the fact that the most distal informative BAC probe for 9p on this particular array is RP11-31M2 at 9p24.2 (4078605–4237207 bp), which is four million base pairs from the end of the chromosome. Subtelomeric FISH for 9p was considered but not performed because of the lack of usable chromosome preparations. Nevertheless, our patient exhibited certain features that are not usually characteristic of trisomy 2p but are sometimes seen in monosomy 9p such as craniosynostosis, midface hypoplasia, upslanting palpebral fissures, and hypoplastic adherent ear lobes (Table I).

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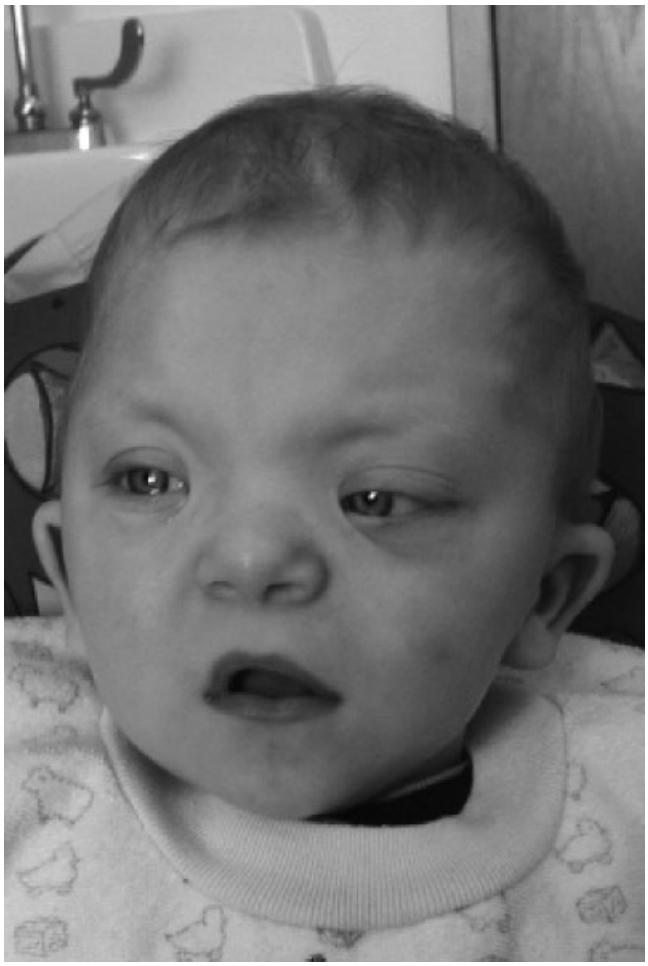

Fig. 1.

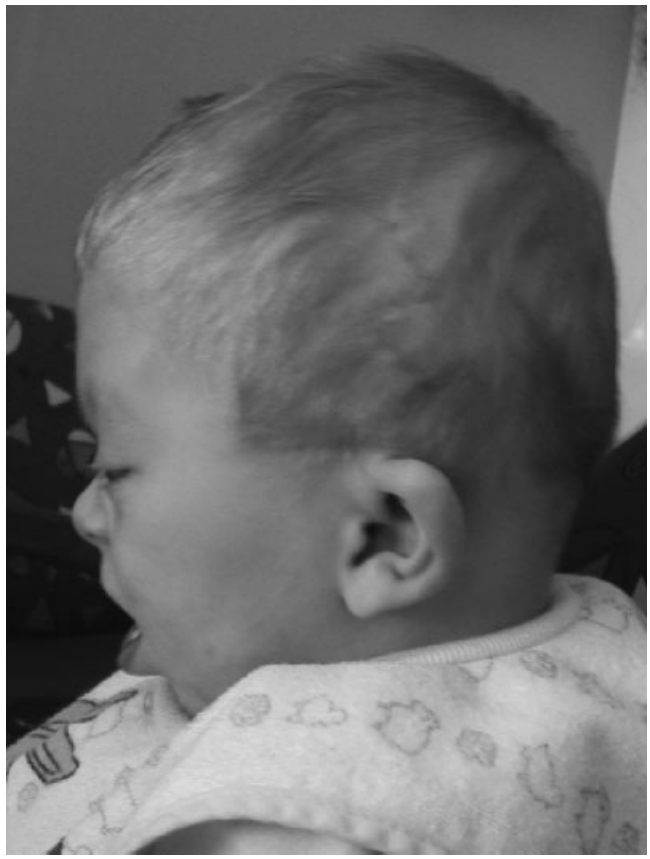

Fig. 2.

Figs. 1 and 2. AP and lateral views of the patient. Note right synostotic plagiocephaly and trigonocephaly, upslanting palpebral fissures, epicanthal folds, left blepheroptosis, orbital hypertelorism, anteverted nares, low-set posteriorly rotated ears, wide philtrum, and short neck.

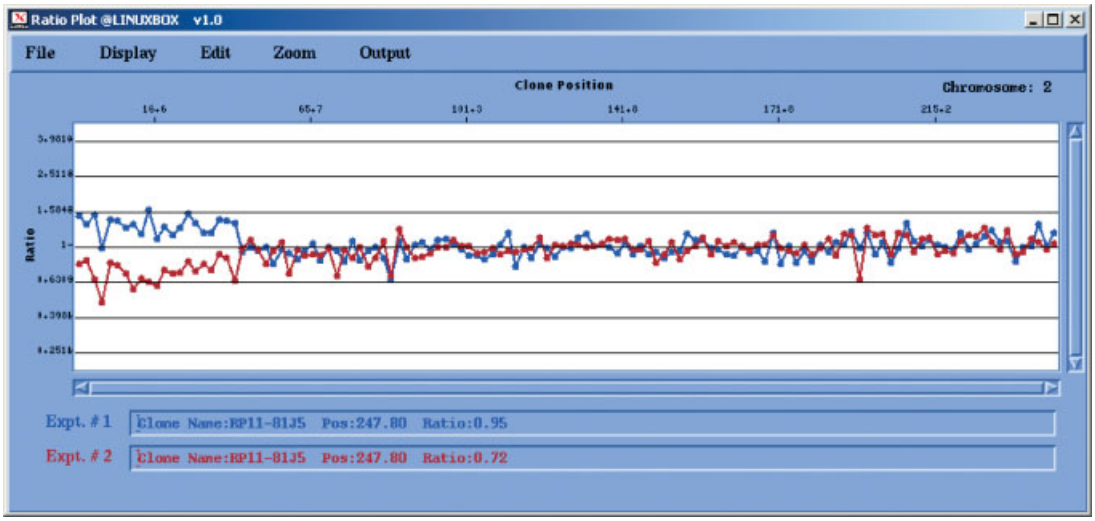

Fig. 3. Chromosome 2 ratio plot with deviations from the expected 1:1 ratio from clone RP11-1N7 (2p25.3) to clone RP11-89F19 (2p22.1). The placement of a blue line over a red indicates gain of material. (SpectralWare software, Spectral Genomics, Inc.).

TABLE I. Comparison of Features Reported in Children With 9p-Syndrome, Partial Monosomy 2p, a Case Report by Fineman et al. [1983], and Present Case

Feature	Commonly associated with 9p-syndrome [Schinzel, 2001]	Commonly associated with trisomy 2p [Lurie et al., 1995]	46,XX,der(9), t(2;9)(p13;p24) [Fineman et al., 1983]	Present case: 46,XY,add(9)(p24)de novo.ish der(9)t(2;9)(p21;p24)(wcp2+,wcp9+) (X* = unique to case)
Craniosynostosis	X			X
Trigonocephaly	X			
Flat occiput	X			
Low hairline	X			X
Microcephaly		X		
Macrocephaly			X	
Midface hypoplasia	X			X
High forehead		X		
Upslanting palpebral fissures	X			X
Epicanthal folds	X	X		X
Hypertelorism		X		X
Dysplasia of the retina		X	X	X
Myopia		X		X
Depressed nasal bridge	X	X	X	X
Bulbous nasal tip				X*
Anteverted nares	X	X		X
Microstomia				X*
Submucous cleft palate		X		X
Micrognathia				X*
Abnormal ear shape or position	X	X	X	X
Hypoplastic adherent lobes	X			X
Redundant nuchal skin				X*
Short neck	X	X	X	X
Widely spaced nipples	X		X	
Sternal abnormalities		X		X
Postaxial hexadactyly of fingers		X		
Wide distal phalanges				X*
Long thin fingers			X	
Short distal phalanges	X			
Tightly abducted hips			X	
Metatarsus adductus and rocker bottom feet			X	
Cryptorchidism	X	X		X
Anteriorly displaced anus			X	
Hypotonia		X	X	X
Absent/poor suck			X	X
Neural tube defect		X	X	
Congenital hydrocephalus		X		
Thin corpus callosum				X*
CHD		X		X
Cardiac situs and looping defects		X		
Bronchopulmonary a/hypoplasia		X		
Diaphragmatic hernia		X		
Nonfunctioning of one kidney		X		
Developmental delay	X	X		X
Seizure disorder		X		X
Neuroblastoma		X		

There are no published reports of children with this translocation. Fineman et al. [1983] described two offsprings of a mother with a balanced translocation involving chromosomes 2 and 9. The features described in their report, as compared to our own case, and features commonly associated with 9p-syndrome [Schinzel, 2001] and trisomy 2p [Lurie et al., 1995] are summarized in Table I. The extent of trisomy 2p in the Fineman et al. [1983] case was greater than that demonstrated in our own patient and may explain the more severe phenotype. Our patient appears to have manifested many of the major features associated with trisomy 2p and monosomy 9p, including craniosynostosis, myopia, congenital heart disease (CHD), cryptorchidism, developmental delay, and hypotonia. Unique to our case are the prominent nasal tip, micrognathia, microstomia, redundant nuchal skin, wide distal phalanges, and thin corpus callosum. Three cases of neuroblastoma with partial trisomy 2p have been reported [Nagano et al., 1980; Say et al., 1980; Patel et al., 1997]. It was postulated that a germline duplication of 2p, giving rise to three copies of MYCN (N-MYC proto-oncogene protein, localized to 2p24.1–24.3), may have predisposed to the development of neuroblastoma [Say et al., 1980]. As a result, we recommended abdominal ultrasonography every 6 months for our patient.

Microarray-based CGH is a high resolution and rapid technique for detecting aneuploidy. The assay is robust and

does not require culture and harvest of actively dividing cells. This significantly reduces the time required to obtain results and eliminates erroneous results due to culture artifacts. Obtaining aneuploidy data at the DNA sequence level provides a rich resource for investigators studying developmental genes. An online catalog for chromosomal aneuploidy could be developed with such data for more accurate genotype–phenotype information. This database would undoubtedly be superior to a cytologic-based compendium that relies on more subjective results obtained by conventional-banding techniques.

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