Genomic microarray analysis identifies candidate loci in patients with corpus callosum anomalies

Abstract—Absence of the corpus callosum is often associated with cognitive deficits, autism, and epilepsy. Using a genomic microarray, the authors analyzed DNA from 25 patients with radiographically confirmed callosal anomalies and identified three patients with de novo copy number changes in chromosome regions 2q37, 6qter, and 8p. Chromosomal deletions and duplications may be a relatively common cause of cerebral malformations.

NEUROLOGY 2005;65:1496–1498

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The clinical profile of patients with agenesis (ACC) or dysgenesis (DCC) of the corpus callosum varies from mildly affected to profound neurodevelopmental impairment and epilepsy. In most cases, the cause of ACC is not known. There is not substantial evidence for recurrence within families, and there are reports of ACC patients with cytogenetic abnormalities.[1] This combination of findings lead us to hypothesize that ACC may be caused by de novo genetic changes. To test this, we screened the first 25 patients enrolled in our study of ACC genetics using a bacterial artificial chromosome (BAC) genomic microarray that contains 2,464 elements and has an approximate resolution of 1.4 Mb.[2] We identified two patients with previously undetected changes in chromosomal copy number. In an additional patient with a known chromosome 8p rearrangement, we readily defined the boundaries of both the duplication and deletion. This novel whole-genome screening may accelerate the discovery of causative mutations for ACC and other cerebral malformations.

Methods. All patients were enrolled in an ongoing prospective study, approved by the UCSF Committee on Human Research, to analyze the genetics and clinical and radiographic findings of patients with ACC. Patients with MRI-documented callosal anomalies were eligible for enrollment. Standardized history and physical examination information was collected, and medical records were reviewed. The MRI images were reviewed and scored for callosal anomalies, involvement of other midline commissures, and other CNS malformations. Additional methods can be found on the *Neurology* Web site at www.neurology.org.

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Results. *Proband 1017-0.* This 12-year-old girl was born at term to a 29-year-old primiparous mother. Early developmental delay and mild craniofacial dysmorphism prompted a genetics evaluation. This demonstrated relative macrocephaly, a cleft palate, short and up-slanting palpebral fissures, anteverted nares, redundant nuchal skin, a bell-shaped chest, and short stature. Laboratory evaluation results were normal, including a high-resolution karyotype. The patient is currently diagnosed with autism spectrum disorder, anxiety, and mental retardation and takes Ritalin for impulsiveness. Recent neuropsychological testing showed a Verbal Intelligence Quotient of 52, a Performance Intelligence Quotient of 49, and a Full Scale Intelligence Quotient (FSIQ) of 47 on the Wechsler Intelligence Scale for Children-Third Edition (WISC-III). MRI analysis revealed near-complete ACC, with only a rudiment of the anterior body evident (figure 1A). Probst bundles were evident, and the ventricles showed colpocephaly. Microarray analysis detected an approximately 2-Mb deletion within 2q37.1 (figure 2A). Repeat analyses confirmed that RP11-69J7 flanked the deletion proximally, both RP11-71H20 and RP11-176L22 were deleted, and RP11-188B21 flanked the deletion distally. Analysis of parental DNA did not detect this deletion (see figure E-1 on the *Neurology* Web site at www.neurology.org).

Proband 1041-0. This 3.5-year-old girl was born at term by sperm donor insemination to a 40-year-old gravida IV para III mother. In the nursery, the patient had hypotonia and poor suck, prompting a head MRI. This detected partial ACC; only the genu and part of the anterior body were present (figure 1B). Colpocephaly, a small anterior commissure, and an absent hippocampal commissure were also evident. She had extensor spasms as an infant and is currently seizure free off anticonvulsants. She had received diagnoses of pervasive developmental delay and cerebral palsy. Developmentally, the patient had global delay: she stood at 16 months, and at 38 months she had an awkward gait and spoke only a few words. On the Bayley Scales of Infant Development at 38 months, she scored more than 3

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Supported by funds from the National Institutes of Health (NS01692) and the UCSF academic senate and carried out in part in the Pediatric Clinical Research Center, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 M01 RR-01271, U.S. Public Health Service.

Disclosure: The authors report no conflicts of interest.

Received May 5, 2005. Accepted in final form August 4, 2005.

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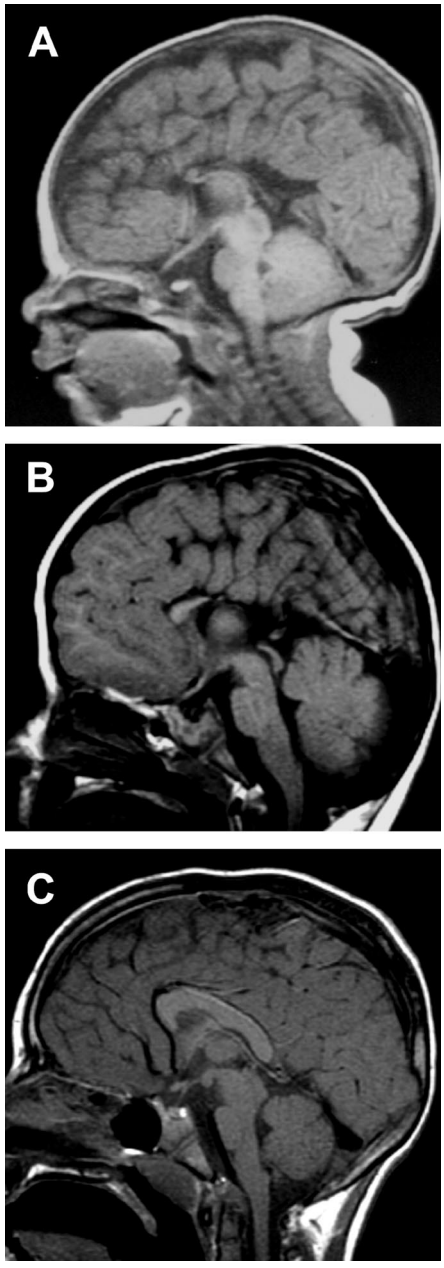

Figure 1. MRI images demonstrating callosal and cortical dysgenesis. (A) Midsagittal T1-weighted image from Patient 1017-0 showing a nearly absent callosum. (B) Midsagittal T1-weighted image from Patient 1041-0. This shows a hypogenetic corpus callosum, with only part of the genu and anterior body present and a small pons. (C) Midsagittal T1-weighted image from Patient 1075-0, demonstrating a dysplastic corpus callosum with a missing rostrum and an abnormally shaped splenium.

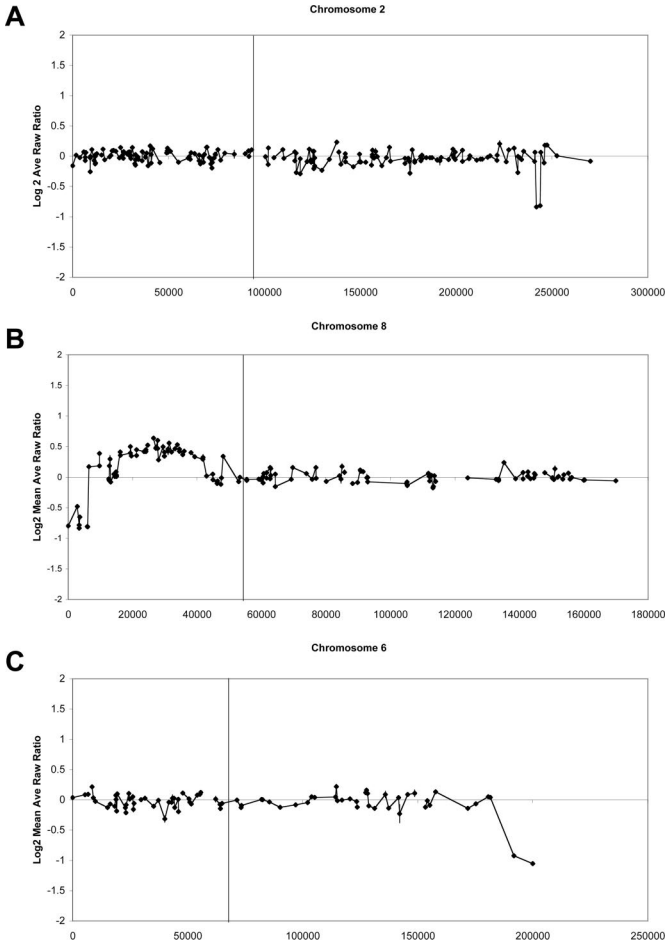

Figure 2. Genomic microarray analysis demonstrates de novo chromosomal changes. Displayed are the affected chromosomes in which two or more bacterial artificial chromosome (BAC) clones demonstrate a consecutive loss or gain of copy number. Chromosomes are displayed graphically from the short arm to the long arm, with the centromere denoted by a vertical line. Numbers along the x-axis correspond to base positions from the February 2003 draft of the human genome. The y-axis displays the log2 ratios of the mean fluorescent intensity of patient to control DNA hybridized at each individual BAC element. A value of −1.0 is equivalent to loss of one copy, and +0.5 corresponds to the gain of one copy. (A) Chromosome 2 from Patient 1017-0 showing deletion of BACs RP11-71H20 and RP11-176L22. (B) Chromosome 8 from Patient 1041-0 demonstrates the 8pter-8p23.1 deletion and the 8p23.1-8p12 duplication. (C) Chromosome 6 from Patient 1075-0, demonstrating a 6q terminal deletion involving the two most distal BACs on the array, CTD2051E21 and RP1-57H24.

SDs below average for her age on both the Psychomotor Development Index and the Mental Development Index. Karyotype: 46 X,X der(8)del(8)(p23.1)dup(8)(p23.1p12). Chromosomal painting verified that the rearranged chromosome was derived entirely from chromosome 8. Normal karyotypes were observed from peripheral blood lymphocytes from the mother and the sperm donor. Array analysis confirmed and defined the boundaries of the deletion (8ptel to BAC RP11-140K14) and the duplication (bounded by BAC RP11-218N24 proximally and BAC RP11-210F15 distally) (figure 2B).

Proband 1075-0. This 12-year-old boy was born at 39 weeks to a 38-year-old mother who took Clomid for conception difficulties, having had four previous miscarriages. Milestones were delayed in early childhood. Recent evaluations demonstrated an FSIQ of 78 (WISC-III) and a Vineland Adaptive Behavior Scales Composite Score of 54. The patient had been treated for anxiety, conduct disorder, and self-mutilating behavior. He is currently taking imipramine. His physical examination results were notable for

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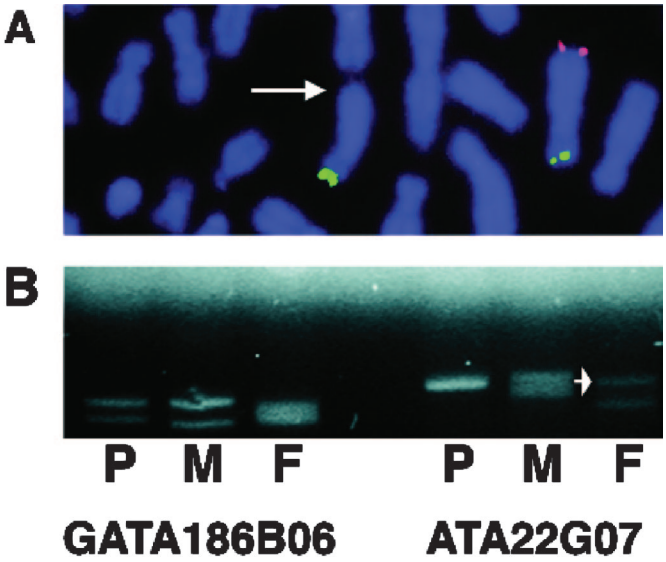

Figure 3. Confirmation and delineation of 6qter deletion in Patient 1075-0. (A) A metaphase chromosomal spread from Patient 1075-0 was hybridized with subtelomeric probes for chromosome 6q (clone VIJyRM2158, red) and 6p (clone 6ptel48, green), confirming a 6qter deletion (arrow). (B) Electrophoretic analysis of PCR fragments amplifying STS markers GATA186B06 and ATA22G07 from the patient (P), mother (M), and father (F). For STS marker GATA186B06, the patient has inherited one allele from each parent. For marker ATA22G07, only one of four possible alleles is present in the patient (which is also seen as the higher molecular weight band in the father [small arrow]), helping to define the proximal boundary of the deletion.

microcephaly and dolichocephaly, and mild craniofacial dysmorphism, including up-slanting palpebral fissures, epicanthal folds, and thickened superior helices. A brain MRI demonstrated a dysplastic corpus callosum with an absent rostrum and a small splenium, missing its normal rounded appearance (figure 1C). High-resolution chromosomes were reported as normal. Microarray analysis demonstrated a chromosome 6q terminal deletion, which spanned BACs CTD2051E21 and RP1-57H24, but distal to BAC RP11-43B19 (figure 2C). This deletion was confirmed by fluorescence in situ hybridization (FISH) (figure 3A). This was found to be a de novo deletion not present in either parent (see figure E-2 on the *Neurology* Web site at www.neurology.org). Using loss-of-heterozygosity analysis, we confined the deletion's proximal boundary to a 3.8-Mb interval bounded by STS markers GATA186B06 and ATA22G07 (figure 3B).

Discussion. We used genomic BAC microarray technology to look for chromosomal copy number changes in 25 well-characterized patients with ACC and found three individuals with de novo chromosome aberrations. For all three patients, we verified these results by a complementary approach and repeating the array analysis. We established strict criteria for our screen, including only individuals for whom two contiguous BACs showed a similar change. Two other individuals met this threshold but were excluded later. One had a duplication on 8p that was present in the father. The other patient had a deletion on 16p; however, no parental DNA was available for comparison. The current generation BAC microarray has 2,464 BAC elements with an approximate resolution of 1.4 Mb. With the availability of higher density arrays,[3] we anticipate that patients with smaller deletions and duplications will be identified in subsequent analyses.

Our findings and those of others suggest that the phenotype of callosal agenesis may be gene dosage dependent. In Mowat–Wilson syndrome, patients have a deletion or a mutation in one allele of the gene ZFHX1B, resulting in ACC, Hirschsprung disease, congenital heart disease, and urogenital anomalies.[4] Males with mutations in the X-linked gene ARX have ACC and lissencephaly, whereas females have mild cognitive impairment and ACC.[5] Additionally, a number of mouse gene inactivation models show gene dosage effects on callosal development including GAP-43, DCC, and Nf1b.[6]

The loci identified in this study (8p, 2q37, and 6qter) have previously been found in association with disorders of the corpus callosum.[1,7,8] Other disorders of cortical development, such as polymicrogyria,[9] and periventricular heterotopia[10] have been found in association with submicroscopic chromosomal deletions and duplications. These findings suggest that screening for de novo chromosomal changes is a clinically valid approach in addition to providing a means to uncovering the mechanisms of these disorders of cerebral development.

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