EMX2-Independent Familial Schizencephaly: Clinical and Genetic Analyses

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Schizencephaly is a human brain malformation distinguished by full-thickness unilateral or bilateral clefts through the neocortex. Heterozygous mutations in the EMX2 locus are reported to give rise to schizencephaly. However, the comprehensive identification of causative genetic loci is precluded by a lack of large pedigrees and genome-wide linkage analyses. We present here a large Turkish pedigree with three individuals with schizencephaly. The similarity of clinical signs in affected individuals strongly suggests an underlying genetic cause; however, genome-wide linkage analysis rules out EMX2 linkage and instead suggests additional candidate loci. These results indicate that genetic forms of schizencephaly are likely to be heterogeneous.

KEY WORDS: familial schizencephaly; brain malformation; epilepsy; polymicrogyria; EMX2

INTRODUCTION

Schizencephaly is a human congenital brain malformation characterized by a full-thickness cleft in one or both cerebral hemispheres [reviewed in Barkovich, 2000; Guerrini and Carrozo, 2001; Battaglia and Granata, 2003]. Cleft lips were first described as fused in a pial-ependymal seam (closed-lip) or separated with the resulting gap filled with cerebrospinal fluid (open-lip) [Yakovlev and Wadsworth, 1946a,b]. Schizencephalic clefts are often found in the perisylvian region and lined with poorly laminated gray matter, polymicrogyria, and heterotopia. Schizencephaly is also associated in some cases with microcephaly, hydrocephalus, or other malformations such as septo-optic dysplasia [Barkovich and Kjos, 1992; Packard et al., 1997; Denis et al., 2000]. The advent of magnetic resonance imaging (MRI) techniques has afforded more descriptive diagnoses of cortical malformations, even at prenatal ages [Denis et al., 2001], and shown that cortical malformations such as schizencephaly are a frequent cause of mental retardation, epilepsy, and motor deficits [Barkovich and Kjos, 1992; Packard et al., 1997; Barkovich, 2000; Denis et al., 2000; Hayashi et al., 2002]. Individuals with schizencephaly can display a variety of clinical signs including developmental delay of cognitive and language functions, motor deficits such as hemiparesis or quadripareisis, hypotonicity, and epileptic seizures [Barkovich and Kjos, 1992; Barkovich, 2000; Battaglia and Granata, 2003]. The severity of clinical features can vary widely among individuals, but bilateral and/or open-lip lesions tend to result in more pronounced symptoms [Barkovich and Kjos, 1992; Packard et al., 1997; Denis et al., 2000].

Although vascular disruptions, cytomegalovirus (CMV) infection, and other environmental insults during early gestation are postulated as causes of schizencephaly [Yakovlev and Wadsworth, 1946a,b; Barkovich and Kjos, 1992; Iannetti et al., 1998; Sener, 1998; Barkovich, 2000], reported cases of familial schizencephaly suggest that genetic components are also involved [Hosley et al., 1992; Hilburger et al., 1993; Haverkamp et al., 1995]. Mutations in the EMX2 transcription factor locus, for example, are associated with schizencephaly in several sporadic and familial cases [Brunelli et al., 1996; Faiella et al., 1997; Granata et al., 1997]. Genetic analysis of additional families with schizencephaly should allow for the identification of additional causative genes, which in turn may provide insights into EMX2 function and/or other aspects of neocortex development. However, additional genetic loci responsible for schizencephaly remain unknown, in part because large pedigrees of affected individuals have yet to be described and severely-affected individuals rarely have children, thus precluding informative genome-wide linkage analysis screens.

Here we identify a large family with three individuals affected by schizencephaly in a stereotyped radiographic pattern. The clinical features of affected individuals are comparatively mild, as all three have relatively preserved intelligence and two have completed primary schooling and started families. Microsatellite marker analysis for this pedigree rules out linkage to EMX2 and suggests linkage to other chromosomal locations. These observations suggest that familial schizencephaly is likely to have multiple genetic causes.

MATERIALS AND METHODS

Subjects

Analyses were performed on three affected individuals, two children, six unaffected siblings, and their parents. Informed consent was obtained from all patients and/or their parents in accordance with protocols approved by the institutional review board.

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board of Boston Children's Hospital. Consent for the use of identifiable photographs was obtained from the family. All affected individuals were examined by a clinical neurologist. Neuroimaging was performed on affected individuals with a Philips 1.5T MRI scanner (Best, the Netherlands) using conventional multiplanar T1- and T2-weighted sequences.

**Linkage Analysis**

Genomic DNA was extracted from peripheral whole blood lymphocytes using previously described protocols (Qiagen, Valencia, CA). A 10 cM average genomewide screen was performed using ~400 fluorophore-labeled PCR primer pairs (ABI Prism Linkage Mapping Set v. 2.5) spanning highly polymorphic microsatellite regions. Additional microsatellite markers as annotated in the human genome (July 2003 version, UCSC genome bioinformatics, http://genome.ucsc.edu) were then analyzed in chromosomal regions of interest using individually designed PCR primers (Sigma-Genosys, The Woodlands, TX) or commercially available microsatellite primer pairs (Invitrogen, Carlsbad, CA). PCR products of patient samples were run on an ABI Prism 3100 genetic analyzer. Alleles were determined using standard software (Genotyper Analysis). Two-point and multipoint logarithm of the odds (LOD) scores were determined using the GeneHunter and Allegro statistical software programs [Kruglyak et al., 1996; Gudbjartsson et al., 2000].

For analysis of linkage to EMX2 and additional candidate loci, data were analyzed for recessive, paternal dominant, and maternal dominant modes of inheritance. For dominant modes of inheritance, a susceptibility allele with frequency 0.001 and penetrance 0.9 was assumed. For recessive modes of inheritance, a susceptibility allele with frequency 0.001 and penetrance 0.99 was assumed. For each marker, four alleles at equal frequencies were assumed.

**Sequencing**

To sequence EMX2 in affected and unaffected individuals, PCR primers were designed to flank the exons and adjacent intron boundaries of EMX2 using standard software (Primer 3). EMX2 sequencing coverage was performed to the same extent as described previously [Brunelli et al., 1996]. EMX2 exons and adjacent intron boundaries were PCR amplified from genomic DNA of affected and unaffected individuals, and PCR products were purified (Qiagen) and sequenced at the Dana-Farber/Harvard Cancer Center High-Throughput DNA Sequencing Facility. Sequence information was compared against annotated EMX2 genomic sequence (July 2003 version, UCSC genome bioinformatics, http://genome.ucsc.edu).

**RESULTS**

**Clinical and Radiological Features**

The pedigree described in this report is illustrated in Figure 1A. The parents deny any relation to one another but grew up in neighboring villages. Although MRI could not be performed on the parents, the clinical examination and mental status of both parents are normal. The parents have six unaffected (II:1–II:3, II:5–II:7) and three affected (II:8, II:9, II:11) living offspring. The parents also had three children that died in the newborn period reportedly from febrile disease and hemiatrophy. Additional signs include dysarthric speech and choreoathetosis in the left hand. Individuals II:9 and II:11 (left to right), with apparent left-sided hemiplegia and hemiatrophy. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The three affected unaffected child each (III:1–III:2). No additional pregnancy losses were reported except for one miscarriage by unaffected individual II:12 coinciding with trauma resulting from a fall.

The three affected individuals display highly similar clinical signs including left spastic hemiparesis and hemiatrophy (Fig. 1B) and seizures characterized by head turning to the left side and left-sided clonic movements with subsequent secondary generalization. Seizures were reported to begin from age 15 (Individual II:9, II:11) to age 20 (Individual II:8). Additional signs include dysarthric speech and choreoathetosis in the left hand. Individuals II:9 and II:11 finished primary school, but individual II:8 did not complete primary school and is illiterate. Verbal IQs for individuals II:9, II:11, and II:8, respectively, were 86, 79, and 64, while performance IQs were 54, 82, and 50. Neuropsychological testing of individuals II:9 and II:11 revealed dyslexia, constructional apraxia, impairment of verbal fluency, dysgraphia, and difficulties in recognizing and comparing figures.
Brain MRIs of affected individuals were remarkably consistent (Fig. 2). Individual II:9 displayed a closed-lip cleft in the right posterior frontal and suprasylvian cortex (Fig. 2A–C, arrows) and contralateral polymicrogyria (arrowheads). Individual II:11 showed a small, open-lip cleft (arrows) and left-side dysplastic cortex (arrowhead). Individual II:8 showed a large, open-lip cleft from the superior portion of the right Sylvian fissure to the mid-body of the right lateral ventricle with polymicrogyria (Fig. 2G–I, arrows), in addition to deep dysplastic infolding of the cortex of the left frontal lobe that approached the frontal horn and was associated with polymicrogyria (Fig. 2H, arrowhead). For each affected individual, MRI examinations also revealed an enlarged right trigone of the right lateral ventricle, a thin posterior body of the corpus callosum, and absence of a septum pellucidum. White matter volume was slightly reduced, most notably in the right hemisphere of individual II:8. Intracranial calcifications, an indication of congenital CMV infection [Iannetti et al., 1998; Sener, 1998], were not detected. All other aspects of the brain appeared normal.

Analysis of EMX2 Linkage

The similarity of clinical and radiological findings in affected individuals strongly suggested a genetic influence. We, therefore, obtained genomic DNA from peripheral-blood lymphocytes from the parents, the six unaffected and three affected offspring, and the two children of affected individuals. A 10 cM genome-wide linkage screen was performed for each individual using microsatellite markers, and data were analyzed assuming recessive, paternal dominant, and maternal dominant modes of inheritance.

Heterozygous EMX2 mutations are implicated in schizencephaly [Brunelli et al., 1996; Faella et al., 1997; Granata et al., 1997]. We, therefore, investigated the probability of EMX2 linkage in this family. Microsatellite markers flanking the EMX2 locus generated maximal multipoint LOD scores <−5.00 for recessive and paternal dominant modes of inheritance, thereby excluding linkage under these models, and <0 for maternal dominant inheritance (Fig. 3A–C). Thus no significant evidence for linkage to the EMX2 locus was identified, although dominant linkage from the mother, albeit very unlikely, could not be completely excluded.
It remained possible, although improbable, that the family harbored an EMX2 mutation with low penetrance that might not be detected by standard linkage analyses. For example, although both parents (I:1, I:2) showed no clinical signs of schizencephaly, one or both parents could be genetic mosaics in which an EMX2 mutation is carried in a fraction of the germline. Moreover, a specific point mutation in the EMX2 homeodomain has been reported where the mother has no clinical signs but can pass the mutated allele and schizencephaly onto her children [Brunelli et al., 1996; Faiella et al., 1997]. We, therefore, sequenced the EMX2 gene of affected individuals. No mutations were identified in the EMX2 coding sequence and >100 bp of flanking intron sequence, including all regions where EMX2 mutations have been described previously [data not shown; Brunelli et al., 1996; Faiella et al., 1997; Granata et al., 1997]. Thus, although it remains possible that one or more unreported, noncoding EMX2 mutations with low penetrance are present in this family, the simpler interpretation of these observations is a lack of significant linkage to the EMX2 locus.

Potential Linkage to Other Genomic Regions

We then searched additional genomic regions for linkage to schizencephaly. Assuming a recessive mode of inheritance, microsatellite markers at Chromosome 8q24.22-24.3 generated a maximal multipoint LOD score of 1.95 and, therefore, could not be ruled out (D8S256-D8S1836; data not shown). No significant LOD scores were detected assuming dominant inheritance, microsatellite markers at Chromosome 5q21.3-23.2 suggested linkage with a maximal two-point LOD score of 2.68 (D5S433-D5S2058; data not shown). Chromosome 8q24.22-24.3 and Chromosome 5q21.3-23.2 thus represent promising regions in which to search for causative loci of schizencephaly.

DISCUSSION

We have described a large pedigree with a familial form of schizencephaly. Radiological features in affected individuals correspond well to previously described findings [Barkovich and Kjos, 1992; Packard et al., 1997; Denis et al., 2000]. However, the clinical features in this family are relatively mild, as two of three affected individuals successfully completed primary school and have started families. The observation that the three youngest surviving children are affected is striking and, at first glance, could implicate environmental causes leading to prenatal injury. However, CMV infection is unlikely as intracranial calcifications were not detected by MRI [Fig. 2; Iannetti et al., 1998; Sener, 1998]. In addition, an environmental predisposition for prenatal injuries resulting from vasculopathy, autoimmune thrombocytopenia, vascular occlusions, or other insults is also unlikely as they would be expected to cause variable clinical and radiological findings [Norman, 1980; Barkovich and Kjos, 1992; Kuipers et al., 1994; Landrieu and Lacroix, 1994; Suchet, 1994; Hahn and Lewis, 2003]. Instead, the consistency of clinical and radiological findings in affected individuals described here, combined with the identification of candidate genomic loci, raise the strong possibility of an underlying genetic cause.

Multiple spontaneous and familial cases of schizencephaly have been linked to heterozygous mutations in EMX2, which were identified by direct sequencing of the EMX2 gene in affected individuals [Brunelli et al., 1996; Faiella et al., 1997; Granata et al., 1997]. EMX2 encodes a homeodomain transcription factor implicated in neural precursor proliferation, cortical plate lamination, and proper positioning of area-specific domains of the developing mammalian neocortex [Bishop et al., 2000; Mallamaci et al., 2000a,b; Heins et al., 2001; Galli et al., 2002; Fukuchi-Shimogori and Grove, 2003; Hamasaki et al., 2004]. In humans, specific EMX2 mutations identified in individuals with schizencephaly are predicted to cause frameshift mutations or interfere with mRNA splicing, while the effects of other identified mutations are less clear. However, “severe” EMX2 mutations tend to correspond to bilateral, open-lip schizencephaly cases, while missense mutations tend to correlate with less severe phenotypes. One form of EMX2 missense mutation was also detected reproducibly in the mothers of affected children, although the mothers showed no overt signs of schizencephaly. In these reports, a large percentage of affected children harbored EMX2 mutations, raising the possibility that EMX2 is the primary, if not sole, genetic locus for schizencephaly [Brunelli et al., 1996; Faiella et al., 1997; Granata et al., 1997].

However, despite several subsequent analyses of individuals with schizencephaly, no additional individuals with EMX2 mutations have been reported, thereby raising the possibility of the existence of other causative loci. Using linkage analysis and sequencing data, we have provided strong evidence against linkage to EMX2 in the pedigree described in this report. Assuming standard modes of inheritance, these results suggest that EMX2 is not the sole genetic cause of familial schizencephaly and that familial schizencephaly is likely to be genetically heterogeneous.

What, then, are the additional genetic loci that can give rise to schizencephaly? The size of the pedigree described here precludes definitively identifying an exact location for a causative gene. However, linkage analysis has indicated two strong candidate regions including Chromosome 8q24.22-24.3 (assuming a recessive mode of inheritance) and Chromosome 5q21.3-23.2 (assuming a dominant mode of inheritance and germlinal mosaicism). To pinpoint conclusive locations of additional causative loci of schizencephaly, analysis of additional pedigrees with familial schizencephaly that do not map to EMX2 is required. Ultimately, the identification of additional loci will help to increase our understanding of the molecular mechanisms that underlie embryonic neocortex patterning and development, in addition to further elucidating the etiology of schizencephaly and other cortical malformation disorders.

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REFERENCES


