dextran may reduce the rate of postendarterectomy stroke, this remains to be established. Hence, we continue to randomize patients into Phase 2 of the study, to determine clinical outcome.

TCD is safe, noninvasive, relatively simple, and inexpensive. If Phase 2 demonstrates that dextran reduces postoperative stroke, then TCD emboli counts may be a useful surrogate for clinical outcome in future trials of antithrombotic agents in the setting of CEA, thereby reducing sample size requirements substantially.

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References


Mutation of the Doublecortin Gene in Male Patients with Double Cortex Syndrome: Somatic Mosaicism Detected by Hair Root Analysis

Mitsuhiro Kato, MD,1 Masayo Kanai, MD,1 Osamu Soma, MD,2 Yuichi Takusa, MD,3 Toshiyuki Kimura, MD,1 Chikahiko Numakura, MD,1 Takasumi Matsuki, MD,4 Shigeki Nakamura, PhD,5 and Kiyoshi Hayasaka, MD1

The molecular basis of double cortex syndrome was investigated in 2 male patients. Magnetic resonance imaging of the patients’ heads showed diffuse subcortical band heterotopia, as is seen in female patients. We found a heterozygous mutation for Asp50Lys or Arg39Stop in both patients. Microsatellite polymorphism analysis revealed that both patients had inherited a single X chromosome from their mothers. Restriction enzyme analysis

From the 1Department of Pediatrics, Yamagata University School of Medicine, Yamagata; 2Department of Development and Neurology, Kobe Children’s Hospital, Kobe; 3Department of Pediatrics, Shimane Medical University, Izumo, Shimane; 4Department of Forensic Medicine, Fukui Medical University, Fukui; and 5Department of Legal Medicine, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan. Received Feb 19, 2001, and in revised form Jul 9. Accepted for publication Jul 9, 2001. Published online Sep 3, 2001; DOI: 10.1002/ana.1231

Address correspondence to Dr Kato, Department of Pediatrics, Yamagata University School of Medicine, lida-nishi 2-2-2, Yamagata 990-9585, Japan. E-mail: mkato@med.id.yamagata-u.ac.jp

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using DNA extracted from the hair roots of each patient showed four different patterns in the combination of cells carrying wild and mutant alleles, which strongly suggest somatic mosaicism. We conclude that somatic mosaic mutations in the doublecortin gene in male patients can cause subcortical band heterotopia, and that molecular analysis using hair roots is a useful method for detecting somatic mosaicism.

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X-linked lissencephaly and subcortical band heterotopia,1 or double cortex syndrome,2 is a brain malformation involving diffuse cortical dysplasia, and is characterized on magnetic resonance images (MRIs) as bilateral continuous symmetric bands of gray matter underlying the cortical mantle (subcortical band heterotopia [SBH]) in female patients. The major clinical manifestations are epilepsy and mild to moderate mental retardation. Affected sons of a mother with SBH show classic lissencephaly (smooth brain), a more severe form of cortical dysplasia. Linkage analysis and subsequent positional cloning have led to the isolation of a novel gene, doublecortin (DCX), mapping to Xq22.3–Xq23.4,4 It has been revealed that females with heterozygous DCX mutations develop band heterotopia, and males with DCX hemizygous mutations develop classic lissencephaly.5 On the other hand, a relatively small number of male patients with band heterotopia has been reported.6 Although the results of one study based on single-stranded conformational polymorphism analysis and direct sequencing has suggested somatic mosaicism for the DCX mutation,7 another study has demonstrated missense mutations of DCX or LIS1, but failed to provide evidence of somatic mosaicism in male patients with band heterotopia.8 The mechanism causing a less severe form in male patients is still unclear.

Here, we report on 2 sporadic male patients with SBH in whom molecular analyses of hair roots revealed somatic mosaic mutations in the DCX gene, and in whom microsatellite polymorphisms were located on the X chromosome.

Patients and Methods
Patient 1
Patient 1 was the first child of unrelated healthy parents. He was born at term in a normal delivery after an uncomplicated pregnancy. His body stature was normal at birth. He showed nystagmus from 2 months of age. At 8 years of age, he had recurrent episodes of loss of consciousness and loss of facial expression. Electroencephalograms (EEGs) revealed focal spike-and-slow-wave discharges in the right occipital area. His seizures were refractory. At 11 years of age, his full scale IQ was 44 (WISC-R). At 12 years of age, MRI revealed SBH, indicating double cortex syndrome (Fig 1A). Results of neurological examination were normal, except for moderate mental retardation. There were no abnormal laboratory findings in biochemical analysis, including liver and renal functions and serum electrolytes. Results of chromosomal analysis were 46XY. EEGs revealed no paroxysmal activity.

Patient 2
Patient 2 was the second child of unrelated healthy parents, whose elder brother showed normal development. Patient 2 was born at term in a normal delivery after an uncomplicated pregnancy. Instability of head control and internal strabismus were noticed at 4 months of age. At 5 months of age, his eyes began to fix and pursue, but neurological examination demonstrated poor head control. His total development quotient was 70 (Japanese Enjoji developmental test). MRI revealed SBH (see Fig 1B). Results of chromosomal analysis were 46XY. EEGs revealed no paroxysmal activity.

Genomic DNA Extraction
After obtaining informed consent from the parents, peripheral blood was taken from the patients and their parents, and...
Hair roots were taken from the patients. Genomic DNA from peripheral blood leukocytes and hair roots was extracted using a DNA extraction kit (Nucleon BACC3 for blood and cell cultures, Amersham, Buckinghamshire, UK) according to the manufacturer's instructions, and according to a previous report.9

DNA Sequencing
All coding regions of the DCX gene were sequenced using genomic DNA from the patients' peripheral leukocytes. Polymerase chain reaction (PCR) products were amplified as described previously,10 and sequence analysis was performed on an automated DNA sequencer (ABI 310; Applied Biosystems, Foster City, CA).

Restriction Enzyme Analysis Using Genomic DNA from Hair Roots
A DNA fragment (exon 2.1) was amplified from the genomic DNA of Patient 1, and another DNA fragment (exon 2.2n) was amplified from the genomic DNA of Patient 2 using a set of primers, 2.2F/2.2R, followed by nested PCR with a set of mismatch primers, 2.2F/n2.2R (5’GCCTGC-

Evaluation of Parental Origin Analyzed by Microsatellite Polymorphisms
Genomic DNA from the peripheral leukocytes of the patients and their parents were analyzed using short tandem repeat systems located on the full length of the X chromosome.11 PCR amplification was performed with an ABI PRISM Linkage Mapping Set-MD 10 Panel 28 (Applied Biosystems) according to the manufacturer's instructions. Control DNA CEPH 1347-02 was used as a reference for allele designation using Gene Scan Analysis v 2.1 software.

Results
DNA sequence analysis of coding regions (exons 2–6) showed that Patient 1 had a G-to-T substitution at nucleotide number 150, which resulted in an asparagine substitution for lysine at codon 50 (Fig 2A). Patient 2 demonstrated a C-to-T transition at nucleotide number 115, which resulted in an arginine-to-stop codon substitution at codon 39 (see Fig 2B). The patients were heterozygous for mutant and wild alleles. Their parents did not carry these mutations, suggesting that the mutations were de novo.

Restriction enzyme analysis of the products from the hair roots of each patient showed four patterns (Fig 3). They represented the product from only the wild allele, the mixed product from a more-wild allele and a less-mutant allele, the mixed product from a less-wild allele and a more-mutant allele, and the product from only a mutant allele. The mutant allele was segregated from the wild allele, suggesting somatic mosaicism.

Microsatellite polymorphism analysis showed that each patient had a single allele of each marker on the X chromosome of the mother (data not shown), indicating that each patient had inherited a single X chromosome from his mother. These results indicated that the patients represented a somatic mosaic mutation, but not a chimera, and suggested that the mutation of the DCX gene located on an X chromosome inherited from the mother occurred during early embryogenesis.

Discussion
We analyzed mutations in the DCX gene in 2 male patients with SBH, and identified two novel heterozygous mutations. Because the patients showed normal karyotypes, it was thought that they were mosaics or chimeras. Microsatellite analysis showed that each patient had a single allele of each marker on the X chromosome of the mother (data not shown), indicating that each patient had inherited a single X chromosome from his mother. These results indicate that neither of the patients are chimeras.

Hair roots have been used as material to confirm somatic mosaicism.12 We therefore performed PCR and restriction enzyme analysis using DNA extracted from

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Fig 3. Electrophoresis of the MboII (A, Patient 1) or AcI (B, Patient 2) digestion fragment of PCR products of exon 2.1 (A) or exon 2.2n (B). Lane 1, size marker digest (A, fX174/Hinc II; B, fX174/Hinf I). Lane 2, normal control. Lane 3, blood sample from the patient. Lane 4, patient's father. Lane 5, patient's mother. Lanes 6–9, hair roots from the patient. (A) The PCR product (394 bp fragment) is normally cleaved by the endonuclease MboII into three fragments of 220, 137, and 37 bp (lanes 2, 4, and 5). The 37 bp fragment in A is faint. The mutant allele deleted a MboII site, producing a 357-bp band and a 37 bp band. A sample obtained from the patient's blood showed both a 357 bp band and a shorter band, indicating heterozygosity. (B) The PCR product (92 bp fragment) is normally cleaved by the endonuclease AcI into two fragments of 72 and 20 bp (lanes 2, 4, and 5). The 20 bp fragment could not be visualized on this gel. The mutant allele deleted an AcI site, producing only a 92 bp band. A sample obtained from the patient's blood showed both 92 bp and 72 bp bands, indicating heterozygosity. Electrophoresis of hair roots from both patients demonstrated four different patterns: a mutated homozygous pattern (lane 6); a normal homozygous pattern (lane 7); an abnormal heterozygous pattern, which showed a thicker mutant band than the normal band (lane 8); and an abnormal heterozygous pattern, in which the normal band is thicker than mutant band (lane 9).

DCX gene may have occurred during early postzygotic division.15

There are conflicting reports as to whether DCX mutations in males with double cortex syndrome indicate mosaicism.7,8 Because mosaicism can vary between tissues, molecular analysis using lymphocytes may not show mutation. On the other hand, hair roots as well as brain are ectodermal derivatives. Hair root analysis would therefore be better than blood analysis for establishing somatic mosaicism of the brain. In addition, there are some SBH patients without DCX mutations in DNA extracted from peripheral lymphocytes, particularly patients with posterior dominant or focal heterotopia.10,16,17 Hair root analysis may also be advantageous for detecting somatic mosaicism localized in one part of the brain.

Factors influencing the severity of brain malformations include mutational genes, location and type (such as missense or nonsense) of the mutation,5,17 distribution of mutation (X chromosome inactivation or somatic mosaicisms), and other modifying agents (such as environment and other genes). Gleson and colleagues analyzed the ratio of mosaicism using quantitative PCR reaction or densitometry of single-stranded conformational polymorphism bands,7 and they suggested that individuals with a higher mutation load are likely to be more severely affected. In our study, Patient 2 showed more severe brain malformation, diffusely thick-band heterotopia, and hypoplastic gyral formation than did Patient 1. We did not estimate the ratio of mosaicism, but the results of restriction analysis using genomic DNA isolated from peripheral leukocytes suggested that there was a larger number of cells carrying mutation alleles in Patient 2 (see Fig 3B, lane 3) than in Patient 1. In addition, it was thought that the nonsense mutation at exon 2 of Patient 2 would have produced a truncated protein that may be toxic and may be related to the severity in Patient 2. Further cases should be analyzed to make clear genotype-phenotype relationships.

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