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SHORT COMMUNICATION

Clinical and genetic analysis of *HLXB9* gene in Korean patients with Currarino syndrome

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Abstract Currarino syndrome (CS) is a rare autosomal dominant disease that has been described as a triad of partial sacral agenesis, anorectal anomalies, and a presacral mass. Mutations in the *HLXB9* gene have been suggested to be the genetic background of CS. In this study, sequence analysis of the *HLXB9* gene was performed in two familial and two sporadic Korean patients showing the clinical features of CS, and two mutations in the *HLXB9* gene were identified only in the two familial cases. One mutation (R295W) has been reported previously, and the other (H260_Q261delinsLELLELE) is novel. Consistent with previous observations, the phenotypic expression of the mutation carriers in the CS families varies from mild to severe, including the complete triad. This study confirms that familial CS patients in Korea have the same genetic background as other ethnicities and reaffirms the phenotype variability among CS patients with the same mutation.

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Introduction

Currarino syndrome (CS; OMIM 176450) is described as a triad of partial sacral agenesis with an intact first sacral vertebra (sickle-shaped sacrum), presacral mass, and anorectal malformations (Currarino et al. 1981). The other abnormalities, such as duplication of the urogenital tract, tethered cord, and different fistulas, might be associated with CS (Emans et al. 2005). CS shows an autosomal dominant inheritance with highly variable expression (Emans et al. 2005; Garcia-Barcelo et al. 2006; Kochling et al. 2001).

In 1998, Ross et al. reported that mutations in the homeobox gene, *HLXB9*, at chromosome 7q36, are the major cause of the CS (Ross et al. 1998). Mutations in *HLXB9* have been identified in almost all cases of familial CS and in approximately 30% of sporadic CS (Belloni et al. 2000; Garcia-Barcelo et al. 2006; Hagan et al. 2000; Kochling et al. 2001; Lynch et al. 2000). There is no genotype–phenotype correlation and extremely high variability of the phenotype can be found in carriers of the mutation, from severe triad to minor sacral abnormalities that are only detectable with radiographs or are even completely asymptomatic (Garcia-Barcelo et al. 2006; Kochling et al. 2001).

Although a few cases of CS have been reported in Korea, there is no genetically proven cases thus far (Kim et al. 1993; Lee et al. 1997). In order to know whether Korean CS patients have the same genetic background as other ethnicities, we performed a mutation analysis of the *HLXB9* gene in four Korean CS patients (two familial and two sporadic) and found two mutations only in the familial cases, respectively.

Patients and methods

Four unrelated patients were enrolled in this study. Two of them (patients 1 and 2) had a family history of suspected CS. For the family study, four sisters (II-2, II-4, II-6, and II-8) of patient 1, and the grandmother (I:2), both parents (II:5 and II:6), two paternal aunts and an uncle (II:2, II:4, and II:7) of patient 2 were tested (Fig. 1). After obtaining informed consent, the genomic DNA was isolated from the peripheral blood leukocytes using a Wizard genomic DNA purification kit according to the manufacturer's instruction (Promega, Madison, WI). The *HLXB9* gene was amplified by a polymerase chain reaction (PCR) using the appropriate primers designed by the authors (available upon request) and a thermal cycler (Model 9700, Applied Biosystems, Foster City, CA). Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) on an ABI Prism 3100 genetic analyzer (Applied Biosystems).

Results

Clinical findings

Patient 1 was a 25-year-old pregnant woman. She had chronic constipation and suffered from recurrent abdominal pain and distension. At age 20, a pre-sacral meningocele, a large bony defect involving the sacral segments S2 to S5, and rectal dilatation were noticed incidentally during a radiological study after a car accident. She was diagnosed with CS and underwent a colostomy due to a megacolon. Two of her four elder sisters were also diagnosed with CS. The eldest sister (II-2) had chronic constipation and a sacral bony defect, and her second son (III-2) also had a sacral bony defect and anorectal stenosis that had been corrected by anoplasty and a colostomy at birth. The youngest sister (II-8) had the complete Currarino triad including sacral bony deformity, presacral meningomyelocele and an anal stricture, and underwent a colostomy.

Patient 2 was a 6-month-old female infant who was admitted due to fever, chill and headache. The CSF culture revealed polymicrobial infection with *Enterobacter cloacae, Citrobacter freundii, Klebsiella pneumoniae, Enterococcus faecalis* and *Enterococcus avium*. Magnetic resonance image (MRI) of the spine showed a presacral mature cystic teratoma with an extension of the spinal canal associated with a lower lining cord and possible tethering to the mass, an anterior bony defect of distal sacrum and a left hydroureteronephrosis. Prominent enhancement along the surface of lumbosacral spinal cord was also observed. Brain computed tomography (CT) showed multifocal low-attenuated lesion in both basal ganglia and right parieto-temporal lobe, widening of the subarachnoid space and increased attenuation in the ventricles and cisterns. These findings were indicative of cerebritis and arachnoiditis. Her parents reported that she had suffered from recurrent abdominal pain and distension. She was finally diagnosed with CS, but had died from sepsis despite receiving antimicrobial chemotherapy. Her

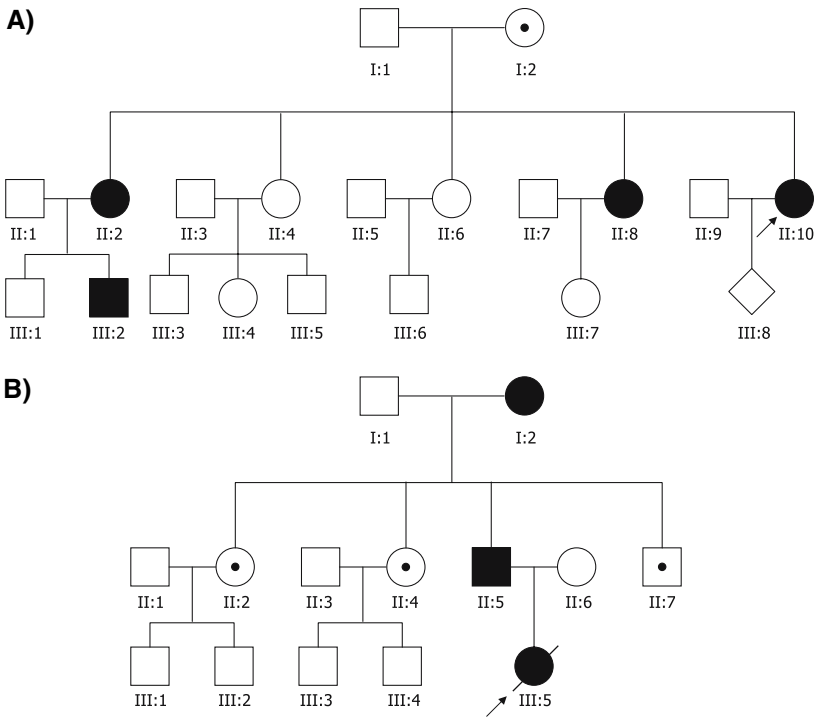

Fig. 1 Pedigrees of two families with Currarino syndrome carrying *HLXB9* gene mutations. **a** Patient 1's family with the R295W mutation. **b** Patient 2's family with the H260_Q261delinsLELLELE mutation. *Circle* female; *square* male; *black symbol* affected; *dot* asymptomatic carrier; *diagonal line* deceased

father (II-5) had chronic constipation and an anal fistula, and her paternal grandmother had a megacolon, which had been corrected by colostomy at age 15. All the other family members were asymptomatic.

Patient 3 was a 6-year-old girl and had a presacral teratoma, sacral defect and a congenital megacolon at birth. Terotoma removal and a rectal wall repair operation were performed at birth. The anoplasty and colostomy was performed at age 2, and colostomy repair was carried out at age 3. No family history was reported.

Patient 4 was a 4-year-old boy with a lipomyelomeningocele, sacral bony defect, imperforate anus, tethered cord and vesicourethral reflux at birth. Anoplasty and removal of lipomyelomeningocele had been performed at birth and at age 4. No family history was reported.

Genetic analysis

Direct sequencing of the whole coding exons and their flanking intronic regions of the *HLXB9* gene revealed a known heterozygous missense mutation (c.883C > T; R295W) and a novel heterozygous deletion–insertion mutation (c.779_781delinsTGGAGCTGCTGGAGCTGG; H260_Q261delinsLELLELE) affecting the homeodomain region in patients 1 and 2, respectively (Fig. 2). Two sisters of patient 1 (II-2 and II-8) had the same heterozygous R295W mutation, while the other two sisters (II-4 and II-6) did not. Patient 2's father (II-5), grandmother (I-2), two paternal aunts (II-2 and II-4) and one paternal uncle (II-7) had the same deletion–insertion mutation. Therefore, three individuals (II-2, II-4, and II-7) in this family were found to be asymptomatic carriers. No mutation was identified in the two sporadic CS cases.

Discussion

The *HLXB9*, a homeobox gene, has three exons encoding a 403-amino acid protein with three significant features: a polyalanine repeat region that shows length polymorphism,

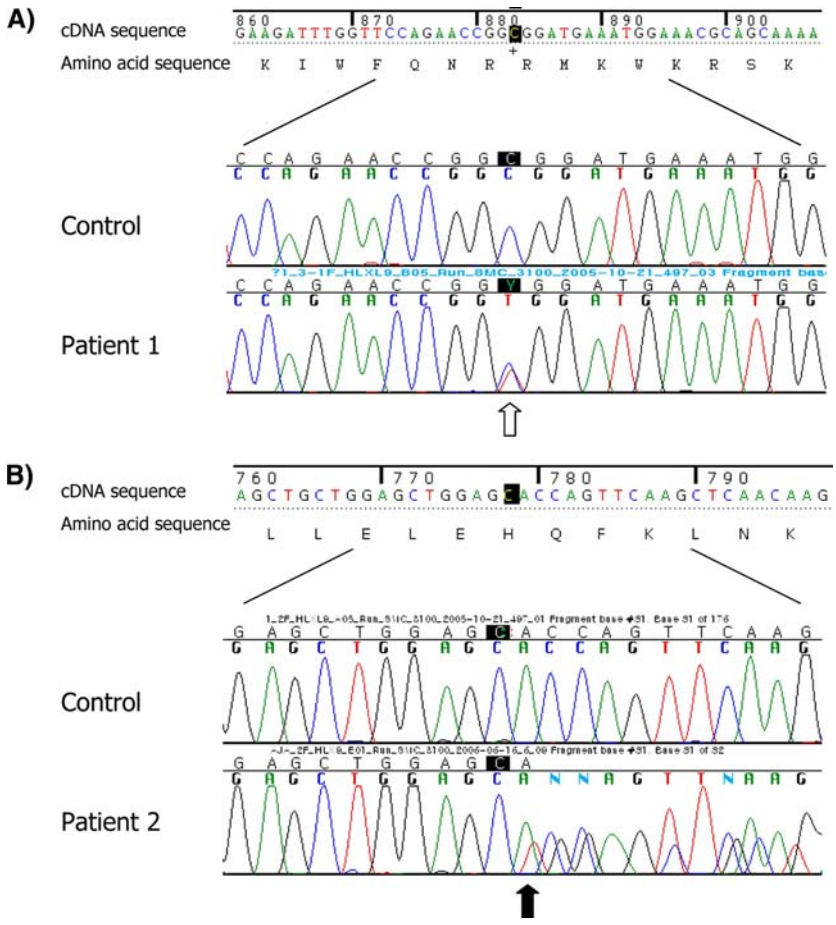

Fig. 2 Direct sequencing analyses of the *HLXB9* gene. **a** A heterozygous missense mutation (c.883C > T; R295W) was identified in patient 1 (*open arrow*) and **b** overlapping peaks from the nucleotide c.779A (*filled arrow*) were noticed due to a heterozygous deletion–insertion mutation (c.779_781delinsTGGAGCTGCTGGAGCTGG; H260_Q261delinsLELLELE) in patient 2

a homeodomain encoded by exons 2 and 3, and a highly conserved region of 82 amino acids upstream of the homeodomain (Hagan et al. 2000). To date, more than 31 CS-causing *HLXB9* heterozygous mutations have been identified (Garcia-Barcelo et al. 2006; Kochling et al. 2001; Wang et al. 2006). All missense mutations are clustered in the homeodomain, whereas the nonsense and frameshift mutations are located mainly on the NH2 terminus of the protein (Kochling et al. 2001). In this study, the first familial case has a previously reported mutation, R295W (Belloni et al. 2000) within exon 3 while the second familial case had a novel deletion–insertion mutation within exon 2. This novel mutation showed a deletion of two amino acids and an insertion of seven amino acids (H260_Q261delinsLELLELE), finally changing two amino acids from H260_Q261 into L260_E261 and adding five amino acids (LLELE) within the homeodomain.

The variability in the manifestations of CS and the high proportion of clinically asymptomatic patients makes it difficult to determine the true incidence of this condition (Hagan et al. 2000). The presence of constipation may lead to a diagnosis of CS early in childhood, even though some of the patients reported did not show any clinical symptoms (Kochling et al. 2001, 1996; O'Riordain et al. 1991). In these clinically asymptomatic patients, the neurological injury, bacterial meningitis or malignant degeneration may be late, serious and often be unguarded complications of the presacral mass (Gaskill and Marlin 1996; Hunt et al. 1977; Tander et al. 1999; Urioste et al. 2004).

Phenotypic variability was also observed among the family members carrying the same mutation in the present study and this phenotypic variability is suggested to be due to a variable gene penetrance or by the effects of other unknown genes or sensitivity to modifications elsewhere in the genome affecting the *HLXB9* protein partners or transcriptional regulators (Belloni et al. 2000; Garcia-Barcelo et al. 2006; Hagan et al. 2000; Kochling et al. 2001).

As previously mentioned, the *HLXB9* mutations have been found in almost all patients with familial CS and in only 30% of patients with sporadic CS. *HLXB9* mutations were not detected in the two sporadic CS patients in this study. Although mutations outside the coding and putative promoter regions as well as somatic mosaicism provide possible explanations, an alternative possibility is genetic heterogeneity. In summary, we can confirm that familial CS patients in Korea have the same genetic background as other ethnicities and reaffirm the phenotype variability among CS patients with the same mutation. Also, the genetic heterogeneity or undetected mosaicism for an *HLXB9* gene in sporadic cases is suggested.

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