A Novel HLXB9 Mutation in a Chinese Family with Currarino Syndrome

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Introduction

Currarino syndrome (CS), first described in 1981,[1] is a congenital malformation typically associated with sacral agenesis, anorectal malformations, and a presacral mass. Patients affected by CS display a phenotypic variability, whereby the spectrum of phenotypes ranges from a severe triad to asymptomatic features.[2,3]

A familial tendency with autosomal dominant inheritance was noted by Yates et al. in 1983.[4] Ross et al. further reported that mutations in the homeobox gene *HLXB9* are the major cause of CS.[5] Mutations in the *HLXB9* gene have been identified in almost all reported cases of familial CS, and in approximately 30% of patients with sporadic CS.[6]

The *HLXB9* gene is essential for proper pancreatic development and for the differentiation of motor neurons in the spinal cord.[7,8] It has 3 exons and encodes a 403-amino acid transcription factor HB9 protein. The HB9 protein contains a homeodomain preceded by a highly conserved 82-amino acid domain and a poly-alanine region.[9]

In this article, we report a new *HLXB9* gene mutation identified in a Chinese family with members suffering from CS, together with the clinical characteristics of the affected individuals.

Patients and Methods

The cases of 2 members of a family diagnosed with CS were analyzed for clinical findings. Blood samples were taken from the patients and their relatives and screened for DNA mutations in the *HLXB9* gene. The *Isl1* and *Lim3* genes, known to be involved in the same pathways as the *HLXB9*,[9,10] were also screened. After having obtained informed consent, DNA was extracted from peripheral blood using a TIANamp Genomic DNA Kit in accordance with manufacturer's instructions (Tiangen Biotech, Beijing, China). Polymerase chain reaction (PCR) amplification and direct sequencing were performed to screen for DNA mutations. The primers used to screen *HLXB9* and *Lim3* genes were as described in the literature.[5,11] The *Isl1* gene primers were designed by us (information available on request). Direct sequencing was performed using an ABI 3730 automatic sequencer (Applied Biosystems, Carlsbad, CA, USA).

Results

Clinical Findings

Patient 1 was an 11-year-old girl. She was admitted due to a recurrent presacral mass for 1 year. She was born with an imperforate anus (low type) and subsequently underwent anoplasty. No magnetic resonance imaging (MRI) was performed at the time, so whether she had a presacral mass or not was not identified. Constipation persisted even after the operation. At the age of 3, a rectal biopsy showed the absence of ganglion cells, consistent with a diagnosis of Hirschsprung's disease. During a radical macrosigmoid operation, a presacral mass was discovered, and she also underwent excision of the mass. Histological examination of the mass showed it to be a dermoid cyst. The presacral mass reoccurred during the follow-up period. MRI of the lumbosacral spine revealed sacrococcy-geal hypoplasia and a presacral mass (6.8 × 6.4 × 9.1 cm; ►Fig. 1), all of which are consistent with the diagnosis of Currarino syndrome. The mass was removed again. A subsequent histological examination determined the mass to be a dermoid cyst.

Patient 2 was a 3-year-old boy, the younger brother of patient 1. He was born with a rectoperineal fistula and consequently underwent anoplasty at the local hospital. Constipation persisted and he was admitted to our hospital. Barium enema suggested the possibility of Hirschsprung's disease, but subsequent rectal biopsy excluded the diagnosis. An MRI of the lumbosacral spine showed a lipomyelomeningocele, a tethered cord, and sacral agenesis (►Fig. 2), all leading to the diagnosis of Currarino syndrome. The child underwent repair of the myelomeningocele and release of the tethered cord. Postoperatively, the patient was still constipated and was managed with enemas for constipation.

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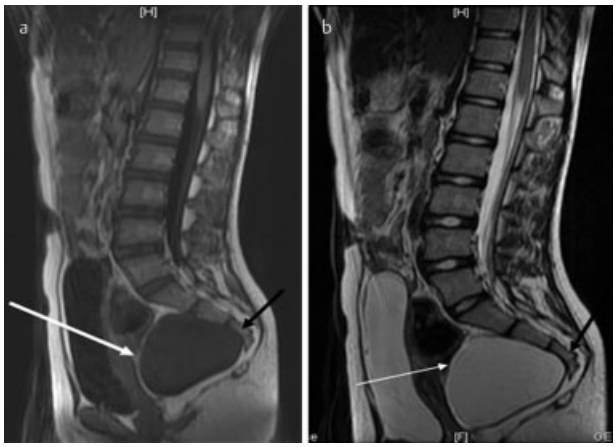

Figure 1 Midsagittal MRIs of Patient 1 (11-year-old girl). (**a**) T1-weighted image; (**b**) T2-weighted image. Partial distal sacral agenesis (black arrow) is present. A large cystic mass (white arrow) is present between the uterus and the sacrum.

Genetic Analysis

Using the methods described above, we identified a novel *HLXB9* heterozygous non-sense mutation (c.552C → G; p.Tyr 184X) affecting the highly conserved domain. The 2 patients, their mother, as well as their maternal grandmother presented with the same heterozygous p. Tyr184X mutation (►**Fig. 3**). The mother and maternal grandmother, who did not show any clinical features and did not undergo any X-ray or MRI examinations, were regarded as asymptomatic carriers. No mutation was present in the other members of the family. The screening of the *Isl1* and *Lim3* genes revealed no mutation in those genes in any members of the family.

Discussion

Currarino syndrome is mainly caused by *HLXB9* mutations attributed to a haploin-sufficiency.[9] To date, a total of 69 *HLXB9* mutations (including cytogenetic anomalies) have been identified.[12–14]

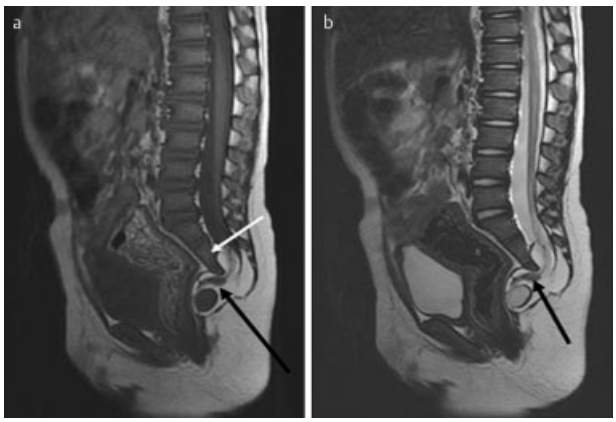

Figure 2 Midsagittal MRIs of Patient 2 (3-year-old boy). (**a**) T1-weighted image; (**b**) T2-weighted image. Partial distal sacral agenesis (white arrow) is present. There is a lipomyelomeningocele (black arrow) from the presacral region to the spinal canal. The spinal cord is tethered to L4.

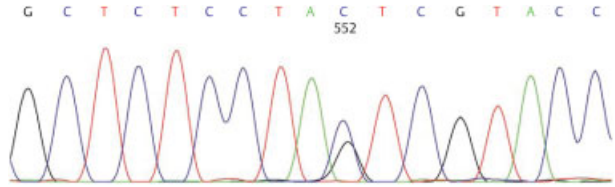

Figure 3 Direct sequencing analysis of the HLXB9 gene. A heterozygous non-sense mutation (c.552C → G; p.Tyr184X) was identified.

In this study, we report a novel *HLXB9* gene mutation (c.552C → G; p. Tyr184X) located in highly conserved domain. The p.Tyr184X is likely to originate as a truncated protein that, if stably translated, would lack the homeodomain region and would possibly affect its DNA-binding and transcription-regulation activities. To our knowledge, this is the first report of familial CS of Chinese origin; it confirms that familial CS patients in China have the same genetic background as other ethnicities.

It is of interest that the presacral mass of patient 1 recurred several years after the first resection. This could be due to an incomplete first resection. Patient 1 also was associated with Hirschsprung's disease. A combination of CS and Hirschsprung's disease has also been reported previously .[13–19] Whether the *HLXB9* gene is also involved in the pathways of Hirschsprung's disease still needs more research.

Constipation is a severe problem in patients with Currarino syndrome, and the true cause sometimes is difficult to elucidate. The constipation of Patient 2 in our study has remained after surgery and can be only managed with enemas. It may be caused by the combination of anorectal malformation, anterior myelomeningocele, and tethered cord.

The phenotypic variability observed in this study among the family members carrying the same mutation can be best explained by the existence of other modifier genes, which may affect the *HLXB9* protein partners or transcriptional regulators.[3,6,9,13] Candidate genes may include those known to be involved in the same pathways as *HLXB9*. We screened the *Isl1* and *Lim3* genes, both of which are regulators of *HLXB9* gene expression during the development of the motor neurons in other vertebrates.[12] In the case of the family studied, however, there were no mutations in the *Isl1* and *Lim3* genes in any family member. It will be necessary to screen more candidate genes to explain the presence of CS.

The identification of the *HLXB9* gene mutation confirms the CS diagnosis, and it is suggested that mutational analysis should be performed in patients suspicious for CS. It is still difficult to offer precise genetic counseling, due to the lack of genotype-phenotype correlations and the variability of expression in carriers.

Conflict of Interest

None

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