Currarino Syndrome and HPE Microform Associated with a 2.7-Mb Deletion in 7q36.3 Excluding *SHH* Gene

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Key Words

Currarino syndrome · Holoprosencephaly · Long-range enhancers · Microdeletion 7q36 · Sonic hedgehog

Abstract

Holoprosencephaly (HPE) is the most common forebrain defect in humans. It results from incomplete midline cleavage of the prosencephalon and can be caused by environmental and genetic factors. HPE is usually described as a continuum of brain malformations from the most severe alobar HPE to the middle interhemispheric fusion variant or syntelencephaly. A microform of HPE is limited to craniofacial features such as congenital nasal pyriform aperture stenosis and single central maxillary incisor, without brain malformation. Among the heterogeneous causes of HPE, point mutations and deletions in the *SHH* gene at 7q36 have been identified as well as extremely rare chromosomal rearrangements in the long-range enhancers of this gene. Here, we report a boy with an HPE microform associated with a Currarino syndrome. Array CGH detected a de novo 2.7-Mb deletion in the 7q36.3 region including the *MNX1* gene, usually responsible for the Currarino triad but excluding *SHH*, which is just outside the deletion. This new case provides further evidence of the importance of the *SHH* long-range enhancers in the HPE spectrum.

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Holoprosencephaly (HPE) is the most common malformation of the human forebrain resulting from incomplete cleavage of the prosencephalon associated with an abnormal development of midline neural structures early in gestation. It is estimated to occur in 1/16,000 live births and 1/250 conceptuses [Dubourg et al., 2007].

Four neuroanatomical classes of HPE are described in decreasing order of severity: alobar, semi-lobar, lobar, and middle interhemispheric variants or syntelencephaly. In 80% of affected individuals, a large spectrum of different craniofacial anomalies is also present [Solomon et al., 2011]. In the clinical subtypes of HPE, microforms are characterized by facial midline defects (e.g. solitary maxillary central incisor, hypotelorism, flat nasal bridge, mid-face hypoplasia, and congenital nasal pyriform aperture stenosis) without neurologic findings [Mercier et al., 2011]. The etiology of HPE is heterogeneous and may result from environmental, metabolic factors, prenatal exposures to drugs or infections. Numerical and structural chromosomal abnormalities as well as gene mutations are also responsible for isolated or syndromic HPE. To date, more than 14 causative genes have been identified [Solomon et al., 2011]. Among them, point mutations and gene deletions of the *SHH* (sonic hedgehog) gene (MIM 600725) at 7q36, responsible for *SHH* haploinsufficiency, were first

identified [Belloni and Roessler, 1996]. *SHH* is the most frequently mutated gene in HPE accounting for approximately 12% of cases [Roessler et al., 2009]. Despite of no clear phenotype-genotype correlation, the mutations of this gene are more related to milder forms of HPE such as microforms than those in other common HPE-related genes [Mercier et al., 2011; Solomon et al., 2012].

The Currarino triad associates partial sacral agenesis with presacral mass and anorectal malformations. Mutations in the gene *MNX1* (*HLXB9*) (MIM 142994), located in 7q36.3, were identified in nearly all patients with a familial form of Currarino syndrome and in 30% of those with a sporadic form [Lynch et al., 2000].

Large subtelomeric deletions in 7q36.3 have also been associated with patients presenting HPE and Currarino syndrome due to the loss of the *MNX1* and *SHH* genes. *SHH* is located 1.2 Mb proximal to *MNX1* [Lynch et al., 1995; Horn et al., 2004]. Chromosomal rearrangements like translocations or inversions with breakpoints mapped in the 5' upstream region of the *SHH* locus have been described in very few patients with HPE [Belloni et al., 1996; Roessler et al., 1997; Lettice et al., 2011]. The 5' upstream 1-Mb genomic region, starting from the *SHH* coding region to *LMBR1* (the limb development membrane protein 1) gene, contains a lot of evolutionarily highly-conserved long-range *SHH* enhancers. These enhancers promote *SHH* expression in key signaling pathways which are fundamental for forebrain, craniofacial, epithelial, and limb development. The specific forebrain enhancers (SBE1–4) are localized within *SHH* introns and 350–500 kb 5'upstream of the *SHH* gene (fig. 1) [Jeong et al., 2006].

We report a case of a boy with a microform of HPE and a Currarino syndrome associated with a 2.7-Mb deletion in the 7q36.3 region. Interestingly, this deletion includes the *MNX1*, but not the *SHH* gene.

Case Report

The proband was born at 37 weeks of gestation by caesarean section from nonconsanguineous healthy parents with no family history of congenital anomalies or developmental delay. No ultrasound abnormality was found during pregnancy. At 31 gestational weeks, the mother was treated by corticosteroid therapy because of a threatened preterm labor.

At birth, the patient was eutrophic [weight: 2,720 g (10–50th percentile), cranial perimeter: 32 cm (10th percentile)]. He presented hypotonia and an acute respiratory distress syndrome. In addition, he had abdominal distension, persistent vomiting and failure to pass meconium in the first 24 h. The exploration of this subocclusive syndrome by ultrasound and MRI scan revealed no digestive abnormalities but a partial sacral agenesis associated with a presacral lipoma, a low-lying spinal cord and an obstructive

megaureter, but no anorectal malformations. A syringomyelic filiform cavity was detected between T8 and T10 vertebrae. These abnormalities suggested an atypical Currarino syndrome.

A craniofacial CT scan revealed a congenital nasal pyriform aperture stenosis of about 5 millimeters (fig. 2a) as well as a single median maxillary incisor (fig. 2b). The pyriform stenosis was surgically treated. A magnetic resonance imaging scan showed no detectable structural brain anomalies.

Hypotelorism and iris, optic nerve and chorioretinal colobomas were also noted. All these clinical and radiological arguments were consistent with a HPE microform. No limb malformations were observed.

A standard G- and R-banding karyotype performed on lymphocyte cultures of peripheral blood was normal, 46,XY. A subtelomeric chromosome study using multiplex ligation-dependant probe amplification (MRC Holland, Amsterdam, The Netherlands) was normal.

Materials and Methods

Array CGH was performed using an 180,000-oligonucleotide microarray (Human Genome CGH Microarray Kit 180K, Agilent Technologies, Santa Clara, Calif., USA) according to the manufacturer's instructions. The average spacing of the probes was 13 kb. DNA from the patient was compared with DNA from 2 other patients with different diseases, according to the loop model [Menten et al., 2006]. Genomic Workbench software standard edition 6.5 (Agilent) was used to interpret the results with the following parameters: aberration detection algorithm 2, threshold 6.0, fuzzy zero, centralization, and moving average window 1 Mb. A copy number variation was noted if at least 3 contiguous oligonucleotides showed an abnormal log₂ ratio (>+0.58 or <-1 according to the Alexa 5 deviation) with a mirror image. The Database of Genomic Variants (<http://projects.tcag.ca/variation/>) was used to compare findings to previously reported studies. Coordinates of copy number variations are based on the GRCh37/hg19 assembly.

A FISH analysis was performed on interphase nuclei and metaphase chromosomes, using RP5-982E9 (Chr7:156,351,383–156,509,576; hg19) localized in the 2.7-Mb deletion.

Results

Array CGH analysis showed a deletion in chromosome band 7q36.3. The 2.7-Mb deletion extends from base 155,686,857 (first deleted oligonucleotide) to 158,384,574 (last deleted oligonucleotide) (NCBI, hg19) from the 7p telomere (fig. 3). No other abnormalities larger than 3 probes were observed, excluding well-known benign copy number variations (Database of Genomic Variants).

The 2.7-Mb deletion was confirmed by FISH analysis on interphase nuclei and metaphase chromosomes. FISH analyses of the parents' peripheral lymphocytes were normal, suggesting that the abnormality detected in the patient was de novo and likely causative of the patient's phenotype.

Fig. 1. Mapping of the deletion in 7q36.3. **a** Schematic representation of the 7q36.3 deletion described in our patient. Known protein-coding genes included in the deletion are reported in blue. The boxed section corresponds to the proximal breakpoint of the presented deletion. **b** Genomic view of the 7q36.3 region close to the *SHH* gene. Translocation breakpoints of T1–T4 (black arrows) in 7q36.3 and respective deletions in derivative chromosome 7 (rearrangements B, C, D, and E) described by Roessler et al. [1997] are reported (solid bars). The breakpoints are approximated in

these cases. Genomic imbalances associated with HPE phenotype are colored in red and with no HPE features in yellow. Chromosomal inversion (7)(q22.1;q36.3) reported by Lettice et al. [2011] is also represented (solid pink bar). The known cis-regulatory enhancers of *SHH* at 7q36.3 are indicated with colored circles: *SHH* floor plate enhancers (SFPE1+2) in purple, brain enhancers (SBE1–4) in blue, epithelial enhancers (MACS1, MRCS1 and MFC4) in yellow, and the limb-specific enhancer (ZRS) is shown in green.

Discussion

In the 2.7-Mb region, 13 genes (7 known protein-coding genes, 2 miRNA genes and 4 other noncoding RNA genes) are listed in NCBI (GRCh37/hg19). Among them, *LMBR1* (MIM 605522), *MNX1* and *DNAJB6* (MIM 611332) are disease-causing genes (fig. 1a).

MNX1, previously known as *HLXB9*, encodes a transcription factor with a homeobox domain. *MNX1* loss-of-function mutations including point mutations, chromosomal rearrangements or whole-gene deletions are associated with Currarino syndrome (OMIM 176450) [Crétolle et al., 2008; Markljung et al., 2012; Holm et al., 2013]. This autosomal dominant disorder was initially

described as a triad of anorectal, sacral and presacral anomalies [Currarino et al., 1981]. Currarino syndrome exhibits variable expressivity and incomplete penetrance [Horn et al., 2004]. A broad clinical spectrum has been reported, and anorectal malformations appear inconstant in Currarino syndrome [Crétolle et al., 2008]. Our patient presents a partial sacral agenesis associated with a presacral mass (lipoma), but no anorectal malformations. These signs can be related to *MNX1* haploinsufficiency.

LMBR1 (MIM 605522) is required for limb formation. A long-range cis-regulatory element located within intron 5 of the *LMBR1* gene governs normal *SHH* expression level, in the posterior zone of polarizing activity region. An evolutionary-conserved region, called the zone of polarizing activity regulatory sequence (ZRS; MIM 605522), is located about 1 Mb upstream from *SHH* and has enhancer activity that directs *SHH* expression in the developing posterior limb bud [Lettice et al., 2003]. ZRS mutations or duplications have been identified causing a wide range of limb malformations [VanderMeer and Ahituv, 2011]. Our patient has no limb abnormalities. In the Database of Genomic Variants, some copy number variations which result in a heterozygous deletion of *LMBR1* are reported [Wong et al., 2007; Pinto et al., 2011]. In the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (<http://decipher.sanger.ac.uk/>), one deletion inherited from a healthy parent (ID 263779) is also reported suggesting that heterozygous deletions of *LMBR1* are unlikely to be involved in a limb phenotype.

Our patient presents a mild holoprosencephaly spectrum phenotype. HPE reported with a 7q36.3 deletion usually includes *SHH* and is linked to its haploinsufficiency [Horn et al., 2004]. Our patient's deletion does not include *SHH*, which is 100 kb away from the proximal breakpoint (fig. 1a). There are very few clinical reports of HPE with cytogenetic rearrangements in 7q36.3 excluding the *SHH* gene. Roessler et al. [1997] reported the clinical features of patients with 4 different reciprocal translocations involving chromosome 7 (T1–T4) in which the breakpoints are in the 7q36.3 region. In these translocations, the centromeric breakpoints also map near *SHH*, but do not interrupt it. Most of these patients exhibit a mild HPE spectrum or a microform (table 1). All T4 patients with balanced translocations or the der(7) have no HPE phenotype (table 1). Although a variable expressivity cannot be excluded, the analysis of all the patients and our present case suggests that the minimal critical region might be situated in a 90-kb segment between T1 and T4 breakpoints [Roessler et al., 1997]. Interestingly, an *SHH*

forebrain enhancer, SBE4, is located in the middle of this segment (fig. 1b).

Recently, Lettice et al. [2011] described a de novo 7q inversion [inv(7)(q22.1;q36.3)] in a child with features of a holoprosencephaly spectrum (hypotelorism, microcephaly and solitary maxillary central incisor) and severe upper and lower limb syndactyly (table 1). MRI scan showed no detectable structural brain anomalies. The author suggested that limb anomalies are due to an enhancer adoption caused by the inversion, relocating the *SHH* gene close to a novel limb bud enhancer located in 7q22.1 [Lettice et al., 2011].

SHH mutations are most often associated with a microform or mild HPE, in contrast to mutations in other common HPE-related genes [Mercier et al., 2011; Solomon et al., 2012]. In the same way, it seems that a HPE phenotype associated with cytogenetic rearrangements occurring in the upstream portion of *SHH* gene is more often mild with predominantly HPE microforms (table 1). However, a broad clinical spectrum is observed, and no precise correlation between the phenotype and the genotype can be currently established. The variable HPE phenotype could be explained in different ways such as the extent of the deletion, an effect of an additional chromosomal imbalance, a possible position effect of the juxtaposed chromosomal regions which could modulate the expression of *SHH* [Lettice et al., 2011], mutations or deletion in another gene [Mercier et al., 2011], or additional environmental effects. Another explanation could be a possible imprinting effect at the *SHH* locus with disruption of an imprinting domain leading to an abnormal phenotype in some individuals, but not in some others [Lukusa et al., 2005].

Overall, in the reported cases above and in our present case, HPE spectrum is most likely due to the 7q36.3 breakpoints dislocating the *SHH* gene from its upstream forebrain enhancers (fig. 1b) [Jeong et al., 2006]. The SBE4 enhancer disruption might play a preponderant role in the HPE spectrum. However, Jeong et al. [2008] also reported a patient with semilobar HPE caused by a rare nucleotide variant located 460 kb upstream of *SHH* resulting in the loss of SBE2 activity. The parents' genotype revealed that the father was an unaffected carrier suggesting that in this case, additional multigenic or multifactorial effects are necessary to explain the HPE phenotype in the proband.

Other *SHH* enhancers are included in the deletion of our patient and in particular the long-range epithelial *SHH* enhancers (MRCS1, MFCS4 and MACS1) (fig. 1b) [Sagai et al., 2009]. The loss of the specific dental placode

Table 1. Holoprosencephalic findings in our patient with de novo 7q36 deletion and patients with chromosomal rearrangements involving 7q36 (reported in Roessler et al. [1997] and Lettice et al. [2011]) and not including *SHH* gene

Patients	T1 [Roessler et al., 1997]		T2 [Roessler et al., 1997]		T3 [Roessler et al., 1997]		T4 [Roessler et al., 1997]		Lettice et al. [2011]	Present case
Chromosomal rearrangements	t(7;9)(q36;q34)		t(6;7)(p21;q36)		t(2;7)(q31;q36)		t(7;17)(q36;p12)		inv(7) (q22.1;q36.3)	arr 7q36.3(155,686,857–158,384,574)×1
Status	balanced	unbalanced der(7)	balanced		balanced	unbalanced der(7)	balanced	unbalanced der(7)	balanced	unbalanced
No. of patients	3	2	1		3	1	1	3	1	1
Brain finding of HPE										
HPE subtype	microform	unknown	unknown		no HPE	microform	no HPE	no HPE	microform	microform
Other brain abnormalities	–	–	mild volume loss		/	arhinen- cephaly	/	/	–	hypoplasia of the pyriform aperture
Craniofacial findings of HPE										
Microcephaly	+ (2/3)	+ (2/2)	–		/	+	/	/	+	+
Hypotelorism	+ (1/3)	+ (2/2)	+		/	+	/	/	+	+
Single median incisor	–	+ (1/2)	–		/	–	/	/	+	+
Other teeth anomalies	+ (2/3)	+ (1/2)	+		/	–	/	/	–	–
Cleft palate	+ (1/3)	+ (1/2)	+		/	–	/	/	–	–
Cleft lip	–	–	+		/	–	/	/	–	+
Flat nose/nasal bridge	+ (3/3)	+ (2/2)	+		/	–	/	/	–	–
Ear anomalies	+ (3/3)	+ (2/2)	+		/	+	/	/	–	+
Ocular anomalies	–	+ (1/2)	+		/	+	/	/	+	+
Unkown = True HPE which was not further categorised in a specific type; + = features present; – = feature absent.										

enhancer MRCS1 may be associated with solitary maxillary central incisor [Lettice et al., 2011]. Involvement of MFCS4 and MACS1 is still uncertain. Furthermore, in mice, the haploinsufficiency of the MFCS4 leads to a normal phenotype [Sagai et al., 2009].

At last, *DNAJB6* encodes a highly conserved member of the HSP40/DNAJ cochaperone family. This family regulates the molecular chaperone activity of HSP70 by stimulating ATPase activity [Fan et al., 2003; Vos et al., 2008]. Mutations in *DNAJB6* have been recently linked to autosomal dominant limb-girdle muscular dystrophy type 1E (MIM 603511) [Harms et al., 2012; Sarparanta et al., 2012]. This disease is characterized by mid-adulthood onset of proximal limb weakness and skeletal muscle pathology [Sandell et al., 2010; Hackman et al., 2011]. At the age of

7 months, as expected, our patient lacks muscular signs. The long-term clinical follow-up will determine if these features are present.

In conclusion, this case provides further evidence of the importance of the *SHH* long-range enhancers in the HPE spectrum. This highlights the potential interest of looking for mutations or disruptions of *SHH* forebrain enhancers and in particular of SBE4 in HPE microforms without *SHH* mutations.

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