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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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 propositus 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 euophic [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 fi-

megaureter 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 [Men-
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 oligonucleo-
tides showed an abnormal log2 ratio (> +0.58 or < -1 according to the Alexa 5 deviation) with a mirror image. The Database of Ge-
nomic 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.
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; Markljug et al., 2012; Holm et al., 2013]. This autosomal dominant disorder was initially...
**Fig. 2.** Craniofacial CT scans of the patient. 
**a** Axial CT scan showing congenital pyriform aperture stenosis. **b** Axial CT scan showing solitary median maxillary incisor (arrow).

**Fig. 3.** Array CGH analysis of the microdeletion in chromosome 7 of our patient. Array CGH profile of chromosome 7 shows a 2.7-Mb heterozygous deletion in 7q36.3. Genomic position of the first deleted (7:155,686,857) and the last deleted oligonucleotides (7:158,384,574) are indicated with black arrows. The *SHH* gene (red circle) is located upstream of the first deleted oligonucleotides.
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 inconsistent 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 Currarino syndrome [Crétolle et al., 2008]. Our patient reported, and anorectal malformations appear inconstant [Horn et al., 2004]. A broad clinical spectrum has been described as a triad of anorectal, sacral and presacral anomalies [Mercier et al., 2011]. 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 (MRC51, MFCS4 and MACS1) (fig. 1b) [Sagai et al., 2009]. The loss of the specific dental placode

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

HPE Microform and 7q36.3 Deletion
Excluding SHH Gene

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].

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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.

Acknowledgement

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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

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<td>t(6,7)(p21;q36)</td>
<td>t(2,7)(q31;q36)</td>
<td>t(7;17)(q36;p12)</td>
<td>inv(7) (q22.1;q36.3) 7q36.3(155,686,857–158,384,574)×1</td>
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<td>arhinencephaly</td>
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<td>+ (1/2)</td>
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<tr>
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Unknown = True HPE which was not further categorised in a specific type; + = features present; – = feature absent.

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