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Short communication

Dental developmental abnormalities in a patient with subtelomeric 7q36 deletion syndrome may confirm a novel role for the *SHH* gene ☆

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ABSTRACT

Studies in mice demonstrated that the *Shh* gene is crucial for normal development of both incisors and molars, causing a severe retardation in tooth growth, which leads to abnormal placement of the tooth in the jaw and disrupted tooth morphogenesis. In humans the *SHH* gene is located on chromosome 7q36. Defects in its protein or signaling pathway may cause holoprosencephaly spectrum, a disorder in which the developing forebrain fails to correctly separate into right and left hemispheres and that can be manifested in microforms such as single maxillary central incisor. A novel role for this gene in the developing human primary dentition was recently demonstrated. We report a 12-year old boy with a *de novo* 7q36.1-qter deletion characterized by high-resolution karyotyping, oligonucleotide aCGH and FISH. His phenotype includes intellectual disability, non-verbal communication, hypospadia, partial sacral agenesis and absence of coccyx, which are distinctive features of the syndrome and mainly correlated with the *MNX1*, *HTR5A* and *EN2* genes. No microforms of

Abbreviations: OFC, occipitofrontal circumference; BERA, brainstem evoked response audiometry; aCGH, array comparative genomic hybridization; CNV, copy number variation; FISH, fluorescence in situ hybridization; ASD, autism spectrum disorder

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holoprosencephaly spectrum were observed; but the patient had diastema and dental developmental abnormalities, such as conical, asymmetric and tapered inferior central incisors. The dental anomalies are reported herein for the first time in subtelomeric 7q36 deletion syndrome and may confirm clinically a novel role for the *SHH* gene in dental development.

Introduction

The classical phenotype of patients with terminal 7q deletions includes developmental delay, microcephaly, genital abnormalities in males, facial anomalies and intellectual disability. Deletions range in size and may extend from 7q32-qter to 7q36-qter. Comprehensive reviews summarized the clinical features of the previously reported patients with different 7q terminal deletions, mostly involving 7q32 (Frints et al., 1998; Lukusa et al., 2005). Nevertheless, only eight of these individuals had isolated 7q36-qter deletions.

In many patients the terminal 7q36 deletion was associated with microforms of holoprosencephaly, sacral agenesis and intellectual disability. The genes *SHH* (Sonic Hedgehog, MIM 600725), *MNX1* (Motor neuron and pancreas homeobox 1, MIM 142994), *HTR5A* (5-hydroxytryptamine receptor 5A, MIM 601305) and *EN2* (engrailed 2, MIM 131310) may be involved with the major clinical features of the syndrome (Cretolle et al., 2008; Dubourg et al., 2007; Millen et al., 1994; Rees et al., 1994). Since these genes are located at 7q36, a more accurate genotype–phenotype correlation would be possible with reports of additional patients who present as their sole rearrangement the deletion of this region.

In this study we report a 12-year old boy with partial sacral agenesis and absence of coccyx, intellectual disability, no signs of holoprosencephaly, diastema, dental developmental abnormalities and a *de novo* 7q36.1 deletion. The extension of the deletion was established by high-resolution karyotyping, oligonucleotide aCGH and FISH. In addition, we discuss his phenotype in relation to the putative genes located in the deleted region and we propose novel roles for the *SHH* gene related to diastema and dental developmental abnormalities such as conical, asymmetric and tapered inferior central incisors.

Materials and methods

This study was approved by the Research Ethics Committee of *Universidade Federal de Minas Gerais*. The written informed consent was undersigned by guardians.

Clinical report

A 12-year-old boy born to healthy unrelated parents is presented (Fig. 1). He has two older healthy brothers. The pregnancy and elective cesarean delivery were uneventful. At birth his weight was 3100 g (10–25th centile), length 49 cm (25th centile) and occipitofrontal circumference (OFC) 33 cm ($\leq$10th centile). He presented hypospadias and hipodysplasia was suspected, so he used double diapers until the age of seven months. Neonatal screening for PKU and hypothyroidism was normal.

Until 6 months he was a very quiet baby. His development was mildly delayed as he sat alone at 12 months and walked independently at 19 months of age. He spoke “mama” for a short time, and then his communication became only non-verbal and gestural.

He was evaluated by a geneticist at 12 months of age and presented weight 8720 g (10th centile), length 71.2 cm (10th centile), OFC 44.1 cm (5th centile), hypotonia, hand-flapping, thin and weak hair, hypospadias with urethral meatus opening in the glans penis and pubic hair. Hearing evaluation at 13 months revealed serous otitis and abnormal BERA with moderate to severe hipoacusia. Repeated evaluation with BERA nine months later was normal. He evolved also with mild hyperopia and astigmatism. Laboratory evaluation at this time showed normal biotinidase activity, blood amino acids, copper and ceruloplasmin, testosterone, 17-α-hydroxyprogesterone, androstenedione and complete blood count. His karyotype was reported as normal. At the age of 13 months, his bone age corresponded to

1.3 years by the Greulich–Pyle Atlas and the lateral neck radiography showed mild adenoid hypertrophy. Cranium tomography, brain nuclear magnetic resonance and electroencephalogram were normal.

His dentition started at four months of age. He had upper premaxillary prominence, forwarded upper teeth with diastema between the superior incisors and the inferior central incisors had developmental abnormalities: they were asymmetric, tapered and conic and the left side was more affected (Fig. 2A). Currently he has full permanent dentition for his age. Radiographically there is no *mesiodens*. His facial profile is typical of a mouth breathing person (Fig. 1).

His growth is normal at age 12, his height is in the 25th percentile, weight in the 10th percentile and OFC in the 10–25th percentile. He is a docile and happy boy, dispersive and a bit hyperactive, spends just one hour per day in a special school and has learning disability. He has control of sphincters since the age of seven.

Multiprobe subtelomeric FISH (*Cytocell* Ltd., Adderbury, UK) was performed at 5 years and 6 months disclosing an isolated subtelomeric deletion of chromosome 7q36 (probe 2000A5). Because of his diagnosis of 7q36 deletion syndrome, radiograph of the lumbosacral was performed at 6 years and 11 months and detected partial sacral agenesis and absence of coccyx (Fig. 2B, C). In addition, clinical exam, echocardiography, electrocardiography, and ions were recently performed and were normal.

Cytogenetic analysis

Chromosome preparations from the patient and his parents were obtained from lymphocyte cultures of peripheral blood. In order to obtain high-resolution chromosomes of the patient, we combined cell synchronization with thymidine and the addition of ethidium bromide. Chromosome analysis was performed after GTG-banding.

Array comparative genomic hybridization (aCGH)

Genomic DNA was isolated from patient lymphocytes using the Qiagen DNA extraction kit (Santa Clara, CA). The investigation of copy number changes was performed by aCGH using the Whole Human Genome CGH Microarray 60K (Oxford Gene Technologies, Oxford, UK) following the manufacturer's protocol. Scanned images of the arrays were processed using the Feature Extraction software (Agilent Technologies, Santa Clara, CA). We applied the Genomic Workbench software (Agilent Technologies) for calling DNA CNV using the Aberration Detection Method 2 statistical algorithm with a sensitivity threshold of 6.7. Duplication or deletion was considered when the $\log_2$ ratio of the Cy3/Cy5 intensities of a given region encompassing at least three probes was >0.3 or <-0.3, respectively. Mapping data were analyzed using the UCSC genome browser — NCBI Build 36, Hg18 (http://genome.ucsc.edu/).

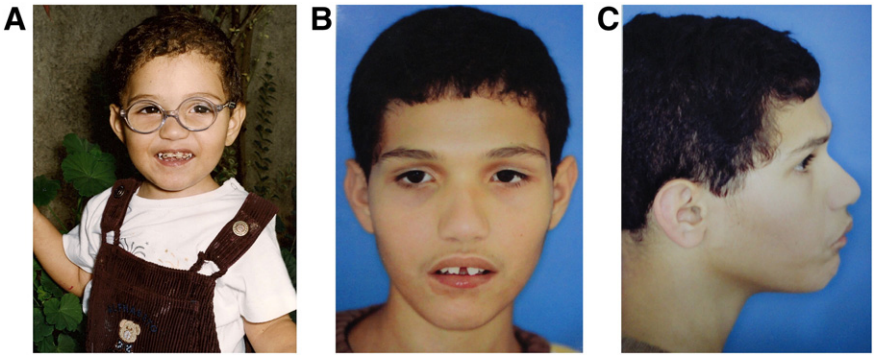

Fig. 1. Facial appearance of the patient. (A) Patient at age 1 year and 4 months. (B, C) Frontal and lateral view at age 11 years showing he had upper premaxillary prominence and forwarded upper teeth with diastema between the superior incisors. Note his facial profile typical of a mouth breathing person.

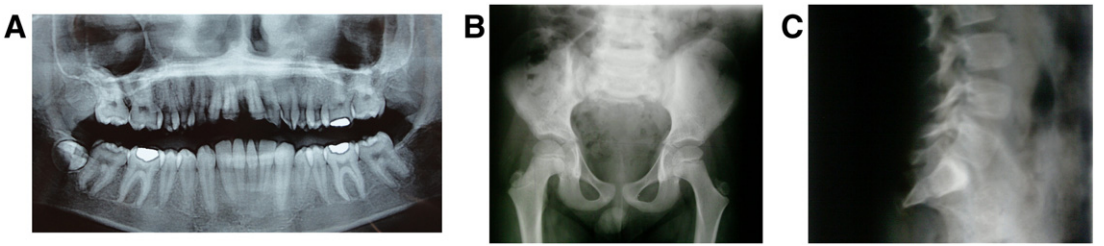

Fig. 2. Dental abnormalities and caudal deficiency sequence of the patient. (A) Panorex X ray showing diastema between the maxillary central incisors and inferior central incisors asymmetric, tapered and conic. (B, C) Frontal and lateral radiographs of lumbosacral spine showing partial sacral agenesis and absence of coccyx.

Fig. 3. High-resolution pair 7 of five patient cells. The normal chromosome 7 is on the left and the abnormal one is on the right and pointed with arrows. On the last pair on the right, the long arm of chromosome 7 is magnified and the deleted region highlighted in the rectangle.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) was performed in the chromosome preparations of the patient and both his parents with the subtelomeric 2000a5 7q probe (Cytocell Ltd., Cambridge, UK)

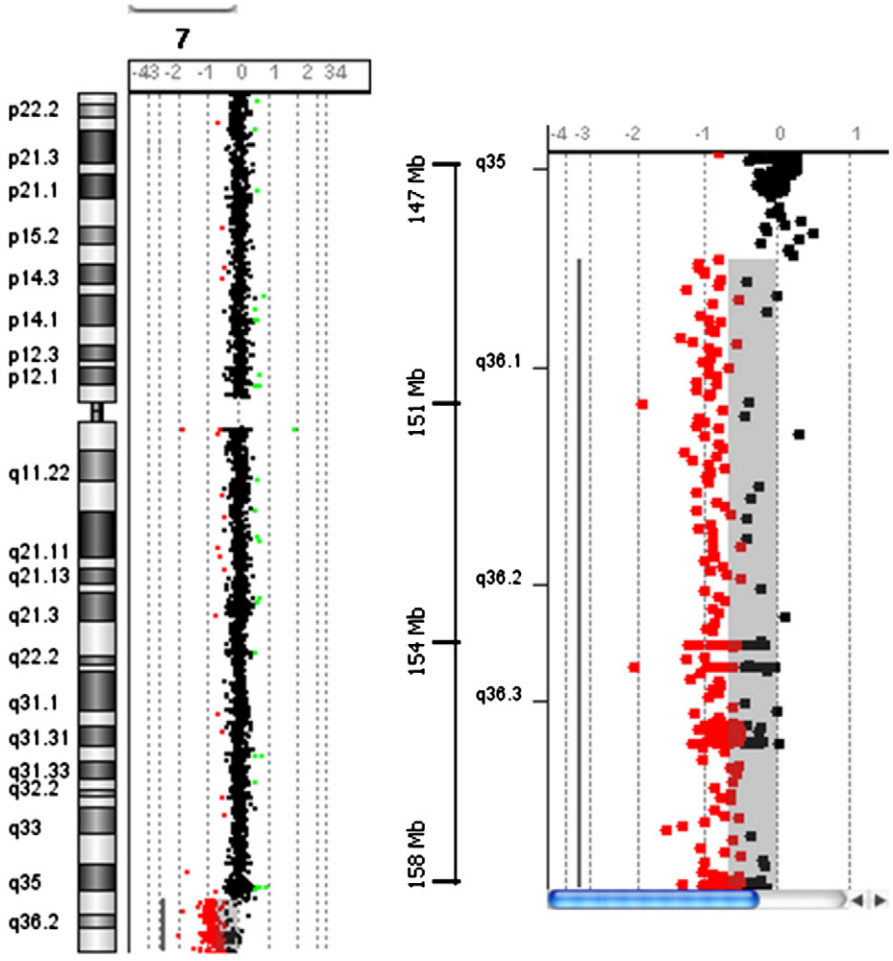

Fig. 4. Copy number profile of chromosome 7 of our patient obtained by oligonucleotide aCGH (60K platform). The patient's ~10.02 Mb 7q36.1-qter deletion is indicated by the red dots on the left panel and shown in detail on the right panel. Images obtained from Genomic Workbench Software (Agilent).

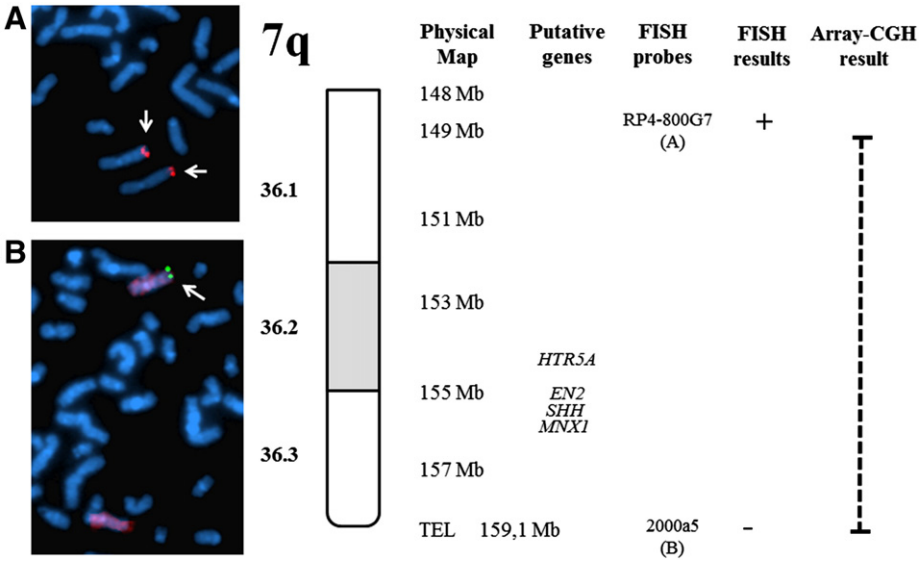

Fig. 5. Characterization of the 7q deletion in the patient. (A) Probe RP4-800G7 (red) hybridized on both chromosomes 7. (B) The subtelomeric 2000a5 probe (green signal) hybridized only to the normal chromosome 7 (arrow) identified by sequential hybridization with a chromosome 7 painting probe (red). Ideogram of chromosome 7q36, physical map, putative genes, FISH probes used in this study and FISH results of the patient are indicated in order, according to the clone placement on the Ensembl database (http://www.ensembl.org). The extension of the deletion according to aCGH result is indicated by the interrupted vertical line. +, signals detected on both chromosomes 7; -, absence of signal on the abnormal chromosome 7.

according to the supplier's instructions. In order to confirm the location of the probe, sequential FISH was performed with a chromosome 7 specific painting probe. FISH with clone RP4-800G7 from proximal 7q36 was performed to confirm the aCGH results. The probe was selected from the Ensembl database (http://www.ensembl.org) and was obtained according to standard procedures.

Results

Chromosome analysis at 600-band resolution evidenced the presence of a cryptic deletion at the terminal part of the long arm of chromosome 7 [46,XY,del(7)(q36.1)] (Fig. 3). Both parents presented a normal karyotype at 400–450 band resolution. Further analysis using oligonucleotide aCGH revealed a chromosomal deletion of about 10.02 Mb spanning from 7q36.1 (probe 0364_152591c7_1_98_s_PSO-60-0036, located at chr7:148,796,048-148,796,107) to 7q36.3 (probe 0364_162258c7_1_125_s_PSO-60-0002, located at chr7:158,821,257-158,821,316) (Fig. 4).

FISH studies in the parents using the subtelomeric 2000a5 7q probe and a whole chromosome 7 painting probe showed signals on both chromosome 7 homologues, indicating no balanced translocations involving 7q36. In the patient, FISH with the probe 2000a5 revealed signals in only one homologue, confirming the monosomy of distal 7q. Therefore, the patient carries a *de novo* terminal deletion. FISH with the RP4-800G7 clone on metaphases of the patient revealed signals on both chromosome 7 homologues, confirming the aCGH results since this probe is located at 148.5 Mb. The results of FISH analysis and aCGH are summarized in Fig. 5.

Discussion

The major clinical features associated with the 7q36 subtelomeric deletion syndrome include developmental delay, intellectual disability, caudal deficiency sequence and microforms of holoprosencephaly. Holoprosencephaly is a structural anomaly of the brain in which there is failed or incomplete separation of the forebrain into distinct hemispheres during early neurogenesis. The phenotypic expression of

holoprosencephaly is extremely variable including severe associated craniofacial anomalies such as cyclopia or proboscis to the so-called microforms: microcephaly, hypotelorism, cleft lip and or/palate, single maxillary central incisor, agenesis of the corpus callosum, iris coloboma, and dysgenesis of the pituitary gland (Cohen, 2006).

The human *SHH* gene was the first holoprosencephaly-causing gene to be identified and mutations and deletions of this gene appear to be the most common genetic cause of human holoprosencephaly identified to date (Dubourg et al., 2007). This gene is located within the HPE3 critical region on 7q36.3 and plays a role in early forebrain and central nervous system development. In families with *SHH* mutations or deletions a wide holoprosencephaly phenotypic spectrum has been observed (Frints et al., 1998). Even within one family, the same *SHH* mutation may be present in individuals with holoprosencephaly, with microforms and asymptomatic (Roessler et al., 1996).

Many reported patients manifested microforms of holoprosencephaly in conjunction with partial sacral agenesis. Sacral agenesis ranges from partial agenesis of the coccyx to the complete absence of the sacrum and lumbar vertebrae (Pang, 1993). Ross et al. (1998) identified causative mutations in *MNX1* (previously known as *HLXB9*) in several hereditary sacral agenesis families. *MNX1* is located 1.2 Mb distal to *SHH*, at 7q36.3, and at least 66 different heterozygous cytogenetic or molecular anomalies at this locus have been identified in Currarino syndrome patients (Cretolle et al., 2008). Currarino syndrome (MIM 176450) is an autosomal dominant congenital malformation characterized by total sacral agenesis below S2, a pre-sacral mass and anorectal anomalies.

There are genes important for brain development and function located at 7q36: *HTR5A*, within 7q36.2, appears to be expressed only in the central nervous system (amygdala, caudate nucleus, cerebellum, hypothalamus, substantia nigra and thalamus) (Rees et al., 1994) and *EN2*, within 7q36.3. A mouse knockout for *En2* presented severe cerebellar hypoplasia, indicating that absence of expression of this gene negatively impacts cerebellar development (Millen et al., 1994). This anatomical phenotype in mouse cerebellum could be comparable with those reported for individuals with autism (Gharani et al., 2004). In addition, human *EN2* maps to a region that has shown a suggestive linkage to autism spectrum disorder (ASD) (AUTS10, MIM 611016). Moreover, some studies have revealed an association between autism and single-nucleotide polymorphisms (SNPs) in the *EN2*, providing stronger evidence that this gene may act as an ASD susceptibility locus (Benayed et al., 2009).

Heart defects were found in only one patient with 7q36.1q36.2 deletion identified by aCGH. Her electrocardiogram showed long QT intervals and sinus tachycardia (Caselli et al., 2008). These authors performed thorough correlations between the cardiac phenotype in this patient and related genes in the 7q36 region, such as *KCNH2* (MIM 152427) and *PRKAG2* (MIM 602743). Since these genes are deleted in our patient, his cardiologic evaluation was performed, but the results were normal.

The literature review suggests that the distinctive phenotype in patients with 7q36 deletion syndrome is developmental delay, caudal deficiency sequence and microforms of holoprosencephaly — despite the absence of the latter in our patient. Diastema and dental developmental abnormalities are, to our knowledge, herein reported for the first time in 7q36 deletion syndrome and may be additional findings related to *SHH* deletion. In fact, three patients have been reported with 7q36 deletions comprising the *SHH* gene and other teeth anomalies, such as unevenly spaced teeth and absence of incisors (Beleza-Meireles et al., 2013; Horn et al., 2004).

Studies in mice demonstrated that the homolog *Shh* regulates growth and determines the shape of the tooth, involving a combination of *Shh* signaling at sites where teeth are required and antagonism in regions destined to remain non-tooth-forming edentulous (diastema regions) (Cai et al., 2011; Cobourne et al., 2004; Dassule et al., 2000; Ohazama et al., 2009). Furthermore, Hovorakova et al. (2011), using a combination of computer-aided three-dimensional reconstructions and whole mount in situ hybridization of mandibles from finely staged wild-type mouse embryos, demonstrated that several *Shh* expression domains sequentially appear in the lower incisor region during early development. Additionally, Nakatomi et al. (2013), using a similar approach with *Evc* knockout mice, showed that the cilial protein *Evc* is required for a spatially balanced *Shh* pathway activation during molar development. The authors concluded that disruptions of the *Shh* pathway could be the primary cause for the variable dental anomalies seen in patients with Ellis–van Creveld (EvC) syndrome (MIM 225500) or Weyers acrodental dysostosis (MIM 193530). These syndromes are caused by mutations in *EVC* (MIM 604831) and *EVC2* (MIM 607261) genes and the dental anomalies found in EvC patients include neonatal teeth, partial

anodontia, conical and microdontic teeth, enamel hypoplasia and delayed eruption of teeth (Kalaskar and Kalaskar, 2012).

Conditional *Shh*- and *Smo*- deficient mice (*Smo* is a membrane protein essential for the transduction of all Hedgehog signals) exhibit morphological aberrations in tooth patterns such as small and abnormally shaped incisors and first molars, abnormal enamel morphology, fused molars and absent dental cord (Dassule et al., 2000; Gritli-Linde et al., 2002). Until recently, the studies on tooth development were conducted mainly in mice. However, Hu et al. (2013) examined the expression profiles and patterns of *SHH* signaling molecules in the developing human embryonic tooth germ. Their results demonstrate an active *SHH* signaling that operates in the developing human tooth and suggest a conserved function of *SHH* signaling pathway during human odontogenesis.

Conclusions

The patient reported in this study has a *de novo* ~10.02 Mb deletion in 7q36.1-qter; a region that harbors 136 genes (counting pseudogenes, hypothetical genes and microRNA — NCBI Map Viewer Build 36.3), comprising 39 genes described in OMIM, including the putative candidates *SHH*, *MNX1*, *HTR5A* and *EN2* (see Supplementary Table 1 for description and function summary of the genes). Our study highlights the value of high-resolution chromosome analysis and/or molecular techniques as an aid tool for the diagnosis of individuals suspected of 7q36 deletion. The application of these techniques may contribute to the diagnosis and description of additional patients with subtelomeric 7q deletion syndrome. Moreover, the report of our patient may confirm a novel role for the *SHH* gene related to diastema and dental developmental abnormalities such as conical, asymmetric and tapered inferior central incisors.

Database linking

Ensembl database — http://www.ensembl.org
UCSC genome browser — http://genome.ucsc.edu/
NCBI — http://www.ncbi.nlm.nih.gov/
OMIM – Online Mendelian Inheritance in Man® – http://www.omim.org/
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2013.10.005.

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