CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Holoprosencephaly, Ectrodactyly, and Bilateral Cleft of Lip and Palate: Exclusion of *SHH*, *TGIF*, *SIX3*, *GLI2*, *TP73L*, and *DHCR7* as Candidate Genes

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Received 3 December 2008; Accepted 9 March 2009

We describe a Brazilian boy with semilobar holoprosencephaly, ectrodactyly, bilateral cleft of lip and palate, and severe mental retardation. The karyotype was normal and the screening for mutations in the genes *SHH*, *TGIF*, *SIX3*, *GLI2*, *TP73L*, and *DHCR7* did not show any change. This rare condition was described previously in seven male patients. Clinical and genetic aspects are discussed.

Key words: holoprosencephaly; ectrodactyly; cleft lip-palate; causative genes

How to Cite this Article:
Zechi-Ceide RM, Ribeiro LA, Raskin S, Pereira Bertolacini CD, Guion-Almeida ML, Richieri-Costa A. 2009. Holoprosencephaly, ectrodactyly, and bilateral cleft of lip and palate: Exclusion of *SHH*, *TGIF*, *SIX3*, *GLI2*, *TP73L*, and *DHCR7* as candidate genes.
Am J Med Genet Part A 149A:1277–1279.

INTRODUCTION

A syndrome of holoprosencephaly, ectrodactyly, and cleft lip and palate was first reported by Hartsfield et al. [1984] in a boy who died at the age 7 days. Similar observations were described later in another six boys. Additional signs such as craniosynostosis, hypertelorism or hypotelorism, microphthalmia, abnormal ears, radial agenesis, genital anomalies, severe psychomotor retardation, and hypothalamic-pituitary dysfunction have been observed [Hartsfield et al., 1984; Van Maldergem et al., 1992; Young et al., 1992; Imaizumi et al., 1998; Corona-Rivera et al., 2000; Abdel-Meguid and Ashour, 2001; König et al., 2003]. Cause remains unknown. All reported cases were male patients and one of them had an apparently balanced de novo t(2;4)(q14.2;q35). Here we describe another boy with this condition. The study of the main determinant genes of HPE was unremarkable. Clinical and genetic aspects are discussed.

CLINICAL REPORT

The propositus (Fig. 1), male, was the 2nd child born to a G3P2A1 normal 29-year-old mother and her normal nonconsanguineous 34-year-old husband. The 1st gestation resulted in spontaneous abortion and the 2nd in a normal girl. Pregnancy was complicated by vaginal bleeding in the 3rd month. There was no history of hyperthermia, high blood pressure, diabetes, or exposure to toxic, traumatic, infectious agents or radiation. Delivery was through cesarean section, at term. Birth weight was 2,900 g (10th centile) and length was 47 cm (3rd centile). Bilateral cleft lip-palate and limb anomalies were noted at birth. Clinical examination at 48 days showed broad nasal bridge with normal inter canthal distance, megalocornea, bilateral cleft lip-palate, prominent ears, ectopic testes, and small penis. The left 2nd and 3rd digits and the right 3rd digit were hypoplastic. Both feet had three toes, large halluces, deep gap between toes 1–2, and complete 4–5 cutaneous syndactyly (Fig. 2). The follow-up of the propositus showed length, weight, and OFC below 3rd centile; inner canthal distance of 2.4 cm (25th centile), and external canthal distance of 7.5 cm (25–50th centile). Clinical examination at age 3 years showed severe psychomotor retardation with poor head control. He could not sit or stand by himself or grasp objects and he could eat only mashed food. Expressive and receptive language development was absent;

Grant sponsor: The State of São Paulo Research Foundation (FAPESP); Grant sponsor: National Council for Scientific and Technological Development (CNPq); Grant numbers: 310659/2006-0; 470996/2006-4301926/2007-7.
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Published online 15 May 2009 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.32844

FIG. 1. Frontal view of the face of the propositus.

however, he was able to recognize his parents and sister. Radiographic examination at age 3 years showed: bilaterally short and bowed 1st and 2nd metacarpi, hypoplastic distal phalanx of the fingers; well preserved centers of the distal tibia, fibula, talus and calcaneus, absence of the lateral and intermediate cuneiform and the navicular absent left 3rd metatarsus, distal fusion of the right 3rd and 4th metatarsi, right hallux phalanges fused, absent 2nd and 3rd toe phalanges, and absent 4th and 5th toe middle and distal phalanx (Fig. 3). Spine films showed absent posterior arch in T1, T2, and T3. Brain MRI showed semilobar holoprosencephaly with fusion of the frontal lobes, absence of the frontal horns of the lateral ventricles, abnormal frontal lobe gyration, callosal agenesis, abnormally modeled and partially fused basal ganglia, and hypoplastic brain stem (Fig. 4A,B).

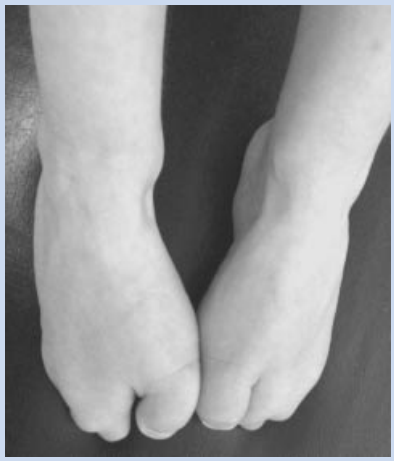

FIG. 2. Clinical aspects of the feet.

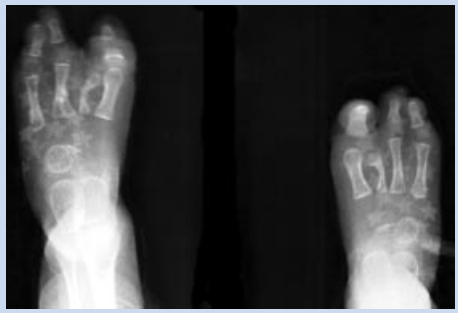

FIG. 3. Radiological findings in the feet.

CYTOGENETIC AND MOLECULAR ANALYSIS

Results of cytogenetic analysis of peripheral blood lymphocytes from the patient, performed using standard techniques and processed by G-banding (550 bands) were normal. Direct sequencing of the entire coding region and splice sites of the *SHH*, *TGIF*, *SIX3*, and *GLI2* genes, as previously described [Ribeiro et al., 2004; Richieri-Costa and Ribeiro, 2006] did not show any causative change. Screening for mutations in the entire coding region and splice sites of the *TP73L* and *DHCR7* gene was also performed, but no mutations were detected.

DISCUSSION

The patient here described has the holoprosencephaly, ectrodactyly, and bilateral cleft lip-palate syndrome (OMIM 300571 [OMIM, 2008]), a rare condition of unknown cause, involving midline and limb field defects. So far, eight sporadic male patients, including the present, were reported. The occurrence of this rare phenotype only in male might point to an X-linked recessive syndrome. However, at the moment, autosomal dominant inheritance with possible lethality in females can not be excluded. Due to the phenotypic overlap with EEC syndrome (OMIM 604292 [OMIM, 2008]), König et al. [2003] analyzed the *TP73L* gene and found no mutation in causative exons. The analysis of this gene in our patient did not show any change. Considering brain midline defects, we also analyzed some of the most common causative genes for holoprosencephaly: *SHH*, *TGIF*, *SIX3*, and *GLI2* but any mutation was found. To date, the only potential causal correlation involving the present condition was found in a previous report on a boy who presented a de novo translocation involving the 2q14 region which harbors the *GLI2* gene [Corona-Rivera et al., 2000]. *GLI2* is one of three vertebrate transcription factors implicated as obligatory mediators of *SHH* signal transduction. Diminished *SHH* signaling is associated with the most common forebrain defect in humans, holoprosencephaly. Up to now, three different mutations in the *GLI2* gene were described leading to a phenotype that includes pituitary anomalies with holoprosencephaly-like manifestations, bilateral cleft lip and palate, and polydactyly [Roessler et al., 2003; Rahimov et al., 2006]. Interestingly, hypothalamic-pituitary dysfunction was also observed in four of the seven previous cases described with the present condition [König et al., 2003] and all patients, including our case,

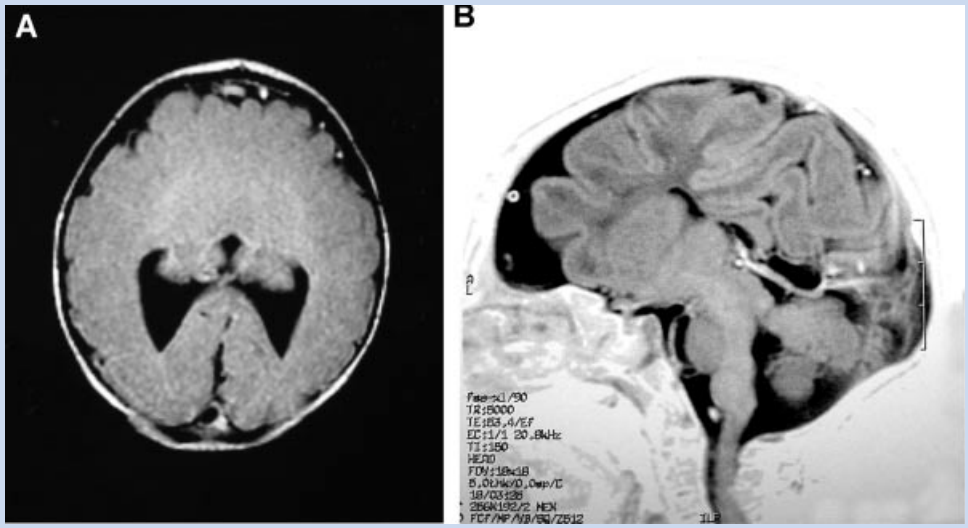

FIG. 4. A,B: Brain MRI.

lacked a classical holoprosencephy facial appearance. In addition, recently Babbs et al. [2007] described a patient with split hand/foot malformation with long bone deficiency (SHFLD) who carries a de novo chromosomal translocation involving the 2q14 region suggesting a novel locus for SHFLD. In our case the analysis of the *GLI2* gene did not show any change but the possibility of the phenotype being caused by microdeletion at 2q14 could not be excluded. We also excluded mutations of other HPE causative genes. The occurrence of manifestations only in males strongly suggests a possible X-linked mechanism not yet determined.

ACKNOWLEDGMENTS

This study was supported by grants from: State of São Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq) (Grant 310659/2006-0; M.L.G.-A. and Grant: 470996/2006-4 and 301926/2007-7; A.R.-C.). Special thanks to Dr. Jeffrey Murray and his staff for the courtesy in performing part of the molecular studies.

REFERENCES

Abdel-Meguid N, Ashour AM. 2001. Holoprosencephaly and split hand/foot: An additional case with this rare association. Clin Dysmorphol 10:277–279.

Babbs C, Heller R, Everman DB, Crocker M, Twigg SRF, Schwartz CE, Giele H, Wilkie AOM. 2007. A new locus for split hand/foot malformation with long bone deficiency (SHFLD) at 2q14.2 identified from a chromosome translocation. Hum Genet 122:191–199.

Corona-Rivera A, Corona-Rivera JR, Bobadilla-Morales L, Garcia-Cobian TA, Corona-Rivera E. 2000. Holoprosencephaly, hypertelorism, and ectrodactyly in a boy with an apparently balanced de novo t(2;4)(q14.2;q35). Am J Med Genet 90:423–426.

Hartsfield JK, Bixler D, DeMyer WE. 1984. Syndrome identification case report 119. Hypertelorism associated with holoprosencephaly and ectrodactyly. Clin Dysmorphol 2:27–31.

Imaizumi K, Ishii T, Masuno M, Kuroki Y. 1998. Association of holoprosencephaly, ectrodactyly, cleft lip/palate and hypertelorism: A possible third case. Clin Dysmorphol 7:213–216.

König R, Beeg T, Tariverdian G, Scheffer H, Bitter K. 2003. Holoprosencephaly, bilateral cleft lip and palate and ectrodactyly: Another case and follow up. Clin Dysmorphol 12:221–225.

OMIM. 2008. Online Mendelian Inheritance in Man, McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and the National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD).

Rahimov F, Ribeiro LA, de Miranda E, Richieri-Costa A, Murray JC. 2006. GLI2 mutations in four Brazilian patients: How wide is the phenotypic spectrum? Am J Med Genet Part A 140A:2571–2576.

Ribeiro L, El-Jaick K, Muenke M, Richieri-Costa A. 2004. *SIX3* and *TGIF* in holoprosencephaly—Mutations, phenotypes, and imaging. How different are they? 54th Annual Meeting of the American Society of Human Genetics, Abstract # 400.

Richieri-Costa A, Ribeiro LA. 2006. Holoprosencephaly-like phenotype: Clinical and genetic perspectives. Am J Med Genet Part A 140A:2587–2593.

Roessler E, Du Y-Z, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A, Muenke M. 2003. Loss-of-function mutations in the human *GLI2* gene are associated with pituitary anomalies and holoprosencephaly-like features. Proc Nat Acad Sci 100: 13424–13429.

Van Maldergem L, Gillerot Y, Vamos E, Toppet M, Watillon P, Van Vliet G. 1992. Vasopression and gonadotropin deficiency in a boy with the ectrodactyly-ectodermal dysplasia-clefting syndrome. Acta Paediatr 81:365–367.

Young ID, Zuccollo JM, Barrow M, Fowlie A. 1992. Holoprosencephaly, telecanthus and ectrodactyly: A second case. Clin Dysmorphol 1:47–51.