Published in final edited form as:

Am J Med Genet C Semin Med Genet. 2010 February 15; 154C(1): 176–182. doi:10.1002/ajmg.c.30234.

Holoprosencephaly and Craniosynostosis: A Report of Two Siblings and Review of the Literature

Manu S. Raam^{1,2}, Benjamin D. Solomon¹, Stavit A. Shalev^{3,4}, and Maximilian Muenke¹

¹ Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland ² HHMI-NIH Research Scholars Program, Howard Hughes Medical Institute, Chevy Chase, Maryland ³ Genetic Institute, Emek Medical Center, Afula, Israel ⁴ Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Abstract

Holoprosencephaly and craniosynostosis are separate conditions that have occasionally been observed to occur simultaneously in the same patient. Here, we compile patients with both conditions that have been documented in the literature thus far; moreover, we report on two additional siblings who have not been previously described. We also compare the clinical features of these patients and discuss the previously hypothesized possibility of an independent association including both holoprosencephaly and craniosynostosis.

Keywords

Holoprosencephaly; craniosynostoses; cranial sutures; receptors; fibroblast growth factor

INTRODUCTION

The adult calvarium is an elegant structure, comprised of eight flat cranial bones that smoothly articulate with each other to create a protective vault surrounding the brain. While it can be tempting to attribute the mechanical and geometric properties of the calvarium entirely to the cranial bones, a proper understanding of these properties must account for the contributions of the calvarial sutures: bands of fibrous connective tissue that prevent premature bone separation and allow for uniform growth of the cranium during brain development [Cohen, 1993]. In particular, as the brain quadruples in mass between birth and 3–4 years of age [Dekaban and Sadowsky, 1978], the outward displacement of cranial bones results in adaptive deposition of bone at the sutural margins to accommodate the increased volume of the cranial cavity [Cohen, 1993; Cohen and MacLean, 2000]. This concept can be appreciated upon examination of skulls affected by craniosynostosis, a disorder in which one or more of the five major calvarial sutures fuse prematurely, limiting local bone growth surrounding the suture and leading to anomalous and asymmetric skull shapes [Cohen, 2005]. Indeed, grossly deformed craniosynostotic skulls have been observed for hundreds of thousands of years, ranging from excavations of prehistoric human skeletons [Gracia *et al.* 2009] to classical descriptions of the Athenian statesman Pericles (c. 495–429 BCE), who was traditionally depicted wearing a tall helmet to conceal the effects of a presumed sagittal synostosis [Cunningham *et al.* 2007; di Rocco 2005].

Craniosynostosis can be divided via several different classification schemes, each of which can prove useful in separating various etiologies. Depending on the number of sutures that fuse prematurely, craniosynostosis cases can be simple (1 suture) or complex (2+ sutures) [Kimonis et al., 2007]. If craniosynostosis occurs due to an intrinsic suture defect, it is termed primary craniosynostosis, while craniosynostosis resulting from another medical condition is designated as secondary craniosynostosis [Kimonis et al., 2007]. Finally, depending on whether craniosynostosis is accompanied by other anomalies, it can be labeled as isolated or syndromic [Kimonis et al., 2007].

Commonly affected sutures in craniosynostosis, ranked from most to least frequent, are the sagittal, coronal, metopic, and lambdoidal sutures [Cohen, 1993; Cohen and MacLean, 2000]. Premature closure of different sutures results in distinctive head shapes, formation of which also depends on the timing and order of synostosis [Cohen, 2005]. For instance, unilateral coronal or lambdoidal synostosis generally causes anterior or posterior plagiocephaly (Greek *plagios*, “oblique”), bilateral coronal synostosis produces brachycephaly (Greek *brachys*, “short/broad”), sagittal synostosis results in scaphocephaly (Greek *skaphe*, “boat-shaped”), and metopic synostosis causes trigonocephaly (Greek *trigonon*, “triangle-shaped”) [Kimonis et al., 2007]. More convoluted alterations in head shape can occur when multiple sutures fuse prematurely, as in the trilobular cloverleaf skull deformity that can result from a variety of complex craniosynostoses [Cohen, 2005]. In some cases, extra care must be taken to distinguish craniosynostotic causes from positional causes of head deformity, particularly in plagiocephaly or with involvement of the metopic and/or sagittal sutures [Huang et al., 1998].

Holoprosencephaly (HPE) and craniosynostosis occur with prevalences of roughly 1:8,000 [Leoncini et al, 2008] and 1:2,500 [Muenke and Wilkie, 2001] live births, respectively. While epidemiologic studies have not been performed to evaluate the prevalence of individuals simultaneously affected by HPE and craniosynostosis, there is a paucity of such patients reported in the literature. Here, we describe 11 patients with concurrent HPE and craniosynostosis, nine of whom have been previously described in the literature and two of whom we present for the first time.

SUMMARY OF PATIENTS

Eleven patients simultaneously affected by HPE and craniosynostosis were identified (see Table I). Previously published photographs of three of these patients are shown in Figure 1. All patients had brain anomalies consistent with HPE, as opposed to microform HPE, with severity ranging from the most severe alobar form to the milder lobar form. The presence of craniosynostosis was established in each patient via clinical examination, radiographic evidence, and/or postmortem autopsy. With regard to specific sutures, the metopic suture was involved in seven patients (64%), the coronal sutures in six patients (55%), the sagittal suture in one patient (9%), and the lambdoidal sutures in two patients (18%). Complete descriptions of head shape were only provided for five individuals (45%); of these, two (18%) were described as having plagiocephaly of an unspecified type.

All patients with available clinical data demonstrated additional craniofacial anomalies. Seven individuals (64%) had confirmed microcephaly. Features of midface hypoplasia were reported in three individuals (27%). Anomalies of orbital spacing were seen in five individuals (45%), three of whom were hypoteloric and two of whom were hypertelorlic. Proptosis was also seen in three patients (27%). Additional craniofacial findings are presented in Table I.

A variety of extracranial findings was observed. Most individuals (73%) displayed some type of limb anomaly, ranging from minor changes, such as clinodactyly, to major changes, such as brachial amelia. Cardiac structural anomalies were observed in three individuals (27%), including septal defects and large vessel abnormalities. Skeletal findings observed in more than one individual included scoliosis, coxa valga, and small vertebral bodies. Three patients (27%) displayed urogenital abnormalities, but none were the same across patients.

Chromosomal analysis was normal or undetermined in all patients except in one individual with a translocation described as t(2;4)(q14.2;q35) [Corona-Rivera et al., 2000]. The locus of the gene *GLI2* (2q14) is located in the cytogenetic vicinity of this translocation, which is of note because loss-of-function mutations in *GLI2* are associated with HPE spectrum findings [Roessler et al., 2003]. Despite this, mapping of the chromosome 2 breakpoint localized it a 3 Mb proximal to the *GLI2* locus, suggesting either long-range disruptive effects involving *GLI2* or the implication of other candidate genes closer to the breakpoint, such as *INSIG2*, a regulator of cholesterol and lipid metabolism located 150 kb distal to the breakpoint [David et al., 2009]. Genetic testing results were only available for two patients, with one individual positive for the c.50T>C = p.Leu17Pro mutation in the signal peptide region of *Sonic Hedgehog* (*SHH*) [Kato et al., 2000], and the other individual negative for clinically significant mutations in the *SHH*, *SIX3*, *ZIC2*, and *TGIF* genes. Family histories were largely unremarkable, although in the family newly reported here, the parents of the probanda and her affected brother were first cousins.

Two patients, who are siblings, have not been previously described and their details are given here. The siblings were born into a family of Arab ancestry, and their healthy parents were first cousins. The younger sibling, henceforth referred to as Patient 1, is female and is depicted in Figures 2a and 2b at seven years of age. Microcephaly was noted *in utero*, and at term, Patient 1 weighed 2760 g (10th centile), with a length of 48 cm (25th centile) and an occipitofrontal circumference (OFC) of 30 cm (<3rd centile). Lobar HPE was diagnosed after birth via brain ultrasound, and head CT during the first year of life showed mild trigonocephaly. At 3.5 years of age, she displayed brachycephaly and a protruding metopic suture, as well as short stature (3–4 standard deviations below the median), microcephaly, profound neurocognitive impairment, cortical blindness, a long face with proptotic eyes, thick auricles, a short neck, and fifth finger clinodactyly. Her karyotype was 46,XX, and direct sequencing revealed no mutations in *SHH*, *SIX3*, *ZIC2*, or *TGIF*.

The elder sibling, henceforth referred to as Patient 2, is male and is shown in Figure 2c at 15 years of age. His parameters at birth are not known, but severe microcephaly was noted starting at four years of age, with an OFC of 43.8 cm (<3rd centile) at age eight. Patient 2's phenotype also included profound neurocognitive impairment, proptosis, nystagmus, and blindness. CT of the head, portions of which are shown in Figure 3, demonstrated holoprosencephaly, possibly of the semilobar type; agenesis of the corpus callosum; widening of the lateral ventricles; and a protruding metopic suture. Features on physical examination included a short philtrum, a high and narrow palate, a hypoplastic scrotum, and tapering, hyperextensible fingers. His karyotype was 46,XY, and no further genetic testing was performed.

DISCUSSION

While all of the patients identified in this review displayed radiographic evidence of HPE, craniosynostosis was confirmed in each patient using different methods, including radiography, postmortem autopsy, and clinical examination. With respect to the latter, it must be stressed that the presence of head deformity is not sufficient to diagnose

craniosynostosis, and careful clinical attention can help distinguish between craniosynostosis and positional causes of head deformity [Huang et al., 1998].

The paucity of concordant cases of HPE and craniosynostosis in the literature raises an important question: is there an independent discrete condition comprising HPE and craniosynostosis? To analyze this question, it is useful to separate craniosynostosis into primary and secondary cases, and consider HPE in conjunction with each.

Secondary craniosynostosis can occur in cases of inadequate brain growth [Huang et al., 1998], which is a hallmark of HPE. This might lead one to expect HPE and secondary craniosynostosis to overlap often, but this is mitigated by the fact that such cases are not frequently accompanied by calvarial deformity, as in primary craniosynostosis, and may thus escape diagnosis [Benson et al., 1996]. Alternatively, Smith and Töndury previously hypothesized that absence of the metopic suture correlated with the absence of the falx cerebri frequently seen in HPE [Smith and Töndury, 1978]. While they identified several HPE patients in which the metopic suture was absent, Cohen points out other patients in which the metopic suture was partially fused or completely patent [Cohen, 2005]. Thus, the precise relationship between HPE and secondary craniosynostosis remains ambiguous.

HPE may also occur in conjunction with primary craniosynostosis. The siblings reported by Camera et al. [1993] were described as clearly having primary craniosynostosis, leading Camera et al. to postulate the existence of a “Genoa syndrome” with autosomal recessive inheritance. Lapunzina et al. suggested that their own patient may represent a severe expression of Genoa syndrome, but recognized that the other malformations in their patient may also suggest a newly recognized syndrome [Lapunzina et al., 2001]. Similarly, the other patients in this review presented with HPE and primary craniosynostosis, but their other anomalies and the diversity of their calvarial malformations shed some doubt on the conclusion that they represent a single common syndrome. We would posit that the newly reported sibs do have a specific causal entity of autosomal recessive inheritance, likely distinct from the others.

A link between HPE and craniosynostosis is also hinted at upon examination of the molecular genetics behind each disorder. Several pathways have been implicated in the pathogenesis of HPE, including the Shh, Nodal, and Bmp signaling pathways, and mutations in a number of genes involved in at least the first two pathways, such as *SHH*, *GLI2*, and *FOXH1*, have been found in humans with HPE [Roessler and Muenke, 2010]. Furthermore, the FGF signaling pathway may be involved in human forebrain patterning, as animal models have implicated Fgf8 in midline telencephalon formation [Gutin et al., 2006; Storm et al., 2006] and, more recently, a hypomorphic *FGF8* allele was found to contribute to the occurrence of HPE in a human kindred [Arauz et al., 2010]. This is of particular significance because the FGF, BMP, and Shh signaling pathways are also implicated in calvarial suture morphogenesis and development of the cranial bones [Kim et al., 1998], while the FGF pathway and mutations in FGF receptors play an essential role in many of the craniosynostosis-associated syndromes, such as Pfeiffer syndrome (*FGFR2*), Apert syndrome (*FGFR2*), Crouzon syndrome (*FGFR2*), and Muenke syndrome (*FGFR3*) [Muenke and Wilkie, 2001]. Thus, at some point in the future, key molecules common to the pathogenesis of HPE and that of craniosynostosis may be detected.

One consideration that may further complicate the molecular relationship, if any, between these two defects is the fact that forebrain morphological defects are caused by the loss of *Fgf8* gene function [Schneider et al., 2001], while the defects in syndromic craniosynostosis are instead caused by gain-of-function mutations in *FGFR* family members, which encode receptors for the FGF8 ligand [Kim et al., 1998; Kimonis et al., 2007]. This raises the

alternate possibility of an inverse relationship between HPE and craniosynostosis. Nevertheless, it is still unclear whether increased flux through the FGF pathway contributes to craniosynostosis outside of the well-described syndromes. Given the rather ambiguous molecular picture, further clarification of the roles of each signaling pathway in HPE and craniosynostosis would be of considerable value in elucidating any relationship between the two conditions.

Acknowledgments

We would like to express our utmost gratitude to the family described in this report for its willingness to participate in this study. Additionally, we thank Erich Roessler for conceptual contributions to the manuscript and Jin S. Hahn for providing radiologic interpretation. This research was supported in part by the Howard Hughes Medical Institute and by the Division of Intramural Research at the National Human Genome Research Institute (National Institutes of Health, Department of Health and Human Services, United States of America).

References

- Arauz RF, Solomon BD, Pineda-Alvarez DE, Wendland J, Gropman AL, Parsons JA, Roessler E, Muenke M. A hypomorphic allele in the *FGF8* gene contributes to holoprosencephaly and is allelic to gonadotropin-releasing hormone deficiency in humans. 2010 In submission.
- Benson ML, Oliverio PJ, Yue NC, Zinreich SJ. Primary craniosynostosis: Imaging features. *Am J Roentgenol*. 1996; 166:697–703. [PubMed: 8623653]
- Camera G, Lituania M, Cohen MM. Holoprosencephaly and primary craniosynostosis: The Genoa syndrome. *Am J Med Genet*. 1993; 47:1161–1165. [PubMed: 8291548]
- Cohen MM. Sutural biology and the correlates of craniosynostosis. *Am J Med Genet*. 1993; 47:581–616. [PubMed: 8266985]
- Cohen MM. Editorial: Perspectives on craniosynostosis. *Am J Med Genet A*. 2005; 136A(4):313–326. [PubMed: 15999346]
- Cohen, MM.; MacLean, RE. Craniosynostosis: Diagnosis, Evaluation, and Management. New York: Oxford University Press; 2000. p. 454
- Corona-Rivera A, Corona-Rivera JR, Bobadilla-Morales L, Garcia-Cobian TA, Corona-Rivera E. Holoprosencephaly, hypertelorism, and ectrodactyly in a boy with an apparently balanced *de novo* t(2;4)(q14.2;q35). *Am J Med Genet A*. 2000; 90:423–426.
- Cunningham ML, Seto ML, Ratisoontorn C, Heike CL, Hing AV. Syndromic craniosynostosis: from history to hydrogen bonds. *Orthod Craniofac Res*. 2007; 10:67–81.
- David D, Marques B, Ferreira C, Vieira P, Corona-Rivera A, Ferreira JC, van Bokhoven H. Characterization of two ectrodactyly-associated translocation breakpoints separated by 2.5 Mb on chromosome 2q41.1–q14.2. *Eur J Hum Genet*. 2009; 17:1024–1033. [PubMed: 19223936]
- Dekaban AS, Sadowsky D. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Ann Neurol*. 1978; 4:345–356. [PubMed: 727739]
- di Rocco C. Craniosynostosis in old Greece: political power and physical deformity. *Childs Nerv Syst*. 2005; 21:859. [PubMed: 16078077]
- Gracia A, Arsuaga JL, Martinez I, Lorenzo C, Carretero JM, Bermudez de Castro JM, Carbonell E. Craniosynostosis in the Middle Pleistocene human Cranium 14 from the Sima de los Huesos, Atapuerca, Spain. *Proc Nat Acad Sci*. 2009; 106(16):6573–6578. [PubMed: 19332773]
- Gutin G, Fernandes M, Palazzolo L, Paek H, Yu K, Ornitz DM, McConnell SK, Hebert JM. FGF signalling generates ventral telencephalic cells independently of SHH. *Development*. 2006; 133:2937–2946. [PubMed: 16818446]
- Hartsfield JK Jr, Bixler D, DeMyer WE. Syndrome identification case report 119: Hypertelorism associated with holoprosencephaly and ectrodactyly. *J Clin Dysmorphol*. 1984; 2(2):27–31.
- Huang MHS, Mouradian WE, Cohen SR, Gruss JS. The differential diagnosis of abnormal head shapes: Separating craniosynostosis from positional deformities and normal variants. *Cleft Palate Craniofac J*. 1998; 35(3):204–211. [PubMed: 9603553]

- Kato M, Nanba E, Akaboshi S, Shiihara T, Ito A, Honma T, Tsuburaya K, Hayasaka K. Sonic Hedgehog signal peptide mutation in a patient with holoprosencephaly. *Ann Neurol*. 2000; 47:514–516. [PubMed: 10762164]
- Kim H-J, Rice DPC, Kettunen PJ, Thesleff I. FGF-, BMP- and Shh-mediated signalling pathways in the regulation of cranial suture morphogenesis and calvarial bone development. *Development*. 1998; 125:1241–1251. [PubMed: 9477322]
- Kimonis V, Gold J-A, Hoffman TL, Panchal J, Boyadjiev SA. Genetics of craniosynostosis. *Semin Pediatr Neurol*. 2007; 14:150–161. [PubMed: 17980312]
- Lapunzina P, Musante G, Pedraza A, Prudent L, Gadow E. Semilobar holoprosencephaly, coronal craniosynostosis, and multiple congenital anomalies: A severe expression of the Genoa syndrome or a newly recognized syndrome? *Am J Med Genet A*. 2001; 102:258–260.
- Leoncini E, Baranello G, Orioli IM, Anneren G, Bakker M, Bianchi F, Bower C, Canfield MA, Castilla EE, Cocchi G, Correa A, de Vigan C, Doray B, Feldkamp ML, Gatt M, Irgens LM, Lowry RB, Maraschini A, McDonnell R, Morgan M, Mutchinick O, Poetzsch S, Riley M, Ritvanen A, Gnansia ER, Scarano G, Sipek A, Tenconi R, Mastroiacovo P. Frequency of holoprosencephaly in the International Clearinghouse Birth Defects Surveillance Systems: Searching for population variations. *Birth Defects Res A*. 2008; 82:585–591.
- Lin C-H, Tsai J-D, Ho Y-J, Lin W-C. Alobar holoprosencephaly associated with cebocephaly and craniosynostosis. *Acta Neurol Taiwan*. 2009; 18:123–126. [PubMed: 19673365]
- Muenke M.; Wilkie, AOM. Craniosynostosis syndromes. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8. New York: McGraw-Hill; 2001. p. 6117-6146.
- Roessler E, Du Y-Z, Mullor JL, Casas E, Allen WP, Gillesen-Kaesbach G, Roeder ER, Ming JE, Altaba ARi, Muenke M. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc Nat Acad Sci*. 2003; 100(23): 13424–13429. [PubMed: 14581620]
- Roessler E, Muenke M. The molecular genetics of holoprosencephaly. *Am J Med Genet C*. 2010; 154C(1) in press.
- Schneider RA, Hu D, Rubenstein JLR, Maden M, Helms JA. Local retinoid signaling coordinates forebrain and facial morphogenesis by maintaining FGF8 and SHH. *Development*. 2001; 128:2755–2767. [PubMed: 11526081]
- Smith DW, Tondury G. Origin of the calvaria and its sutures. *Am J Dis Child*. 1978; 132:662–666. [PubMed: 665591]
- Storm EE, Garel S, Borello U, Hebert JM, Martinez S, McConnell SK, Martin GR, Rubenstein JLR. Dose-dependent functions of *Fgf8* in regulating telencephalic patterning centers. *Development*. 2006; 133:1831–1844. [PubMed: 16613831]
- Thomas M, Donnai D. Bilateral brachial amelia with facial clefts and holoprosencephaly. *Clin Dysmorphol*. 1994; 3(3):266–269. [PubMed: 7981864]
- Zimpfer A, Miny P, Dombrowski U, Tolnay M, Meyer P, Bruder E. Upper limb amelia, facial clefts, holoprosencephaly, and interrupted aortic arch. *Fetal Pediatr Pathol*. 2007; 26:169–176. [PubMed: 18075831]

Biographies

Manu S. Raam, B.S.E. is a trainee in the HHMI-NIH Research Scholars Program, which is a medical student training program jointly administered by the Howard Hughes Medical Institute and the National Institutes of Health. He is a member of the Muenke lab and is involved in investigating the genetics of holoprosencephaly.

Benjamin D. Solomon, M.D. is a trainee in the Combined Pediatrics and Medical Genetics Residency Program, based at the National Human Genome Research Institute. He is a member of the Muenke lab and is involved in investigating the genetics of holoprosencephaly.

Stavit A. Shalev, M.D. is the director of the Genetic Institute at the Emek Medical Center in Afula, Israel, providing genetic services to approximately one million residents in northern Israel. She has a research interest in delineating characteristics of genetic disorders among the local populations in this area.

Maximilian Muenke, M.D. is the chief of the Medical Genetics Branch at the Division of Intramural Research in the National Human Genome Research Institute. He has a longstanding interest in elucidating the genetics of holoprosencephaly as well as syndromic forms of craniosynostosis, including Muenke syndrome.

Figure 1.

Photographs of patients who have previously been described in the literature: **(a)** proposita described by Camera *et al.* (1993) [Camera et al. 1993]; **(b)** proposita described by Lapunzina *et al.* (2001) [Lapunzina et al. 2001] at 15 days of age, with particular attention called by those authors to the down-slanting palpebral fissures, midfacial hypoplasia, coronal craniosynostosis, low-set ears, facial asymmetry, and prominent metopic fontanel; **(c)** propositus described by Corona-Rivera *et al.* (2000) [Corona-Rivera et al. 2000]. All images used with permission of John Wiley & Sons, Inc.

Figure 2. Photographs of the newly described Patients 1 and 2: (a) frontal view of Patient 1, (b) 45° view of Patient 1, and (c) 45° view of Patient 2. Note the proptosis in both patients, the brachycephalic appearance of Patient 1 when compared to Patient 2, and the prominent metopic ridging in Patient 2.

Figure 3. Axial head CT sections of the newly described Patient 2: **(a)** showing midline brain anomalies consistent with HPE, possibly of the semilobar type; **(b)** showing the prominent metopic suture and trigonocephalic head shape.

Table 1
Comparison of phenotypic characteristics in patients with features of both holoprosencephaly and craniosynostosis.

	Hartsfield 1984	Camera 1993 (1)	Camera 1993 (2)	Thomas 1994	Corona-Rivera 2000	Kato 2000	Lapunzina 2001	Zimpfer 2007	Lin 2009	This report (1)	This report (2)
Gestational age (w)	36	“Term”	Fetal demise, 21 w	Fetal demise, 24 w	“Term”	“Term”	35	Fetal demise, 19 w	33	“Term”	NS
HPE type	Lobar	Semilobar	Semilobar	Alobar	Semilobar	Lobar	Semilobar	Alobar	Alobar	Lobar	Semilobar
Head shape	“Distorted”	Brachycephaly, plagiocephaly	NS	NS	Scaphocephaly	NS	Plagiouricephaly, triphyllocephaly	NS	NS	Brachycephaly, trigonocephaly	Trigonocephaly
Synotic sutures	Coronal, metopic	Coronal, lambdoidal	NS	All except occipital sutures	Metopic	NS	Coronal	Metopic	All except sagittal	Coronal, metopic	Metopic
Method of determining synostosis	CE, R, PMA	CE, R	R	PMA	CE, R	NS	CE, R, PMA	PMA	CE, R	CE	CE
Fontanelles	Anterior absent, posterior open	NS	NS	NS	NS	NS	Supernumerary metopic fontanelle	Tiny anterior fontanelle	NS	NS	NS
Microcephaly	+	+	NS	+		+	–	NS	+	+	+
Other craniofacial findings	Hypertelorism, downslanting palpebral fissure	Hypotelorism, upslanting palpebral fissures	NS	Widely spaced orbits	Depressed nasal bridge, low-set and posteriorly rotated ears	Hypotelorism, low nasal bridge, hypoplastic nasal septum, macroglossia	Midface hypoplasia, downslanting palpebral fissures, nasal hypoplasia, low-set ears	Maxillary hypoplasia, low-set ears, proptosis	Hypotelorism	Proptosis, thick auricles	Proptosis, nystagmus, short philtrum, high/narrow palate
Limbs anomalies	Ectrodactyly, adactyly, absent radius, bowed ulna	Fifth finger clinodactyly, small hands/feet, cone-shaped epiphyses, hypoplastic phalanges	NS	Brachial amelia	Ectrodactyly, syndactyly, absent phalanges, hypoplastic metatarsals, abnormal creases	–	Hypoplastic nails/phalanges, absent phalanges, flexion deformities of hips	Brachial amelia	–	Fifth digit clinodactyly	Tapering, hyperextensible fingers
Cardiac anomalies	–	–	NS	–	–	–	Moderate aortic coarctation, PDA	Interrupted aortic arch, VSD	PFO	NS	NS
Skeletal anomalies	–	Slender long bones, small vertebral bodies, coxa valga, delayed bone age	NS	–	Subluxated joints, pectus excavatum, thoracolumbar scoliosis, coxa valga	–	Scoliosis, small vertebral bodies, hypoplastic 12th ribs	Thoracic kyphoscoliosis	–	NS	NS
Urogenital anomalies	–	–	NS	–	–	Micropenis, cryptorchidism	Uterine hypoplasia, hydronephrosis	“Meanderform” right ureter	–	NS	Hypoplastic scrotum
Other findings	–	–	NS	–	Hypertrichosis, capillary hemangioma,	Joint contractures at knees, ankles	Nevus flammeus, anteriorly placed anus,	Keratocornea	–	Short stature, cortical	Blindness

	Hartsfield 1984	Camera 1993 (1)	Camera 1993 (2)	Thomas 1994	Corona-Rivera 2000	Kato 2000	Lapunzina 2001	Zimpfer 2007	Lin 2009	This report (1)	This report (2)
Family history	Paternal cousins: ectrodactyly	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Mother: gestational diabetes	Parental consanguinity	Parental consanguinity
Chromosomal analysis	46,XY	“Normal”	“Normal”	NS	46,XY,t(2;4)(q14.2;q35)	46,XY	46,XX	Normal copy number for 13, 18, 21, X, Y (FISH)	46,XX	46,XX	46,XY
Genetic testing results	–	–	NS	–	–	SHH: c.50T>C= p.Leu17Pro	–	–	–	(–) for SHH, SIX3, ZIC2, TGIF	–
Reference	[Hartsfield Jr. et al. 1984]	[Camera et al. 1993]	[Camera et al. 1993]	[Thomas and Donnal 1994]	[Corona-Rivera et al. 2000]	[Kato et al. 2000]	[Lapunzina et al. 2001]	[Zimpfer et al. 2007]	[Lin et al. 2009]	–	–

Symbols/Abbreviations: +: present, –: absent, CE: clinical examination, FISH: fluorescent *in situ* hybridization, NS: not specified, PDA: patent ductus arteriosus, PFO: patent foramen ovale, PMA: postmortem autopsy, R: radiography, VSD: ventricular septal defect, w: weeks.