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Holoprosencephaly-polydactyly syndrome: in search of an etiology

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Abstract

Holoprosencephaly-Polydactyly (HPS) or Pseudotrisomy 13 syndrome are names conferred to clinically categorize patients whose phenotype is congruent with Trisomy 13 in the context of a normal karyotype. The literature suggests that this entity may be secondary to submicroscopic deletions in HPE genes; however a limited number of investigations have been undertaken to evaluate this hypothesis. To test this hypothesis we studied a patient with HPE, polydactyly, and craniofacial dysmorphologies consistent with the diagnosis of Trisomy 13 whose karyotype was normal. We performed mutational analysis in the four main HPE causing genes (*SHH*, *SIX3*, *TGIF*, *ZIC2*) and *GLI3*, a gene associated with polydactyly and Fluorescent *in-situ* hybridization (FISH) to search for microdeletions in these genes and two candidate HPE genes (*DISP1* and *FOXA2*). No mutations or deletions were detected. A whole genome approach utilizing array Comparative Genomic Hybridization (aCGH) to screen for copy number abnormalities was then taken. No loss or gain of DNA was noted. Although a single case, our results suggest that coding mutations in these HPE genes and copy number anomalies may not be causative in this disorder. Instead, HPS likely involves mutations in other genes integral in embryonic development of the forebrain, face and limbs. Our systematic analysis sets the framework in which to study other affected children and delineate the molecular etiology of this disorder.

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Keywords

holoprosencephaly; polydactyly; pseudotrismy13; forebrain; cerebral cortex; limb anomalies; *SHH*; *SIX3*; *TGIF*; *ZIC2*; *GLI3*; array CGH

1. Introduction

Holoprosencephaly (HPE) is a major clinical feature in the Holoprosencephaly-Polydactyly Syndrome (HPS) and results from inadequate division of the forebrain into two separate cerebral hemispheres. The etiologies of HPE are diverse, comprising genetic [3,6,9,11,12], environmental [5] and cytogenetic factors [1,2]. Dominant *de novo* mutations and undetected microdeletions have been suggested to account for HPS [4]. However, only a limited number of investigations have been undertaken to address these later theories.

We sought to ascertain the molecular etiology behind the phenotype in a patient with HPS and a normal karyotype, beginning our investigation with known HPE genes and extending our analysis using a whole genome approach.

2. Patient

This 14-month-old male was born at 35 weeks of gestation weighing 3,655 grams, to non-consanguineous parents. The family history was unremarkable and the mother related no exposure to drugs or medical complications such as pregestational diabetes during her pregnancy. A second trimester prenatal ultrasound was consistent with HPE. At birth his head circumference was 39.5 cm (>98th percentile) and magnetic resonance imaging (MRI) revealed semilobar HPE with a large extra-axial interhemispheric loculated cyst and agenesis of the corpus callosum (Fig. 1A, B). A ventriculoperitoneal shunt was placed at three months of age after development of progressive hydrocephalus.

Physical examination at 14 months of age revealed macrocephaly, bilateral epicanthal folds and exotropia, hypoplastic ala nasae and a capillary hemangioma over the forehead (Fig. 1C). No microphthalmia, micrognathia, cleft lip/palate or external genitalia abnormalities were detected. Pre- and postaxial polysyndactyly involved all extremities, with a total of 26 digits (Fig. 1D–I). His right hand had 6 digits (a post-axial pedunculated digit) and syndactyly involving the 4th and 5th digits (Fig. 1D, E). His left hand had 8 digits, with syndactyly of digits 1 to 4, 5 and 6 and a cleft with the fused digits (Fig. 1F). Bilateral Sydney lines were noted (not shown). The right and left feet each had 6 digits (Fig. 1 G, H) His right foot exhibited a broad bifid great toe (Fig. 1I).

Neurologic examination showed severe motor delay and generalized hypotonia. Echocardiogram and renal ultrasound were normal. Radiographs of the limbs are not available. Blood was sent for chromosomal analysis and quantitative cholesterol determinations

3. Materials and methods

3.1 Karyotype

Metaphase chromosomes were prepared from stimulated peripheral lymphocytes and analyzed by high resolution banding techniques.

3.2 Mutation analysis

The exons of the HPE genes (*SHH*, *ZIC2*, *SIX3*, and *TGIF*) and the polydactyly gene, *GLI3* were sequenced for mutations as previously described [3,6,11,12,8].

3.3 Fluorescent in situ hybridization studies

A panel of FISH probes, using bacterial artificial chromosome (BAC) clones, was used to detect microdeletions in four known HPE genes [*SHH* (7q36), *TGIF* (18p11), *ZIC2* (13q32) and *SIX3* (2p21)] and two candidate genes [*DISP1* (1q41) and *FOXA2* (20p11)] [2]. For each gene, a BAC clone containing sequences of the gene was selected from NCBI and Ensembl databases and obtained from BACPAC Resources (Oakland, CA). BAC clone DNA was prepared and fluorescently labeled using nick translation as previously described [7]. Correct chromosomal location of each of the BAC clones was confirmed using standard FISH mapping [7]. The presence of sequences of each gene in the corresponding BAC clone was confirmed by PCR of targeted gene exons (data not shown). A strategy using 3 different fluorescent dyes (Cy5, Cy3, and FITC) for each BAC clone was adopted so that all 6 probes could be visualized and analyzed concomitantly in a single experiment. FISH analysis of chromosomal preparations from the patient was performed using the probe panel as described earlier [2]. 20 metaphase spreads were scored.

3.4 Microarray Comparative Genomic Hybridization(aCGH)

An aCGH was performed using utilizing the Agilent® 44B, Human Genome CGH kit allowing an average probe spatial resolution of 35 Kb. Patient DNA was labeled and cohybridized against a reference DNA pool made of 10 normal male donors. Array slides were scanned using an Agilent® microarray scanner, data were extracted from images with Feature Extraction 8 software (Agilent®), and CGH profiles were generated using CGH-analytics 3.2 software (Agilent®). Gains or losses were considered if present at least on two tailing probes to avoid false positives.

3.5 Serum Cholesterol analysis

Serum cholesterol and 7-dehydrocholesterol were quantified.

4. Results

High resolution GTG banding revealed a normal 46, XY karyotype (Fig. 2A). Mutation analysis did not detect any mutations in the exons or intron-exon boundaries of *SHH*, *ZIC2*, *SIX3*, *TGIF* or *GLI3* genes. The multiprobe FISH analysis did not detect deletions or duplications of the six known or candidate HPE genes tested (Fig. 2B). We then chose a whole genome screening approach to look for gains or losses of DNA copy number. However, none were observed involving two or more tailing probes (Fig. 3). We specifically noted no quantitative changes in *FBXW11* or *GLI3*. Cholesterol studies were normal.

5. Discussion

The molecular mechanisms underlying the HPS brain, craniofacial and limb phenotypes in this entity are unclear. In order to determine the molecular mechanisms responsible for HPE other disorders with similar phenotypes should be considered in the differential diagnosis and ruled out. The major considerations involved in the differential diagnosis involve disorders with HPE, limb anomalies and those with both, taking into consideration phenotypic heterogeneity in a number of disorders. This disorders involve chromosomal aberrations, classic HPE and those disorders with polydactyly.

We began our search for the molecular basis of HPS in our patient based upon HPE. Because HPE is a major diagnostic criterion for this syndrome we first screened for coding mutations in four main genes known to cause HPE (*SHH*, *ZIC2*, *SIX3*, and *TGIF*). We then tested for deletions and duplications in these genes as well as in two candidate genes involved in the Sonic Hedgehog signaling pathway (*DISP1* and *FOXA2*). No abnormalities were identified,

therefore we used aCGH for a pan-genomic screening for DNA gains and losses. No genomic copy number changes were detected. In particular, the 5q35.1 cytoband (*FBXW11* gene locus) which was elegantly reported as duplicated in one case of HPE with preaxial polydactyly, was not duplicated in our patient [9]. Next, we focused on *GLI3* as a candidate gene because the polydactyly was more characteristic of that seen in patients with *GLI3* mutations such as Greig cephalopolysyndactyly or Pallister-Hall syndromes than typically seen in Trisomy 13. No mutations in the coding sequence were found in *GLI3*, suggesting that coding mutations in this component of the hedgehog signaling pathway is unlikely to be causative. Cholesterol studies were normal, ruling out Smith-Lemli-Opitz syndrome, in which HPE is observed in approximately 5% of cases and exhibit a similar limb phenotype.

Explanations for our results include: the mutation is intronic in the genes we screened or does not involve the genes we analyzed; copy number aberrations do not lead to HPS or the resolution of the FISH and array probes were not sufficient for their detection; the developmental anomalies seen in this syndrome results from an elaborate interplay between genetic and environmental factors which are yet to be determined.

Our results underscore the huge void in our knowledge of the underlying molecular mechanisms leading to HPS, as well as a number of other developmental disorders involving multiple anatomic structures. The complexity of these molecular mechanisms in this disorder is exemplified by the brain and limb malformations. At first the molecular mechanisms underlying HPS appears counter-intuitive because classical HPE appears to be due to a loss-of-function whereas polydactyly results from gain-of-function. Possible explanations for this paradox include: a single gene mutation results in the dysregulation of a signaling pathway which exhibits bifunctional characteristics leading to gain-of-function in some embryonic structures and loss-of-function in other structures during development. This may result because the brain and limb have different evolutionary origins and although they may use the same molecular pathways during embryonic development their response to a given signaling pathway differs. Alternatively, the phenotype is not the result of a single gene mutation (monogenic) but involves mutations in two different yet undetermined genes (digenic) such as described in other human diseases including HPE [10].

Although definitive conclusions regarding the molecular etiology can not be made based upon a single case, our approach to the clinical workup of patient's with this HPS sets the stage for the evaluation of other children with this syndrome. Additional patients are needed to elucidate the genetics leading to the abnormal brain, face and limb phenotypes in HPS. Advances in our understanding of brain, face and limb development may also provide new candidate genes and novel avenues in which to direct research efforts and diagnostic testing of this elusive syndrome.

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Figure 1.

The radiographic and clinical phenotypes. MRI of the head revealed: (A) a large cystic region (red arrow) and agenesis of the corpus callosum on sagittal sections and (B) a single ventricle containing a large dorsal cyst (red arrow) on the axial view. Some separation of the two cerebral hemispheres can be seen in the axial view. The radiographic findings are consistent with semi-lobar HPE. (C) Frontal view of the face reveals a midline frontal capillary hemangioma (red arrowhead), bilateral epicanthal folds, exotropia, and hypoplastic ala nasae. (D,E) The right hand exhibits 6 digits (a post-axial pedunculated digit) and syndactyly of digits 4 and 5. (F) The left hand has 8 digits, syndactyly of digits 1 to 4, 5 and 6 and a cleft between the fused digits. (G,H) The left foot has 6 digits, the supernumerary digit is a small pedunculated. (I) the right foot also has 6 digits and a broad bifid great toe.

Figure 2.

Cytogenetic studies. (A) A high-resolution karyotype without evidence of chromosome 13 aberrations. (B) Multicolor fluorescence *in situ* hybridization analysis performed on metaphase chromosomes. A panel of six probes (BAC clones) containing four HPE genes (*SHH*, *ZIC2*, *SIX3*, *TGIF*) and two candidate genes (*DISP1*, *FOXA2*) were used and image processed. No deletions were noted. (The probe or BAC clone name is under the gene name for easy correlation)

Figure 3.

CGH array results (chromosome 13). Genome wide analysis did not reveal any gain or loss of chromosomal material implying 2 or more tailing probes on chromosome 13, as well as on all the other chromosomes (data not shown).