The Deceptive Family Tree: Genetic Counseling Complexities in Holoprosencephaly

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Goals and Objectives

- Review the clinical and genetic aspects of Holoprosencephaly (HPE)
- Review modes of inheritance of HPE
- Discuss timing and modalities for prenatal diagnosis of HPE
- Review the complexities of genetic counseling with case examples
- Discuss ongoing research and recent findings
Holoprosencephaly
Guest Editors: Paul S. Kruszka, Benjamin D. Solomon, Maximilian Muenke
What is Holoprosencephaly (HPE)?

HPE results from failure or incomplete separation of the forebrain in early gestation. It is characterized by the degree of forebrain separation:

1. Alobar (most severe)
2. Semilobar
3. Lobar
4. Middle inter hemispheric variant
5. Septoproptic types
6. Microform HPE
Holoprosencephaly


31 days

37 days

51 days
Craniofacial findings in patients with holoprosencephaly-spectrum disorders

Alobar holoprosencephaly
Prevalence of holoprosencephaly

All conceptions

Unaffected conceptions: 249/250 conceptions

Affected conceptions: 1/250 conceptions

Fetal demise: 39/40 affected conceptions

Affected liveborns: 1/10,000 overall conceptions

Other epidemiological information:
- No clear racial/ethnic predilection
- Possible slight female preponderance in some subgroups

Causes of holoprosencephaly

- Mutations in “main HPE genes” (SHH, ZIC2, SIX3, TGIF1)
- Submicroscopic chromosomal alterations (e.g., as ascertained by microarray)
- Unknown/possible environmental influences (e.g., increased risk due to maternal diabetes mellitus)
- Syndromic (e.g., Smith-Lemli-Opitz syndrome)
- Mutations in other genes
- Cytogenetic anomalies (e.g., trisomy 13, triploidy)

Multiple modes of inheritance

- Autosomal dominant (non syndromic single gene)
- Autosomal recessive (Smith Lemli Opitz)
- Chromosomal (Trisomy 13 or microarray abnormality)
- Multifactorial (teratogenic /maternal diabetes)
Trisomy 13 (Patau syndrome)
Prognosis in classic HPE

- Developmental delay present in almost 100% of patients
- Increased risk for seizures and pituitary dysfunction
- 50% of infants die by 4 months of age; 90% by 1 year
PRENATAL CONSULTATION: framing

• The physician/genetic counselor framing of medical prognosis is very important:

• Parents given a prognosis of a chance for a reasonably good survival (positive framing) were more likely to choose resuscitation than those told of the chance of death and disability (negative framing)

Prenatal imaging of HPE

- Early ultrasound: 11-12 weeks can identify some severe cases

- Anatomy ultrasound at 18-20 weeks can identify almost all classic cases

- Fetal MRI at 17 weeks + helps to refine diagnosis: improved visualization over ultrasound; increased risk for incidental findings
Ultrasound image of holoprosencephaly (HPE) at 16 weeks of gestation: (left image) Single large monoventricle and (right image) proboscis are evident in the fetal head.
**Figure 2** Alobar holoprosencephaly with cup-shaped holosphere and a dorsal cyst, 17 week fetus. Sagittal T2 (a) and axial T2 (b) images. The monoventricle communicates with the dorsal cyst (*). The thalami and the midbrain form a contiguous mass, consistent with a dysplasia at the diencephalic mesencephalic junction (arrow, a).

Unique to fetal evaluation/diagnosis of Holoprosencephaly

- Findings are not always 100% clear
- Gray area leaves room for interpretation
- Provider dependent explanations
- Imaging is our main tool to guide us in terms of which additional testing to recommend
- **Timing is an issue**: patients may be considering abortion based on the findings and discussion
- No fetal intervention available but delivery planning may be important
- High anxiety patient population
Prenatal screening/testing for HPE

• When a genetic etiology is known or suspected:
  1. Chorionic Villus Sampling (CVS) at 10-12 weeks gestation
  2. Amniocentesis at 15+ week

• When etiology is unknown and/or invasive testing is declined:
  
  Option for first and second trimester maternal serum screening/NIPT to screen for aneuploidy and other associated syndromes if diagnostic testing declined (low estriol on MSS in fetuses with SLO)
(a) Amniocentesis

1. A sample of amniotic fluid can be taken starting at the 14th to 16th week of pregnancy.

2. Biochemical tests (tests for genetic disorders) can be performed immediately on the amniotic fluid or later on the cultured cells.

3. Fetal cells must be cultured for several weeks to obtain sufficient numbers for karyotyping (testing for chromosomal disorders).

(b) Chorionic villus sampling (CVS)

1. A sample of chorionic villus tissue can be taken as early as the 8th to 10th week of pregnancy.

2. Karyotyping and biochemical tests can be performed on the fetal cells immediately, providing results within a day or so.
How do we decide what testing to offer?

- US → MRI findings → hypothesis/short gene list
- Multidisciplinary input / TEAMWORK
- Family and patient history is key
- Timing (decision making versus delivery planning)
- Laboratory costs, accuracy and turn around time
# Commercially Available Testing for HPE

<table>
<thead>
<tr>
<th>Diagnostic center</th>
<th>Method</th>
<th>Genes tested</th>
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</thead>
<tbody>
<tr>
<td>ARUP Laboratories</td>
<td>Targeted capture NGS panel; CGH</td>
<td><em>DISP1, FGF8, FOXH1, GLI2, NODAL, PTCH1, SHH, SIX3, TDGF1, TGIF1, ZIC2, FGF8 next generation sequencing; CGH</em></td>
</tr>
<tr>
<td>Blueprint Genetics</td>
<td>WES panel</td>
<td><em>CDON, FGF8, FGFR1, FOXH1, GLI2, GLI3, NODAL, PTCH1, SHH, SIX3, TGIF1, ZIC2</em></td>
</tr>
<tr>
<td>GeneDx</td>
<td>Sanger sequencing/MLPA</td>
<td><em>SHH, SIX3, TGIF1, ZIC2</em></td>
</tr>
<tr>
<td>Invitae</td>
<td>Targeted capture NGS panel</td>
<td>Primary panel: <em>GLI2, SHH, SIX3, TGIF1, ZIC2</em>; add-on preliminary-evidence genes: <em>CDON, FOXH1, NODAL, PTCH1</em></td>
</tr>
<tr>
<td>Muenke Laboratory at NIH</td>
<td>Sanger sequencing</td>
<td><em>SHH, SIX3, TGIF1, ZIC2</em></td>
</tr>
<tr>
<td>Prevention Genetics</td>
<td>Targeted capture NGS panel; CGH</td>
<td><em>CDON, DLL1, DISP1, FGF8, FOXH1, GAS1, GLI2, NODAL, PTCH1, SHH, SIX3, TDGF1, TGIF1, ZIC2 (TDGF1 and DLL1 not tested with CGH)</em></td>
</tr>
<tr>
<td>The University of Chicago Genetic Services Laboratories</td>
<td>Targeted capture NGS panel</td>
<td><em>CDON, FGFR1, PTCH1, SIX3, TGIF1, FGF8, GLI2, SHH, STIL, ZIC2</em></td>
</tr>
<tr>
<td>OSHU Knight Diagnostic Laboratories</td>
<td>Targeted capture NGS panel</td>
<td><em>CDON, DISP1, DLL1, FGF8, FOXH1, GAS1, GLI2, NODAL, PTCH1, SHH, SIX3, TGIF1, ZIC2</em></td>
</tr>
</tbody>
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CGH = comparative genomic hybridization; MLPA = multiplex ligation-dependent probe amplification; NGS = next-generation sequencing; WES = whole exome sequencing.

*List of laboratories may not be complete.

Four most common HPE associated genes

Genetic Counselors can help

• Board certified masters degree professionals with specialized training in medical genetics and counseling
• Available for counseling preconception or early pregnancy to review all screening/testing options
• Provide family and medical history review
• Psychosocial assessment: patient’s anxieties, thoughts and beliefs, family dynamics
• Counseling for abnormal screening or testing results
• Understanding of ultrasound abnormalities and additional testing options
• Resources for patient
• Assisting with care management
Genetic counseling challenges

- Variable expressivity: One person with genetic change may present entirely different than another person with the exact same change.
- Incomplete Penetrance: The probability of a genetic variation revealing any phenotype at all.
- Laboratory availability (not all offer prenatal testing) costs and turn around times.
- Counseling for recurrence risks in future pregnancies.
Incomplete Penetrance

Solomon et al., American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 154C:133–141 (2010)
Case report

• The mother of the proband was born with multiple craniofacial malformations including hypotelorism, midface hypoplasia, and bilateral cleft lip/palate.

• Proband: semilobar holoprosencephaly confirmed by brain magnetic resonance imaging and facial abnormalities

• Additional significant findings of the proband include microcephaly, hypotonia, temperature dysregulation, developmental delay, seizure disorder, and feeding tube dependence
Retinal Coloboma
Positive for SHH

Bilateral Cleft Lip / Palate
Hypotelorism
Positive for SHH Variant

Bilateral Cleft Lip / Palate
Semi-lobar Holoprosencephaly
Deleterious variant in SHH gene

Case Report: unaffected parents with first child presenting with semilobar holoprosencephaly

No variant in ZIC2

ZIC2 c.975_976dupCG
Droplet Digital™ polymerase chain reaction (ddPCR)

Next Generation Sequencing and Droplet Digital™ polymerase chain reaction (ddPCR)

Hu et al., 2018
Summary of 20 Families with “de novo” variants

### Table 1 Summary of targeted capture BAM files analysis of parental mosaicism in families with putative de novo variants detected in probands and confirmation analysis by ddPCR

<table>
<thead>
<tr>
<th>Family</th>
<th>Variant</th>
<th>Read count in proband (%)</th>
<th>Read count in parents F (%)</th>
<th>Mosaicism confirmation by ddPCR (%)</th>
<th>Clinical findings: proband (relative(s) and/or controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td>SXC c.338G&gt;A (pathogenic)</td>
<td>F 0.5% = 0/361 M 9% = 37/406</td>
<td>F (not tested) M (13%)</td>
<td>Lobar HPE, corpus callosum dysgenesis, hydrocephalus, spina bifida, seizures, developmental delay. (Deceased sib with alobar HPE, not tested for this variant.)</td>
<td></td>
</tr>
<tr>
<td>Family 2</td>
<td>SHH c.766G&gt;T (pathogenic)</td>
<td>F 10% = 10/99 M 0% = 0/85</td>
<td>F (13%) M (not tested)</td>
<td>Proband has HPE. (Sib AM3808 confirmed as affected and c.766G&gt;T, p.(E256*) positive by CLIA testing at GeneDx; One detection among healthy controls in Kaviar.)</td>
<td></td>
</tr>
<tr>
<td>Family 3</td>
<td>FGFR1 c.1880G&gt;C (pathogenic)</td>
<td>F 3% = 26/840 M 0.1% = 1/827</td>
<td>F (3.7%) M (not tested)</td>
<td>Semilobar HPE, corpus callosum dysgenesis, diabetes insipidus, growth hormone deficiency, bilateral cleft lip/palate, microtia, cryptorchidism, ectodactyly both hands and feet. (Multiple affected sibs testing positive for this variant.)</td>
<td></td>
</tr>
<tr>
<td>Family 4</td>
<td>ZIC2 c.975-976dupCG (pathogenic)</td>
<td>F 0.1% = 0/1291 M 1.54% = 19/1233</td>
<td>F (not tested) M (2.1%)</td>
<td>Normal karyotype, alobar HPE, hypotelorism, depressed nasal root. (Mother has microform findings, microcephaly, hypotelorism, learning disability.)</td>
<td></td>
</tr>
<tr>
<td>Family 5</td>
<td>ZIC2 c.1076-1G&gt;A (pathogenic, splice site)</td>
<td>F 0.3% = 1/342 M 0% = 0/195</td>
<td>F (0.1%) M (not tested)</td>
<td>Semilobar HPE. Incomplete family history.</td>
<td></td>
</tr>
</tbody>
</table>

A alternative allele; ACMG American College of Medical Genetics and Genomics; BL blood sample; ddPCR Droplet Digital™ polymerase chain reaction; F father; HPE holoprosencephaly; LCL lymphoblastoid cell line; M mother; R reference allele

aConfirmed by Sanger sequencing in a CLIA lab

Variants correspond to these transcripts: NM_000193.2 (SHH), NM_007129.3 (ZIC2), NM_005413.3 (SXC), NM_023110.2 (FGFR1)


cHu et al., 2018
Three Types of Mosaicism
Human Germ Cell Line Development

Campbell et al. The American Journal of Human Genetics 95, 173–182, August 7, 2014
Germline Mosaicism in Seizure Disorders

<table>
<thead>
<tr>
<th>Family No.</th>
<th>Proband Phenotype</th>
<th>Mutation</th>
<th>Parent with Mosaicism</th>
<th>Sample Type, % Mosaicism</th>
<th>Phenotype of Parent with Mosaicism</th>
<th>No. of Affected Siblings</th>
<th>Phenotype of Affected Sibling, Mutation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dravet syndrome</td>
<td>SCN1A p.R101W</td>
<td>Father</td>
<td>Blood, 29.6; saliva, 16.7</td>
<td>Unaffected</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Dravet syndrome</td>
<td>SCN1A p.S1516*</td>
<td>Mother</td>
<td>Blood, 17.6</td>
<td>Febrile seizure</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>Dravet syndrome</td>
<td>SCN1A p.I1483MFS*18</td>
<td>Father</td>
<td>Blood, 30.6; saliva, 24.1</td>
<td>Febrile seizure</td>
<td>1</td>
<td>Dravet syndrome, SCN1A heterozygote</td>
</tr>
<tr>
<td>4</td>
<td>Developmental and epileptic encephalopathy</td>
<td>SCN8A p.L1331V</td>
<td>Father</td>
<td>Blood, 12.0; saliva, 4.7</td>
<td>Febrile seizures plus†</td>
<td>1</td>
<td>Febrile seizures plus,† focal seizures, learning difficulties, SCN8A heterozygote</td>
</tr>
<tr>
<td>5</td>
<td>Developmental and epileptic encephalopathy</td>
<td>KCNT1 p.R950Q</td>
<td>Father</td>
<td>Blood, 10.8; saliva, 14.0</td>
<td>Mild autosomal dominant nocturnal frontal lobe epilepsy</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>Developmental and epileptic encephalopathy</td>
<td>KCNQ2 p.V567D</td>
<td>Mother</td>
<td>Blood, 1.4; saliva, 1.5</td>
<td>Unaffected</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>Epilepsy with myoclonic–tonic seizures</td>
<td>SLC6A1 p.A334P</td>
<td>Mother</td>
<td>Blood, 8.0; saliva, 9.3</td>
<td>Unaffected</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>Developmental and epileptic encephalopathy</td>
<td>GNB1 p.A326T</td>
<td>Father</td>
<td>Blood, 7.8</td>
<td>Unaffected</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>Developmental and epileptic encephalopathy</td>
<td>CACNA1A p.A713T</td>
<td>Mother</td>
<td>Blood, 6.4; saliva, 8.5</td>
<td>Unaffected</td>
<td>1</td>
<td>Developmental and epileptic encephalopathy, CACNA1A heterozygote</td>
</tr>
<tr>
<td>10</td>
<td>Developmental and epileptic encephalopathy</td>
<td>DNM1 p.R237W</td>
<td>Father</td>
<td>Blood, 4.5; saliva, 4.1</td>
<td>Unaffected</td>
<td>1</td>
<td>Developmental and epileptic encephalopathy, DNM1 heterozygote</td>
</tr>
<tr>
<td>11</td>
<td>Developmental and epileptic encephalopathy</td>
<td>SYNGAP1 p.L150VFS*6</td>
<td>Inferred</td>
<td>ND</td>
<td>Unaffected</td>
<td>1</td>
<td>Developmental and epileptic encephalopathy, SYNGAP1 heterozygote</td>
</tr>
<tr>
<td>12</td>
<td>Epilepsy with myoclonic–tonic seizures</td>
<td>KIAA2022 p.R322*</td>
<td>Inferred</td>
<td>ND</td>
<td>Unaffected</td>
<td>1</td>
<td>Epilepsy with myoclonic or atonic seizures, KIAA2022 heterozygote</td>
</tr>
</tbody>
</table>

* NA denotes not applicable, and ND not detected.
† Febrile seizures plus are febrile seizures that occur after the age when these seizures usually occur (3 months to 6 years) or when there are concurrent afebrile tonic–clonic seizures.
Resources for patients and providers

• Fetal Medicine Institute: genetic counseling, fetal MRI and ultrasound, Neurology consultation, Geneticist consultation
  www.childrensnational.org/departments/fetal-medicine-institute

• NIH: paul.Kruszka@nih.gov and mamuenke@mail.nih.gov

• Support groups and websites:
  • www.familiesforhope.org
  • http://www.hperesearch.org
HoPE Conference, Dallas 2016
Conclusions

- Holoprosencephaly is routinely diagnosed prenatally
- Genetic counseling should be performed pre- and post-genetic testing
- After excluding trisomy 13, much of the genetic and environmental basis of holoprosencephaly is unknown
- Inherited holoprosencephaly is incompletely penetrant
- *De novo* can be gonadal mosaicism
- Genetic counseling with case examples is complex and will be become complex with time