

## Holoprosencephaly: An Antenally-diagnosed Case Series and Subject Review

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### Abstract

**Introduction:** Holoprosencephaly (HPE) is an uncommon congenital failure of forebrain development. Although the aetiology is heterogeneous, chromosomal abnormalities or a monogenic defect are the major causes, accounting for about 40% to 50% of HPE cases. At least 7 genes have been positively implicated, including *SHH*, *ZIC2*, *SIX3*, *TGIF*, *PTCH1*, *GLI2*, and *TGDF1*. **Clinical Picture:** Twelve antenatally- and 1 postnatally-diagnosed cases are presented in this study. These comprised 6 amniotic fluid, 3 chorionic villus, 2 fetal blood, 1 peripheral blood, and 1 product of conception. **Outcome:** The total chromosome abnormality rate was 92.3%, comprising predominantly trisomy 13 (66.7%). There was 1 case of trisomy 18, and 3 cases of structural abnormalities, including del13q, del18p, and add4q. **Conclusion:** Despite the poor outcome of an antenatally-diagnosed HPE and the likely decision by parents to opt for a termination of pregnancy, karyotyping and/or genetic studies should be performed to determine if a specific familial genetic or chromosomal abnormality is the cause. At the very least, a detailed chromosome analysis should be carried out on the affected individual. If the result of high-resolution karyotyping is normal, Fluorescence in situ hybridisation (FISH) and/or syndrome-specific testing or isolated holoprosencephaly genetic testing may be performed. This information can be useful in making a prognosis and predicting the risk of recurrence.

Ann Acad Med Singapore 2008;37:594-7

**Key words:** Chromosomes, Genes, Karyotyping, Trisomies

### Introduction

Holoprosencephaly (HPE) is an uncommon congenital developmental defect of the forebrain structures to divide into separate hemispheres and ventricles during embryogenesis. The prevalence rate of HPE is estimated to be between 1 in 11,000 to 1 in 20,000 live births, and 1 in 250 during early embryogenesis.<sup>1</sup> The ratio of females to males is 2:1.

Although there is a continuous spectrum of craniofacial malformations from severe dysmorphism to normal, the condition can be classified into 3 major anatomic brain categories. In the most severe and predominant form, the lobar form, there is a total absence of the 2 cerebral hemispheres due to the complete failure of septation into the left and right halves, resulting in a single ventricle with fused thalami, arrhinencephaly and agenesis of the corpus callosum. The semilobar form has varying degrees of

hemispheric formation but the frontal lobes are fused. There is usually an absence of the olfactory lobes and the corpus callosum is at best partially formed. The lobar form has a near-normal appearance, characterised by an almost complete separation of the cerebral hemispheres but the olfactory lobes and corpus callosum may be incompletely developed. As a result of these malformations, the mid-facial areas of HPE patients are frequently disturbed, with a range of phenotypes from cyclopia to near normal.

Although the aetiology is heterogeneous, chromosomal abnormalities or a monogenic defect are the major causes, accounting for about 40% to 50% of HPE cases.<sup>2</sup>

An earlier study presented 7 cases of HPE diagnosed at the former Kandang Kerbau Hospital, Singapore.<sup>3</sup> In this study, we present an additional 13 cases of antenally- or postnatally-diagnosed HPE, between 2000 and 2007, with a brief review of the molecular developments since then.

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## Materials and Methods

Between April 2000 and March 2007, 13 specimens were received for karyotyping at the Singapore General Hospital (SGH) on the basis of an abnormal ultrasound finding/clinical assessment that included HPE. Twelve cases were diagnosed antenatally, and 1 postnatally. This comprised 6 amniotic fluid, 3 chorionic villus, 2 fetal blood, 1 peripheral blood and 1 product of conception. Two of these specimens were from Singapore and the rest were from Malaysia.

### Amniotic Fluid and Chorionic Villus Samples (CVS)

These specimens were set up using the *in situ* culture technique. Briefly, the cell suspension of each case was plated onto 22 x 22 mm sterile glass coverslips within a 35-mm petri dish. Five cultures were set up per case, and were harvested after about a week from initiation.

### Product of Conception Sample

Five cultures were established using the *in situ* coverslip technique and were harvested one-and-a-half weeks after.

### Blood Samples

Bloods were set up using the suspension culture method, with 2 to 3 cultures established per case. The cultures were harvested after 48 and 72 hours of incubation.

### Fluorescence In Situ Hybridisation (FISH)

FISH with telomere and LSI probes for 18pter/qter (Cytocell, UK) and 13q14/q34 (Vysis Inc., IL, USA) was performed in accordance with the manufacturers' recommendations.

## Results

Among the 12 antenally-diagnosed cases, the women were aged 24 to 43 years, with a mean age of 31.6 years. Of the 13 cases, 12 had an abnormal karyotype (92.3%). The predominant chromosomal abnormality was trisomy 13, comprising 8 cases (66.7%). All the other karyotypic abnormalities involved single cases, including 1 trisomy 18, and 3 cases with different structural abnormalities. These included deletion of 13q (Fig. 1), deletion of 18p (Fig. 2), and additional material on 4q (Fig. 3). FISH confirmed the rearrangements of 18p- and 13q-. The single normal karyotype case had a 46,XY constitution.

## Discussion

The leading cause of holoprosencephaly is a chromosomal disorder, comprising 40% to 50% of all documented cases.<sup>1,4</sup> Trisomy 13 alone accounts for 75% of the abnormal karyotypes, with trisomy 18 constituting the bulk of the remaining cases, along with the occasional cases of triploidy and trisomy 21.<sup>5,6</sup> The total abnormality rate in our series

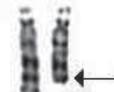


Fig. 1. Partial karyotype showing a terminal deletion of chromosome 13 at band q31.

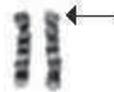


Fig. 2. Partial karyotype showing a terminal deletion of chromosome 18 at band p11.31.

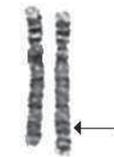


Fig. 3. Partial karyotype showing additional material at band q33, resulting in monosomy for bands distal to this breakpoint.



Fig. 4. Ultrasound image showing alobar brain with fused thalami and single horseshoe shaped ventricle.

was much higher at 92.3%. This difference can largely be explained by the smallness of our study sample. But in keeping with the literature, trisomy 13 was the most prevalent cause of HPE, accounting for 66.7% of the total abnormality rate, in contrast to trisomy 18 with only a single case. In our experience, trisomy 18 fetuses rarely present with HPE. The extra gene copy can disrupt normal metabolic functioning.<sup>4</sup>

In this study, the structural abnormalities included deletion 13q, deletion 18p, and loss of distal 4q. At least 7 genes have been positively implicated in HPE: *SHH*, *ZIC2*, *SIX3*, *TGIF*, *PTCH1*, *GLI2* and *TDGF1*.<sup>7</sup> Mutations of *SHH*, mapped to 7q36, are the most common.<sup>8</sup> None of our cases appeared to involve the 7q36 band. Along with *SHH*, *ZIC2*, *SIX3*, and *TGIF* are expressed early during embryonic gastrulation, between the 18th to 28th day of gestation.<sup>1</sup> Many of these genes interact with one another, or act as a precursor upstream. Disturbance to any upstream gene function can cause a cascade of events that can result in HPE.

Before the putative genes were identified, Wilson et al<sup>9</sup> reviewed cases of HPE associated with trisomy 13, partial

trisomy 13, and deletions of 13q. The authors erroneously ruled out deletion or duplication of single chromosome 13 bands as the cause of HPE. However, they did note that altered timing of forebrain development, leading to a reversion to a more primitive brain structure, is related to an imbalance of chromosome 13 regions. It is now known that heterozygous loss of *ZIC2* at 13q32 via deletion or mutations of this zinc finger transcription factor can lead to brain malformations due to haploinsufficiency.<sup>10</sup> Our case with the terminal loss of 13q31 effectively resulted in the heterozygosity of *ZIC2*. FISH showed that the deletion extended to at least band q34 with the proximal q14 band conserved. The abnormality was *de novo*. Of interest, while loss of *ZIC2* function leads to severe central nervous system (CNS) abnormalities, most of these patients have mild facial anomalies, contradicting DeMyer et al's "the face predicts the brain" rule,<sup>11</sup> which applies to about 80% of cases. Croen et al<sup>5</sup> had earlier noted that while severe facial phenotypes often predicts lobar or semilobar HPE, a mild facies may still herald an alobar brain. The fetus in our study had alobar HPE (Fig. 4).

One fetal blood karyotype showed a terminal deletion of 18p, confirmed by telomere FISH studies. This rearrangement was also *de novo*. HPE is associated with rearrangements involving the short arm of chromosome 18 resulting in loss of a part or the whole arm. The *TGIF* (*TG-interacting factor*) gene, an atypical homeodomain protein located at the holoprosencephaly critical region HPE4 at 18p11.3 may act as a repressor of retinoic acid-regulated gene transcription whose loss of function may lead to excessive retinoic acid exposure.<sup>12</sup> Studies in mice and humans have shown that retinoic acid exposure can cause CNS abnormalities resembling HPE.<sup>13</sup> On the other hand, *TGIF* may also act as a co-repressor of the *SMAD2* protein. The latter has been linked to the Nodal signalling pathway and defects in Nodal-related genes are known to cause cyclopia.<sup>14,15</sup> Gripp et al<sup>12</sup> detected 4 heterozygous missense mutations most likely to lead to loss of *TGIF* function. These mutations, together with the documented association of HPE and loss of 18p, suggest that HPE4 is an autosomal dominant disorder due to haploinsufficiency. Chow et al<sup>3</sup> reported a case with alobar HPE with a 46,XY,i(18q)(q10;q10) karyotype. Their case may similarly be explained by a loss of *TGIF* in 18p.

The peripheral blood with the unbalanced 46,XY,add(4)(q33) karyotype was from a 2-year-old child diagnosed as having either septo-optic dysplasia or HPE. Discrimination between the two is sometimes difficult as septo-optic dysplasia somewhat resembles lobar holoprosencephaly.<sup>16</sup> Indeed, most authors agree that this syndrome may be within the spectrum of HPE. Although the additional material was not determined, the

rearrangement resulted in monosomy for the segment 4q33->qter and partial trisomy of the unknown region. The rearranged segment may harbour an as yet unidentified candidate gene for HPE.

The aetiology of the disease in the case with the normal karyotype was not determined. As mentioned earlier, the causative factors of HPE are manifold, of which chromosomal disorders account for not more than 40% to 50%. Mutations (missense, nonsense, deletion, insertion, and frameshift) seen in the putative genes account for only a very small proportion of all HPE cases.<sup>17</sup> Apart from chromosomal abnormalities and genetic defects, the remaining cases of HPE are thought to be environmentally- and/or teratogenic-derived, including insulin-dependent maternal diabetes, alcohol consumption and cholesterol synthesis inhibitors.<sup>2,4</sup> While HPE is not associated with advanced maternal age,<sup>18</sup> maternal insulin-dependent diabetes is thought to account for as many as 4% to 8% of HPE cases.<sup>4</sup> Other non-genetic causes include exposure to high maternal intake of alcohol early in pregnancy, and exposure to teratogens. These varied causes attest to the extreme heterogeneity of the disease. Since disturbance of brain development resulting in HPE occurs before day 28 of embryonic life, basically any factor that causes perturbation to forebrain development at the critical phase of the mid- to late-gastrulation period of embryogenesis can result in HPE. With the above case with the normal karyotype, there was no indication of fetal alcohol syndrome, history of maternal diabetes or exposure to alkaloids, cytomegalovirus (CMV) infection or other teratogens.

In some instances, HPE has been associated with triploidy. Chow et al<sup>3</sup> reported 1 case of triploidy in their series. In our experience with triploid miscarriages, none presented with HPE.

The fear of recurrence in a family with a history of HPE is the reason why karyotyping and/or mutational studies are of great importance. The recurrence risk depends on the underlying aetiology of the condition. While both autosomal dominant and recessive conditions have been reported, incomplete penetrance or an incomplete form or microform can make the analysis of familial occurrence problematic. Generally, if the cause is due to a *de novo* monogenic defect of any of the putative genes, the risk is just slightly above that of the general population risk, owing to the possibility of mosaicism in the parents. Direct transmission from a mildly affected mosaic mother to her 2 offspring has been reported.<sup>19</sup> The same risk applies to *de novo* chromosome abnormalities such as deletions. In the majority of cases, deletions are *de novo*, such as with our cases. However, if it is due to an inherited single gene mutation or a familial chromosomal rearrangement involving HPE loci, the risk may be as high as 50%. The recurrence risk if HPE is due

to an inherited autosomal recessive condition may be as high as 25%. Because of the manifold causes and the high incidence of HPE as a result of reasons other than chromosomal and genetic disorders, the family history must be carefully studied. With such sporadic occurrence, the risk is about 6% in non-chromosomal cases. Finally, an abnormal karyotype like trisomy 13 suggests a low recurrence risk because it is due to a random malsegregation error during meiosis. This is about twice the maternal age-related risk for recurrence of that trisomy.

Generally, for live born cases, the prognosis is poor in terms of survivability and quality of life, depending on the HPE severity and the associated medical and neurological complications. The degree of mental retardation varies from mild to severe, with the IQ ranging between 25 and 75, although normal or borderline intelligence have been reported.<sup>20</sup> Perhaps the strongest correlation is a direct relationship between the severity of facial anomalies and increased mortality. Individuals with cyclopia or ethmocephaly have a median survival that is rarely beyond 1 week. On the other hand, life expectancy is not compromised in cases with isolated HPE. Mildly affected children may exhibit few symptoms and live a normal life.

Despite the poor outcome of an antenatally-diagnosed HPE and the likely decision by parents to opt for a termination of pregnancy, karyotyping and/or genetic studies should be performed to aid counselling. The cost of these tests is estimated at not a lot higher than a couple of thousand dollars collectively, but the cost to parents of not knowing the cause of the condition in the proband or the recurrence risk is immeasurable in terms of anxiety. The SGH Birth Defect Registry recorded 5 cases of antenatally-diagnosed HPE over the 8-year period, of which only 2 were karyotyped. This reveals a low take-up rate of karyotyping as a follow-up to abnormal ultrasound findings. Given the incidence of HPE of between 11,000 and 20,000, the number of liveborn HPE cases in Singapore over the past 8 years would be in the region of 1.9 to 3.5 per annum, or about 15 to 28 cases. While no data is available to us on the number of cases that were karyotyped at the other hospitals, it is likely that many cases may not have been adequately followed up. Although both of the antenatally-diagnosed structurally abnormal cases in this study were *de novo*, prenatal or preimplantation genetic testing may be an option in future pregnancies if a specific familial genetic or chromosomal cause can be identified. At the very least, a detailed chromosome analysis should be carried out on the affected individual. If the results of high-resolution karyotyping are normal, syndrome-specific testing or isolated holoprosencephaly genetic testing (e.g., *SHH*,

*ZIC2*, *SIX3*, *TGIF*) may be performed. This information can be useful in making a prognosis and predicting the risk of recurrence.

#### Acknowledgements

The authors would like to thank Ms Lau Lai Ching, Ms Fiona Liaw, and Ms Teng Li Min from the Cytogenetics Laboratory for their excellent bench work support. The authors would also like to thank senior staff nurse Tham Yim Leng for her invaluable help with the statistics from the SGH Birth Defect Registry.

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