Holoprosencephaly (HPE) is a clinically and genetically heterogeneous malformation of the forebrain development. The most severe form is called alobar HPE, where there is failure of division of the telencephalon into two cerebral hemispheres with a single ventricle. These children can present with severe craniofacial anomalies such as cyclopia, or premaxillary agenesis. Less severe forms of HPE, such as semilobar or lobar HPE can present with mild facial dysmorphism such as hypotelorism, iris coloboma, absent or abnormal upper labial frenulum, single maxillary central incisor, or cleft palate. Some patients with these forms of HPE may have no obvious craniofacial anomalies.

Most cases of HPE are sporadic but familial forms have been described. These usually show autosomal dominant inheritance with reduced penetrance and variable expression. At the present time 12 loci have been identified and genes at four loci identified. These include Sonic Hedgehog (SHH) gene at 7q36, ZIC2 at 13q32, SIX3 at 2p21, and TGIF at 18p11.3. Mutations in these genes have been identified in sporadic and autosomal dominant forms of HPE.

Mutations in SHH account for a significant proportion of autosomal dominant HPE. Although sporadic forms of HPE are more frequent than familial forms, SHH mutations have been identified more frequently in familial (autosomal dominant) HPE than sporadic HPE. SHH is a homologue of the Drosophila hedgehog (hh) gene, which is a segment polarity gene. In mice have shown that shh is expressed in a large number of tissues including notochord, ventrolateral midbrain, ventral forebrain, gut endoderm, branchial arches, posterior distal limb mesenchyme, testis, and penis. In Drosophila the hh protein is the ligand for a transmembrane receptor called patched (Pch). In the absence of hh protein Pch inhibits another transmembrane receptor called smoofened (Smo); When hh binds to Pch, Smo is released from inhibition, which activates other intracellular signalling pathways. This hh/patched signalling pathway is conserved from Drosophila to mice. In humans the downstream target genes for SHH include the GLI factors, and the WNT and BMP gene families.

We describe a family in which several members over two generations were found to have a missense mutation in SHH with remarkable variability of expression.

CASE REPORT

Figure 1 shows the pedigree of this family. The family was ascertained following the identification of HPE in the index case (III:3) by antenatal ultrasound.

Case 1 (III:3)

This fetus was the product of the third pregnancy of non-consanguineous white parents. A detailed fetal anomaly scan at 20 weeks gestation had shown alobar HPE. The pregnancy was terminated at 21 weeks gestation and postmortem examination showed a male baby with microcephaly, hypotelorism, premaxillary agenesis, and alobar HPE (fig 2). Fetal karyotype was normal. A novel missense mutation in SHH was identified in fetal DNA. This mutation had resulted in a substitution of thymine for an adenine residue at nucleotide position 263 of SHH, resulting in the substitution of the amino acid aspartic acid for valine at position 88 of the SHH peptide.

Case 2 (III:4)

This is an older sibling of case 1. He was born at term with a birth weight of 2.94 kg (9–25th centile) and a head circumference of 34 cm (9–25th centile). He had a right sided cleft lip, cleft palate, inferior iris coloboma, sensorineural hearing loss, and single palmar creases. He had feeding difficulties with failure to thrive, global developmental delay, and postnatal development of microcephaly with his head circumference running parallel to but 2–3 cm below the 0.4th centile. At 3 years of age he was assessed using the Schedule of Growing Skills II. This showed that he was functioning at about the 18–24 months level in all areas except visual skills (wearing glasses) where he was functioning at an age appropriate level. When this assessment was repeated at the age of 36 months his locomotor, manipulative, speech and language, and interactive/social skills were at the 24 months level. His visual skills were at the 48 months level, and his hearing and language and self care/social skills were only at the 18 months level.

Abbreviations: ADHD, attention deficit hyperactivity disorder; CNS, central nervous system; CT, computed tomography; HPE, holoprosencephaly; MRI, magnetic resonance imaging; Pch, patched transmembrane receptor; Smo, smoofened transmembrane receptor; SHH, sonic hedgehog; TGIF, TGF-like factor; SIX3, Sin3 related HMG box; ZIC2, zinc finger gene.
level. A cranial magnetic resonance imaging (MRI) scan at this time was normal.

At the age of 6 years and 3 months he is hypotonic and clumsy, with mild learning difficulties. He also has problems with polydipsia and polyuria and drinks at least four litres of fluid a day. He has significant problems with enuresis but his early morning plasma and urine electrolytes and osmolality are normal. He has a clinical diagnosis of attention deficit disorder that has responded well to low dose methylphenidate (10 mg morning, 5 mg midday) with improvement in his functioning at school. Assessment using the NEPSY showed an attention executive score on the 21st centile, sensorimotor score on the 2–10th centile, and memory score on the 2nd centile. Genetic testing showed that he also had the Asp88Val \( SHH \) mutation that had been identified in his sibling with alobar HPE (figure 3).

Case 3 (III:9)
This child is the maternal first cousin of cases 1 and 2. He was born at 34 weeks gestation by emergency caesarean section. His birth weight was 2.2 kg (50th centile) and his head circumference 28 cm (0.4th centile). He was noted to have hypospadias at birth. Postnatally his head circumference fell below the 0.4th centile and continued to grow 2–3 cm below the 0.4th centile. His height grew along the 0.4th centile and his weight just below the 0.4th centile. At 9 months his development was thought to be normal but at the age of 3 years concerns were raised about his development, particularly with regard to language. He was found to have notable hypotelorism. A Griffith assessment at 3 years and 9 months showed delays, particularly in speech and language where he performed at less than a 2 year level. He performed at a 2–2.5 year level for all other areas.

The Griffith assessment was repeated at the age of 45 months. His gross motor skills were at the 39 month level, personal social skills at 27 months, speech/language at 22 months, eye–hand coordination at 28 months, performance at 40 months, and practical reasoning at the 26 months level. His understanding and level of attention limited the assessment. He was unable to complete an NEPSY assessment as he had an attention/executive score of <1st centile, with very poor auditory attention and memory skills. His main difficulties were around hyperactivity, impulsive behaviour, and poor concentration, which impaired his performance in the classroom. He responded well to a trial of low dose methylphenidate (10 mg morning, 5 mg midday) with significant improvement in his abilities to follow instructions and complete tasks at school. He did not have a cranial MRI scan but genetic testing confirmed that he too had the Asp88Val \( SHH \) mutation. (figure 4)

Case 4 (II:4)
The mother of cases 1 and 2 had learning difficulties as a child and also had problems with concentration. On examination, her head circumference was 2 cm below the 0.4th centile. She had mild hypotelorism and a high arched palate. She was also shown to have the familial \( SHH \) mutation (figure 4).

Case 5 (II:5)
The mother of case 3 had a history of mild learning difficulties and received some extra support at school. On examination her head circumference was 51 cm (<0.4th centile) and her height was 149 cm (0.4–2nd centile). She had mild hypotelorism and a high arched palate. She was also shown to have the Asp88Val \( SHH \) mutation (figure 4).

Case 6 (II:2)
This was the younger sister of cases 4 and 5. She was being treated for systemic lupus erythematosus. Her head circumference was 51 cm (<0.4th centile) and her height was 149 cm (0.4–2nd centile). She had mild hypotelorism and a high arched palate. She was also shown to have inherited the familial \( SHH \) mutation like her two older sisters.

Case 7 (II:1)
This was the youngest sister of cases 4, 5 and 6. She had a history of moderate learning difficulties requiring education at a special school and lived with her mother. She had longstanding torsion dystonia, right facial hemiatrophy and a
ties. No further information was available about this man.

from the maternal grandfather (I:1), who had died in his for-
therefore concluded that the mutation had been inherited
but shown not to carry the familial

Sonic Hedgehog mutation 295

amino acid residue aspartic acid is located in the N-terminal
signalling domain (SHH-N). The phenotypic

alterations in biological activity of

Although the functional effects of the Asp88Val change have
not yet been studied, this mutation presumably leads to
alterations in biological activity of SHH-N. The phenotypic

variability in this family has been described in smaller
kindreds previously, but to our knowledge this is one of the
largest kindreds described. In this family the same SHH mu-
tation was associated with alobar HPE in the index case but HPE
“microforms” in other family members. Craniofacial malfor-
mations that are seen in individuals with normal neuroim-
ing, who are at risk of having children with HPE are called
HPE “microforms”. These include microcephaly, ocular hypo-
telorism, iris coloboma, mid face hypoplasia, congenital nasal
pyriform aperture stenosis, absent or abnormal upper labial
frenulum, and single central maxillary incisors. In case 2 the
ocular hypotelorism was an HPE microform as he had normal
brain imaging and was shown to have inherited the familial
SHH mutation. Case 3 also had hypotelorism but cranial CT or
MRI scans were not performed and he could have had a mild
form of HPE (semilobar or lobar). The microcephaly that was
seen in cases 3, 4, and 5 was likely to have represented an HPE
microform, but CNS imaging was not performed in these
cases. Developmental delay and learning difficulties can also
be considered microforms of HPE, as they can be seen in indi-
viduals with normal neuroimaging, who are at risk of having
affected children with HPE (cases 2–5 and 7). In this family
the phenotype ranges from very mild microcephaly and no
clinical symptoms to an infant with alobar holoprosencephaly.

Both children with the SHH mutation had attention difficul-
ties. These have not previously been described as a microform
of HPE, but may in fact just be a delay in maturation as both
children improved significantly with age.

Non-penetrance has also been described in autosomal
dominant forms of HPE. Obligate mutation carriers in such
families may be asymptomatic with no craniofacial anomalies.
This has been confirmed in families with autosomal dominant
HPE with SHH and SIX3 mutations. Given the intrafamilial
clinical variability in kindreds carrying an SHH mutation, we
speculate that the other gene acting in the same or different
developmental pathways might act as modifier for expression
of the HPE spectrum. Interestingly the HPE patients with an
SHH mutation were also identified with an alteration in a sec-
ond gene which acts on brain development. Because of the
non-penetrance in some HPE families, genetic counselling can
be extremely difficult. Autosomal dominant HPE is estimated
to have a penetrance of 70%. This means that the risk of ana-
omical HPE or microforms of HPE in the offspring of an obli-
gate carrier of autosomal dominant HPE is about 35% (16–21% would be expected to have a severe form of HPE and
around 13–14% would have mild HPE or microforms of HPE).

Those with severe forms such as alobar or semilobar holopros-
encephaly may be identified antenatally by fetal ultrasound
scanning, but those with mild HPE or HPE microforms cannot
be identified in this manner. These children, as shown by the
manifestations in this family, may have a number of signi-
ficant clinical problems and yet normal cranial morphol-
ogy. These problems may include developmental delay, learn-
ing difficulties, cleft lip and palate, and microcephaly. Follow-
ing the birth of a child with HPE, the parents need to be
examined carefully for the microforms of HPE in order to pro-
vide them with an accurate recurrence risk for HPE or HPE
microforms in another pregnancy. Although mutation analy-

sis is not routinely available for autosomal dominant forms of
HPE at present, this family illustrates the importance of com-

bined clinical and molecular assessment to facilitate correct
diagnosis and the provision of appropriate genetic coun-
selling.

Figure 3 Case III:4.

stabile scoliosis. She also had in toeing of her right leg with
sensory disturbance. Her cranial computed tomography (CT)
scan was normal. She was thought to have an autosomal
dominant form of dystonia as she had three maternal uncles
who also had torsion dystonia. She tested positive for the
dominant form of dystonia as she had three maternal uncles
scan was normal. She was thought to have an autosomal

Figure 4 Case III:9 and his mother (II:5). Another child in this
family also has microcephaly, hypotelorism, and similar attention
difficulties but has not been tested.

DISCUSSION

This family highlights the intrafamilial variability of expres-
sion of an identical missense mutation in SHH (Asp88Val). The
amino acid residue aspartic acid is located in the N-terminal
signalling domain (SHH-N) at an invariant position in the

www.archdischild.com