

## ONLINE MUTATION REPORT

# First occurrence of aprosencephaly/atelencephaly and holoprosencephaly in a family with a *SIX3* gene mutation and phenotype/genotype correlation in our series of *SIX3* mutations

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**A**prosencephaly/atelencephaly (AP/AT) refers to rudimentary prosencephalon, while holoprosencephaly (HPE) is a failure of telencephalon cleavage.<sup>1</sup> The question of whether AP/AT results from a disruptive lesion or a growth failure is still debated.<sup>1,2</sup> At least six causal genes, namely *SHH*, *ZIC2*, *SIX3*, *TGIF*, *PATCHED*, and *CRIPTO* (*TDGF1*), are linked to HPE and to ventral and dorsal patterning defect of the neural tube.<sup>3-7</sup>

We report the first occurrence of AP/AT and HPE in a non-consanguineous family with three affected sibs, carrying a maternally inherited mutation of the *SIX3* gene. A phenotype/genotype correlation in the series of seven *SIX3* mutations found in our cohort of 210 HPE probands is provided.

## METHODS

Systematic sequencing of the entire coding region of the *SHH*, *ZIC2*, *TGIF*, and *SIX3* genes has been performed in a series of 210 non-chromosomal HPE probands including 56 patients reported previously.<sup>8</sup> Probands were included in the study after brain imaging, karyotyping, and detailed clinicopathological studies, referring to the protocol for fetuses.<sup>9</sup> Mutational analyses were performed as detailed elsewhere.<sup>10</sup>

## RESULTS AND DISCUSSION

Of the 210 patients screened, seven had mutations in the *SIX3* gene but none in other HPE causing genes. The findings are illustrated in figs 1, 2, and 3. HPE was associated with AP/AT in three affected sibs carrying a maternally inherited mutation of *SIX3*.

Aprosencephaly/atelencephaly (AP/AT) refers to a rare forebrain malformation defined by a lack of prosencephalic derivatives.<sup>11</sup> In aprosencephaly the prosencephalon is absent, while in atelencephaly it exists as a rudimentary medial vesicle resembling the diencephalon but without the lateral telencephalic vesicles. Holoprosencephaly (HPE) results from incomplete cleavage of telencephalic vesicles. The only constant finding between the three major forms of HPE (alobar, semi-lobar, and lobar) is the continuity of the dorsal telencephalon over the midline (personal observation). Interestingly, craniofacial malformations similar to HPE are found in AP/AT and involve the fronto-nasal eminence (ranging from cyclopia/synophthalmia, cebocephaly, and midline cleft to mild hypotelorism or normal face).<sup>11-13</sup>

Our neuropathological findings in the probands of family 'A' fulfil classical criteria for AP/AT and HPE. Classically in AP/AT and HPE, the midbrain and hindbrain are said to be normal. However, Florell *et al*<sup>12</sup> stress the existence of abnormal cerebellar hemispheres with "a peculiar mesenchymal proliferation" seen only within the central nervous system. These changes have been linked to the "glomeroid vasculopathy" described in an encephaloclastic vasculopathy

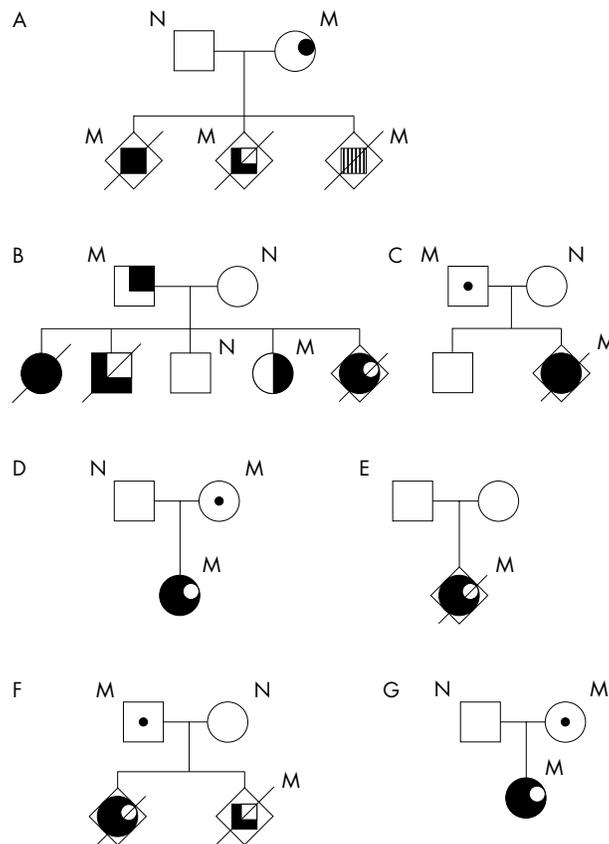
## Key points

- Aprosencephaly/atelencephaly (AP/AT) and holoprosencephaly (HPE) belong to a group of severe forebrain malformations. Neither the occurrence of AP/AT and HPE in the same family nor a common causative gene have ever been documented.
- This is the first report of the occurrence of AP/AT and HPE in a family with three affected sibs carrying a maternally inherited mutation of the *SIX3* gene. Phenotype/genotype correlation in this series of *SIX3* mutations found a preferential association of HPE with eyes abnormalities.
- Our neuropathological and mutational analysis with phenotype/genotype evaluation provides strong evidence for a link between AP/AT and HPE and the role of the *SIX3* gene in the genesis of AP/AT.
- In HPE with eye disorders and no other malformations, we suggest that mutational analysis should start with *SIX3* gene screening.
- *SIX3* plays an essential role in the patterning of the anterior fate of neuroectoderm and in craniofacial and visual field development.

leading to hydrancephaly.<sup>11</sup> We consider thick meninges with persistent embryonic vascular dural plexus to be a common finding in HPE and AP/AT.

During CNS development, sequential specification along anteroposterior (rostrocaudal) and dorsoventral axes occurs even before neural tube closure in Carnegie stage 9. This requires a balance between anteriorising and posteriorising signals ending with the differentiation of the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon) following a general scheme common to vertebrates.<sup>14</sup> By Carnegie stage 11,<sup>15</sup> the rostral most part of the prosencephalon gives rise to the telencephalon medium followed caudally by the diencephalon. At this stage, optic vesicles originate laterally from the diencephalon wall. It is now recognised that the optic primordial appears earlier during development (by Carnegie stage 8) as a single retinal field at the rostral end of the neural plate. The prechordal plate is believed to be responsible for suppressing the median part of the retinal field leading to the emergence of two

**Abbreviations:** AP/AT, aprosencephaly/atelencephaly; HPE, holoprosencephaly; WG, weeks gestation

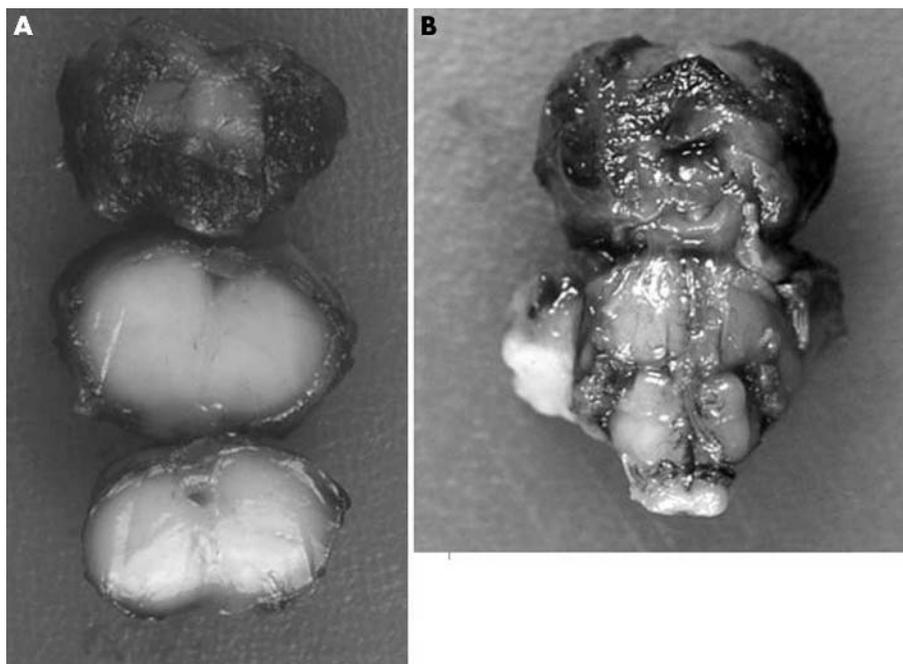


**Figure 1** *SIX3* mutations in HPE: representative pedigrees showing probands and relatives. Filled symbols indicate alobar HPE with synophthalmia or cyclopa. Three quarters filled symbols indicate alobar or semi-lobar HPE without synophthalmia. Half filled symbols indicate minor features of the HPE spectrum with hypotelorism, single upper incisor, and mental retardation. Quarter filled symbols indicate isolated hypotelorism. M, *SIX3* mutation carrier; N, no mutation. (A) Familial case with missense mutation in the SIX domain leading to p.Val92Gly. The last fetus showed cyclopa with atelencephaly (hatched symbol). In all cases, pregnancies were terminated because of CNS malformations. The mother had isolated hypotelorism and abnormal palate. Patient 1, the proband, a 23 weeks gestation (WG) male fetus, had alobar HPE with cyclopa, frontal encephalocele, and no visceral malformations. Patient 2, a 13 WG male fetus, had alobar HPE and arrhinia, with exophthalmos and small bilateral gingival clefts. He had no encephalocele or visceral malformations. Patient 3, a 22.5 WG male fetus, had extreme microcephaly with cyclopa, atelencephaly, sloping forehead, and absent frontonasal eminence, but no other external or visceral malformations. Neuropathological examination disclosed a forebrain reduced to a rudimentary single medial vesicle weighing 9 g (normal for age: 90 g) without telencephalic vesicles or olfactory tracts. A single optic nerve was identified as well as a pituitary stalk (fig 2). Histological evaluation (fig 3) of the rudimentary forebrain disclosed a diencephalic pattern with a well defined third ventricle and thalamic and hypothalamic nuclei. In the brain stem, the cortico-spinal tracts were lacking, but the inferior olivaries were well developed as was the cerebellum. In addition, meninges were thick and hypervascularised. The median eye displayed severe retinal dysplasia with posterior retinal coloboma. (B) Familial case with GG duplication in the SIX domain (c.556\_557dup) creating a frameshift leading to a nonsense mutation 63 amino acids downstream in the homeodomain region already described.<sup>8</sup> The family confirms the recurrence of alobar HPE with synophthalmia (two fused eyes) and proboscis. (C) Familial case with 35 bp duplication in the SIX domain (c.542\_576dup) creating a frameshift leading to a nonsense mutation 58 amino acids downstream in the homeodomain region. The female fetus presented synophthalmia and 11 bilateral ribs. The mutation was inherited from the father who did not present any feature of the HPE spectrum. (D) A 2 year old girl had bilateral cleft, infantile spasms, and semi-lobar HPE, without visual disorders. She had a missense, maternally inherited mutation in the SIX domain (c.605C→T) leading to p.Thr202Ile. The mother has no features of the HPE spectrum. (E) Termination of pregnancy at 32 WG of a female fetus because of alobar HPE and median cleft palate. Visceral and ocular evaluation were unremarkable except for hypoplastic optic nerves. In this fetus a missense mutation in the homeodomain (c.692C→G) leading to p.Pro231Arg was found. DNA samples from her parents were not available. (F) The first pregnancy was terminated because of alobar HPE at 15 WG. The female fetus presents a new missense mutation in the homeodomain (c.769C→T) leading to p.Arg257Trp. In this family, recurrence of semi-lobar HPE led to the termination of the next pregnancy at 22 WG. In both cases, fetal autopsy confirmed HPE without eye disorder or visceral malformation. The mutation was inherited from the father who has no features of the HPE spectrum. (G) A 1 year old girl had alobar HPE with no severe ophthalmological abnormalities (detailed phenotypic data were not available for this patient). She had a maternally inherited missense mutation in the SIX domain (c.518A→C) leading to p.His173Pro. The mother had no clinical signs of the HPE spectrum.

bilateral optic primordial. Lack of inhibition of the median component leads to a single eye (cyclopa) and to its variant synophthalmia. The neurohypophysial anlage rises from the ventral diencephalon and may be deficient in AP/AT as well as in HPE. The future cerebral hemispheres become identifiable during Carnegie stage 14, rising laterally from the telencephalon medium. In terms of timing, AP/AT may be considered as an early failure of the neural tube in forming a normal prosencephalon, while HPE results from the incapacity of the telencephalon to split into two hemispheric vesicles. One can postulate that in addition to cyclopa, HPE

may result from lack of suppression of a presumptive “medial telencephalic field”.

The aetiology of AP/AT is still debated. Classically, AP/AT has been reported as a sporadic event due to an encephaloclastic process.<sup>1,2</sup> AP/AT was associated twice with a chromosomal aberration (46,XY,del(13)(q22q31) and 45,XY,-13/46,XY,r(13)).<sup>11</sup> In our family, the mutation of *SIX3*, an HPE causing gene, supports the hypothesis of a link between AP/AT and HPE. The *SIX3* gene has been isolated from the HPE2 region on chromosome 2p21.<sup>16,17</sup> This gene belongs to the human *SIX* family which contains several



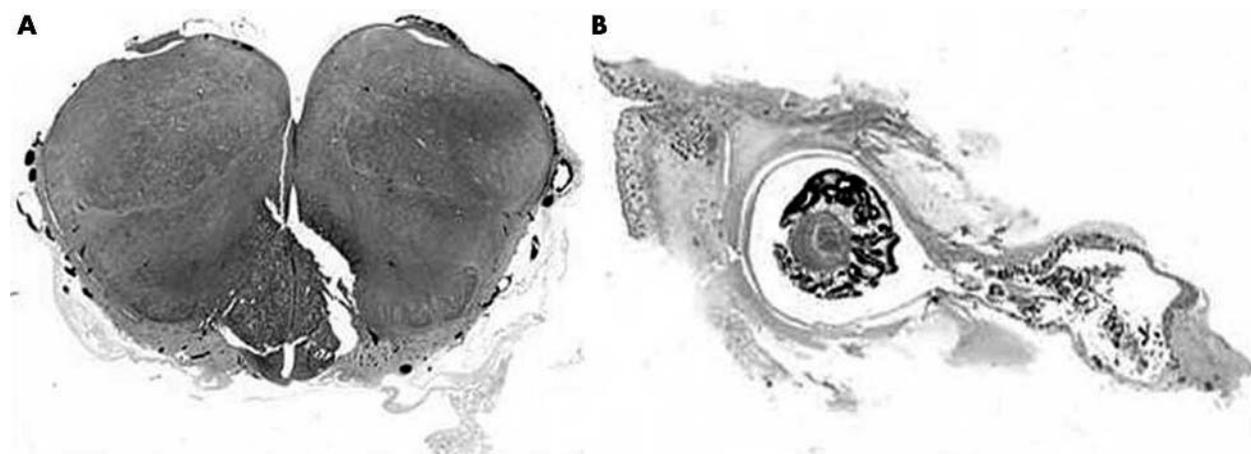
**Figure 2** Neuropathological findings in patient 3. (A) Coronal sections and (B) basal view of the brain. Note the absence of telencephalic vesicles, replaced by a rudimentary medial brain vesicle.

genes (*SIX1/SIX2*, *SIX3/SIX6*, and *SIX4/SIX5*) exhibiting strong amino acid sequence homology.<sup>18</sup> This amino acid sequence of the *SIX3* gene has mainly two DNA binding regions called the SIX domain and the homeodomain. Both share a complete amino acid sequence homology with the mouse *Six3* gene and almost complete homology with the mouse *optix*, a *Drosophila* gene (expressed in eye primordia).<sup>16 17 19</sup> In addition, the mouse *Six3* gene has been found to be expressed in the retina and lens of developing eyes as well as in the hypothalamus and pituitary.<sup>20</sup> In human, expression of *SIX3* has only been studied in the retina and was detected from embryonic weeks 5–7 until adulthood.<sup>19</sup> Interestingly, in *Six3*<sup>-/-</sup> mice the prosencephalon is severely truncated, telencephalic vesicles are lacking, and eyes and olfactory placodes are malformed.<sup>21</sup>

Our mutational analysis of the *SIX3* gene in 210 HPE patients found seven *SIX3* mutations in both highly

conserved regions, two frameshift, and five missense. The new frameshift mutation led to a truncated protein in the homeodomain. This is additional evidence for the involvement of the *SIX3* gene in HPE. The five new missense mutations concerned both the SIX domain and the homeodomain. Interestingly, another missense mutation, c.Arg257Pro, has been also reported in association with semi-lobe HPE, microphthalmia, and iris coloboma.<sup>17</sup> The missense mutations could be related to HPE for two main reasons: they are localised in highly conserved regions (residues Pro231 and Arg257 which are conserved in the *optix* gene) and there is adequate segregation between the mutation and the associated malformations in all tested families (the mutation was not found in 100 normal chromosomes from unrelated Caucasian individuals).

Genotype/phenotype correlations in our series of seven *SIX3* mutations with HPE found three cases with a spectrum



**Figure 3** (A) Histological findings of the medial vesicle showing a characteristic pattern of the diencephalon. Note the presence of a third ventricle and thalamic and hypothalamic nuclei and note the thick hypervascularised meninges. (B) Histological findings of the eye showing retinal dysplasia and posterior cystic retinal coloboma.

of eye anomalies (from synophthalmia/cyclopia to hypotelorism). Eye abnormalities are consistent with the pattern of *SIX3* expression during embryonic development in mice or in humans. Anterior encephalocele found in one case with HPE may be linked to abnormality of ectomenchymal tissue of the cephalic pole. In contrast to other HPE causing genes (*SHH*, *ZIC2*, and *TGIF*),<sup>22</sup> in this series no other malformations, usually associated with the limbs and heart, were found. We suggest that mutational analysis starts with screening of the *SIX3* gene in cases of HPE associated with severe eye disorders but no other malformations.

In conclusion, the occurrence of HPE and AP/AT and our mutational and neuropathological analysis provides strong evidence of a link between AP/AT and HPE which may form part of a continuum of forebrain patterning defects. These results support the hypothesis that *SIX3* is central in the patterning of the anterior fate of the neuroectoderm by inhibiting posteriorising (diencephalic) signals, including, but probably not limited to, Wnt signalling.<sup>23</sup> Further studies, especially of the timing and pattern of *SIX3* gene expression during early human development, should help understanding of the mechanism of AP/AT and HPE.

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