

SUPPLEMENTARY MATERIAL

Clinical data

In Family one, the first ascertained case was a five year old boy, born at 41 weeks gestation by Caesarean section due to difficult labour because of large head size. His birth weight was 3.39 kg (25-50th centile), length was 51 cm, and OFC was 39 cm (>99.6th centile). Positive findings on examination included frontal bossing, upturned nose, large ears and generalized hypotonia. Postnatal brain MRI revealed partial agenesis of the corpus callosum, a well-defined cystic lesion within the left lateral ventricle near the foramen of Monro, see Figure 1. The lesion had a similar signal intensity to the white matter on T1 but displayed bright signal intensity on T2, findings consistent with subependymal cyst. Although ventriculomegaly was apparent, there was no evidence of CSF permeation to suggest active hydrocephalus. In addition, there was right periventricular heterotopia and enlarged cisterna magna. Follow up revealed persistence macrocephaly and a repeat MRI at age nine months showed evolving leukomalacia in both cerebral hemispheres more on the left with diffuse ventriculomegaly sparing the fourth ventricle. Sagittal T2 was suggestive of aqueduct stenosis. He underwent endoscopic third ventriculostomy with fenestration of pineal cyst. However, he developed tonic clonic epilepsy one month postoperatively with only partial response to dual therapy with carbamazepine and keppra. His development was grossly delayed: he only walked at age four years and age five years his vocabulary is limited to 10 words. His most recent growth parameters at five years of age are OFC 54 cm, height 114 cm, and weight 34 kg. Parents are Sudanese first cousins with three normal children and one deceased similarly affected daughter.

In Family one available records on the affected sister revealed that she was born with multiple congenital anomalies in the form of Dandy-Walker malformation, hydrocephalus necessitating a ventriculo-peritoneal shunt placement, ventricular septal defect, cleft lip and palate (Bilateral cleft lip and palate) and epilepsy with abnormal EEG (persistent spikes) started at age of 10 days. Brain MRI revealed a large posterior fossa cyst with atrophy of the cerebellum and partial agenesis of the vermis, suggestive of Dandy Walker Malformation. It also revealed heterotopia in both cerebral hemispheres, posteriorly kinked midbrain, atrophic pons, and a large interhemispheric cyst communicating with the third and lateral ventricles. A follow up MRI showed evidence of an enlarged and disfigured ventricular system, giving the appearance of a monoventricle with absent septum pellucidum, findings suggestive of holoprosencephaly, see Figure 1. The ventricular system appeared to be

separated from a dorsal cyst by thin layers. She passed away because of multiple complications of her surgery and no DNA was available.

In Family two, the first affected was a male infant. At 20 weeks of gestation, he was noted on antenatal ultrasound to have holoprosencephaly. Further ultrasound scanning confirmed alobar holoprosencephaly, midline cleft lip, microcephaly and cardiac abnormality (double outlet right ventricle, ventricular septal defect and possible aortic atresia). He was born at term but died shortly after birth. He also had cyclopia. The parents declined post-mortem examination. He had a normal male karyotype and no evidence of 22q11 deletion.

In Family two the next pregnancy was an affected female infant. Early antenatal ultrasound scans showed probable holoprosencephaly, with the cranium being almost entirely fluid filled and only brain stem structures present. No other abnormalities were reported. She died a few hours after birth. Facial features included ears completely low set and joining together under the chin area and no eyes as such but disrupted tissue beneath a proboscis. The parents declined post-mortem examination. The baby had a normal female karyotype.

The parents are British-Pakistani first cousins. Their first baby was a male infant born at 33 weeks. He had an intra-cranial haemorrhage at age 24 days attributed to haemorrhagic disease of the new-born. There was no underlying structural brain abnormality. This appeared to cause secondary hydrocephalus and cerebral atrophy. He had severe developmental delay and epilepsy. He was subsequently diagnosed with extra hepatic biliary atresia and died aged two years from a chest infection shortly after gastrostomy operation. No DNA was available and chromosome analysis had not been performed. The couple then had an intrauterine death at 17 weeks gestation, and then a healthy son.