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Evidence for *SHH* as a candidate gene for encephalocele

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Introduction

An encephalocele is a congenital malformation involving protrusion of the intracranial contents through a cranial defect. These contents can include cerebral spinal fluid, meninges, or brain tissue. They can be categorized by anatomic location, with the most common being in the occipital region (Kiymaz *et al.*, 2010). The frequency of encephaloceles has been estimated to be approximately 1–5/10,000 live births (Siffel *et al.*, 2003). Although the causes are largely unknown, a number of environmental factors have been reported as being associated with encephalocele, including maternal nutrition deficiencies, aflatoxin exposure, advanced paternal age, and long intervals between pregnancies (Alexiou *et al.*, 2010).

Perturbations of Shh signaling resulting in under or overexpression can result in abnormal early brain development (Himmelstein *et al.*, 2010; Ekker *et al.*, 1995). Heterozygous loss-of-function mutations in the *Sonic Hedgehog* gene cause holoprosencephaly, a disorder resulting from incomplete or failed forebrain separation early in gestation (Roessler *et al.*, 1996). Conversely, mouse models show that activation of the Sonic hedgehog pathway is associated with neural tube defects. Loss-of-function mutations in negative regulators of Shh signaling, such as *Ptch1*, *Sufu*, *Rab23*, *Tulp3*, and *PKA* result in increased activity of the Shh pathway, which can be associated with exencephaly and neural tube defects (Murdoch and Copp, 2010).

Shh signaling has been shown to regulate neural plate bending in the mouse embryo. There are three points of initiation in neural tube closure, which differ in terms of the morphology and location of neural induction. Neural tube closure at these initiation points may involve either median hinge points (referring to the morphologic type of neural tube folding) or dorsolateral hinge points (DLHP) (again, specifying the pattern of neural tube closure, in which the type of bending that occurs is termed dorsolateral bending) (Shum and Copp, 1996). Local release of the Shh-N peptide inhibits DLHP formation and is therefore a physiologic inhibitor of dorsolateral bending, preventing neural tube closure (Ybot-Gonzalez *et al.*, 2002).

We report a patient with an encephalocele who was found to have a *de novo* duplication including the *SHH* gene, which was initially ascertained by microarray. Although encephalocele is not a primary anomaly of neural tube closure, this malformation could arise

secondary to abnormal signaling from an inappropriately patterned neural tube (Murdoch and Copp, 2010).

Clinical report

The patient (Fig 1) was delivered via normal spontaneous vaginal delivery at 31 weeks gestation to a 27 year old G4P2012 woman with a prenatal history concerning for a fetal occipital encephalocele, which was first noted on prenatal ultrasound at approximately 18 weeks gestation. The fetus was noted to have a large encephalocele with the lateral atria and choroid plexus located outside of the fetal skull. Ultrasound also reported to demonstrate abnormal facies with large orbits and a dysplastic mandible. The mother underwent amniocentesis, which showed a normal male chromosome complement (46, XY on routine G-banded karyotype), but a subsequent 105K oligonucleotide array comparative genomic hybridization (CombiMatrix) (showed an approximately 0.4 Mb duplication in the area containing *SHH*: arr 7q36.3 (154,977,855–155,372,260) x3 (hg 18). The duplicated region (Fig 2) includes regulatory regions as well as the genes *CNPY1* and *RBM33*, which are not known to be associated with human disease. Zebrafish knockdown models of *CNPY1* demonstrate midbrain-hindbrain boundary defects and impaired FGF signaling (Hirate and Okamoto, 2006). However, duplication of *CNPY1* has been reported in a normal control (Park *et al.*, 2010). Duplication of *SHH* has not been previously reported in normal controls. Further testing of the parents demonstrated that this duplication was *de novo*.

The mother's prenatal history was remarkable for a previous abortion at about two and a half months of gestation. The fetus had no anomalies noted. The mother also had a history remarkable for a deep venous thrombosis in the immediate postpartum period after a previous delivery. A thrombophilia work up at that time revealed absent beta2-glycoprotein-1 and low protein C and S, which could have been contributing factors to her DVT. The mother's family history was negative for bleeding or clotting disorders. She was treated with Coumadin until approximately 2 months prior to conception, and was started on Lovenox during the affected pregnancy. She had not been taking a multivitamin or folic acid prior to pregnancy. There was no other known history of drug or chemical exposure during the pregnancy. There was no consanguinity and no previous history of fetal abnormalities.

At the time of delivery the infant was noted to have a large occipital encephalocele. He was taken to the NICU for further workup and observation. The infant started to have seizures within the first six hours of life and passed away after three days secondary to respiratory failure, in accordance with the chosen avoidance of life sustaining measures.

Other than the encephalocele, no other anomalies were identified. The infant was described as having normal facial features. Postmortem computerized tomography (CT) showed a large posterior encephalocele (autopsy was declined). There were no other congenital anomalies found; physical examination and CT findings included normal limbs, skeleton, and eyes. Specifically, there was no polydactyly or other limb abnormalities, which have been previously associated with *Shh* overexpression (Gunther *et al.*, 1994). The placenta was not sent for pathologic examination.

Discussion

We describe a patient with an occipital encephalocele who was found to have a *de novo* duplication in a 0.4 Mb region containing *SHH*. Encephalocele is an uncommon congenital malformation, and the etiologies are not well understood. Increased *SHH* signaling has been shown to be associated with an elevated risk of neural tube defects. Occipital encephalocele is a common feature in patients with Meckel-Gruber Syndrome with evidence in mouse models that these malformations are related to abnormal *SHH* signaling (Cui *et al.*, 2011).

Although the malformations in this model relate to a decrease in SHH signaling, abnormalities of signaling in either direction (as SHH is expressed in the neural tube for floor plate specification) could nonetheless contribute to encephalocele.

While this duplication may be related to the congenital anomalies, there is strong evidence that the genomic anomaly would not be sufficient alone. There have been several reports of trisomy of the long arm of chromosome 7 but only 18 reported cases in one series of pure partial trisomy without additional monosomy of other chromosomes (Scelsa *et al.*, 2008). There is wide phenotypic variation among patients with these mutations. Novales *et al.* initially categorized phenotypes, which was revised by Scelsa *et al.* based on location of the duplicated region. Scelsa *et al.* describes four different groups of phenotypes, with group 1 having the entire arm duplicated, group 2 with duplication of large segments with proximal breakpoints, group 3 with interstitial duplications of various sizes, and group 4 with more distal duplications (and presumably including *SHH*). The duplication of the presented patient would therefore fall into group 4, described phenotypically with macrocephaly, frontal bossing, small nose, low-set ears, and developmental delay (Scelsa *et al.*, 2008). Of note, there are no case reports of patients with these duplications presenting with encephalocele or neural tube defects.

However, on a biological basis, we postulate that the duplication could be the etiology for this patient's encephalocele. Sonic hedgehog is required for ventral cell types within the spinal cord. Gunther *et al.* identified a new mouse mutation termed *open brain (opb)*, resulting in neural tube defects and alterations in gene expression along the spinal cord. In the ventral spinal cord of affected mouse embryos, Shh expression was greater than in the wild-type embryos (Gunther *et al.*, 1994). This overexpression of the gene may be a contributing factor to the phenotype in these mice. It is important to note however that most of the mutant embryos also had limb, axial skeleton and eye abnormalities, which were not seen in this patient.

Though little is known about the function of the other genes in the duplicated interval, *CNPY1* is interesting, given the fact that in zebrafish, this gene appears to be related to midbrain-hindbrain boundary signaling (Hirate and Okamoto, 2006). Duplication of *CNPY1* has been reported in one normal control though, making it less likely to be the sole underlying cause. It is entirely possible that the patient's phenotype is related to overexpression of multiple genes (as well as regulatory elements) in the duplicated region. Admittedly, the cause may also be entirely unrelated to the duplication. In addition, it is possible that this phenotype could be related to the maternally inherited duplication of chromosome 15 although this is less likely given the lack of phenotypic features in the mother.

Other confounding variables in this case could be the mother's clotting disorder. Unfortunately the placenta was not sent for pathology at the time of delivery, which could have indicated disruption or dysfunction. There have been no published reports linking protein C, protein S or beta2-glycoprotein-1 deficiencies to neural tube defects. She was also taking Coumadin prior to pregnancy. There are reports of fetal warfarin syndrome with occipital encephalocele, although this has been reported only when the medication is taken during the first trimester (Chen, 2008). This mother had discontinued use of this medication approximately two months prior to conception. She was transitioned to Lovenox, which does not cross the placenta and is not predicted to have a similar fetal effect. Neural tube defects have been reported in mice embryos when they received Lovenox *in vitro*, but there would have to be significant placental dysfunction or disruption for this to occur *in vivo* (Uysal *et al.*, 2005).

In summary, this is a unique case of a patient with an occipital encephalocele, which may be caused by a *SHH* duplication. Further investigation into such duplications would be important in future research into the cause of encephaloceles and related malformations.

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Fig. 1.

Fig. 2.

Fig. 3.