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SIX3 Deletions and Incomplete Penetrance in Families Affected by Holoprosencephaly

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Abstract

Holoprosencephaly (HPE) is failure of the forebrain to divide completely during embryogenesis. Incomplete penetrance has not been reported previously in *SIX3* whole gene deletions which are known to cause HPE. Both chromosomal microarray and whole exome sequencing (WES) were used to evaluated families with inherited HPE. Two families showed inherited deletions that contain *SIX3* and were incompletely penetrant for HPE. Using WES, we ruled out parental mosaicism, a *SIX3* hypomorph, and clinically significant variants in genes that are known to interact with *SIX3* as causes of incomplete penetrance. We demonstrate the importance of molecular cascade testing in families with HPE and we answer important questions about incomplete penetrance.

Keywords

holoprosencephaly; *SIX3* deletion; incomplete penetrance

INTRODUCTION

Holoprosencephaly (HPE) is failure of the developing forebrain to separate at the midline during the third and fourth weeks of gestation. Occurring in 1 in 250 embryos and in approximately 1 in 10,000 livebirths, this malformation is the most common forebrain embryonic anomaly (Matsunaga & Shiota 1977). HPE is classified by the degree of separation of the cerebral hemispheres with alobar being the most severe anomaly characterized by a single ventricle and no interhemispheric fissure. Least severe is middle

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DISCLOSURE

The authors have nothing to disclose.

interhemispheric fusion variant (MIHV) where the posterior frontal and parietal regions are not completely separated. Clinical manifestations may include facial dysmorphic features, cognitive impairment, seizures, motor impairment, ophthalmologic findings, hypothalamic dysfunction such as diabetes insipidus and difficulty with temperature regulation, and feeding problems.

The etiology of HPE can be environmental exposures such as maternal diabetes, genetic, or a combination of both. In this report, we focus on two families with inherited deletions that include the *SIX3* gene. *SIX3* is a transcription factor that is involved in multiple biological processes including regulation of *Sonic Hedgehog (SHH)* expression, transcriptional repression of BMP, Wnt, and Nodal targets, and interacting with Geminin to determine cell fate during forebrain development (Del Bene et al. 2004; Geng et al. 2008; Inbal et al. 2007; Kobayashi et al. 2001; Lagutin OV 2003; Liu et al. 2006).

In humans, variants within *SIX3* are incompletely penetrant and have variable expressivity, even within families with the same variant (Solomon et al. 2009). Multiple families have been found with truncating and missense mutations (Lacbawan et al. 2009) and there has been a report of an individual with a *SIX3* deletion with microcephaly without HPE (Rosenfeld et al 2010); however, there has not been a report of an inherited deletion of the entire *SIX3* gene. Here we present two families affected by HPE and inherited deletions that contain *SIX3* and use whole exome sequencing to test hypotheses for incomplete penetrance.

METHODS

Study participants were ascertained through the National Human Genome Research Protocol 98-HG-0249 (Genetic Analysis of Brain Disorders). Clinical evaluation was conducted by clinical geneticists and neurologists, and computerized tomography (CT) or magnetic resonance imaging (MRI) determined brain structure. Sanger sequencing was performed for the genes *SHH, ZIC2, SIX3*, and *TGIF* using the ABI Prism BigDye Terminator Cycle Sequencing Kit version 1.1, ABI 3100 Avant sequencer; Applied Biosystems, Foster City, CA, USA, further details are available on request. For family 1, copy number variation (CNV) was determined with chromosomal microarray using the Illumina HumanExome BeadChip-12v1_A (Illumina Inc. San Diego, CA), single nucleotide polymorphism (SNP) chip (~250,000 markers). Samples with calls below Illumina's expected 99% SNP call rates were excluded. For family 2, evaluations for CNVs were performed with a SNP microarray (Reveal^{SM} SNP Microarray) through Laboratory Corporation of America. Whole exome sequencing was completed on family 1 as previously described (Kruszka et al. 2015). Whole sequencing data was evaluated with VarSifter (Teer et al. 2012) and in house perl scripts. Copy number variation data was ascertained from the whole exome sequencing data using EXCAVATOR2 (v.1.1) (D'Aurizio et al. 2016) and eXome-Hidden Markov Model (XHMM) (Fromer et al. 2012).

WES data was filtered for variants that segregated with the HPE phenotype in those individuals with the deletions containing *SIX3.* Variants were further filtered for candidate modifier genes from the *SIX3* pathway (see Supplementary Table 1).

RESULTS

Patients

Family 1 has multiple affected individuals with HPE as seen in Figure 1. The first child to be diagnosed with HPE, II.5 (Figure 1 and 2C), presented at birth with a cleft lip, at 6 months with infantile spasms and subsequently with diabetes insipidus. Physical examination (Figure 2C) showed microcephaly, bilateral epicanthal folds, coloboma of the left iris, and moderate spastic diplegia. CT of the brain showed lobar HPE and agenesis of the corpus callosum. II.8 (Figure 2D) was born at 37.5 week gestation with and clinical evaluation showed lobar HPE (CT brain), microcephaly, agenesis of the corpus callosum, choanal stenosis, and diabetes insipidus (Figure 2D). II.9 and II.10 are monozygotic twins (Figure 2E). II.9 was born with hydrocephalus, severe hypotelorism, and midline cleft lip (Figure 2E). At two days of age, a CT scan showed alobar, enlarged ventricles, and bilateral micropthalmia. The second twin, II.10, was born with microcephaly, hypotelorism, cebocephaly, absent philtrum, and a thin vermilion border of the upper lip (Figure 2E). An ultrasound demonstrated enlarged lateral ventricles, suggestive of HPE. II.10 developed respiratory problems and seizures soon after birth and died at 14 days of age. II.1 and II.2 are healthy without known intellectual disability (formal cognitive testing not done). II.7 was a stillbirth at 21 weeks gestation and had a cleft lip but brain imaging was not performed.

In Family 2 (Figure 2), the first individual affected by HPE was II.2 who was born with alobar HPE based on brain MRI and later died at age 6 months (Figure 2H). II.3 presented with premaxillary agenesis, semi-lobar HPE, and infantile seizures (Figure 2I). The family also has an unaffected daughter (II.1, Figure 2G). Neither parent has clinical features of HPE or any known family history of HPE-like phenotypes (father pictured in Figure 2F).

Genetic testing

II.8 was tested using Sanger sequencing for the genes *SHH, SIX3, TGIF1 and ZIC2*, which was negative, followed by chromosomal microarray which found a 3.5 Mb microdeletion on chromosome 2p21 (Supplementary Figure 1). The microdeletion contained 24 genes (Supplementary Figure 1), of which only *SIX3* is known to be associated with HPE. Subsequent chromosomal microarray analysis was done on I.1, I.2, and II.2. I.2 (Figure 2A and 2B) was found to be a carrier of this 3.5 Mb deletion and I.2 and II.2 were found to be negative (other family members were not tested). Whole exome sequencing completed on I. 1, I.2, II.1, and II.8 confirmed the 3.5 Mb deletion in I.1 and II.8 using EXCAVATOR2. Additionally, II.1 was found to have the 3.5 Mb deletion using EXCAVATOR2; we confirmed the deletion in II.1 with a complementary method, XHMM. We confirmed the deletion in II.1 with an orthogonal approach by using informative SNPs to see a Mendelian error. For the 8 informative SNPs within the deletion region where the mother was heterozygous, II.1 and II.8 were homozygous for the same SNP due to inheriting the paternal deletion and inheriting the same maternal allele.

Molecular testing in Family 2 began with chromosomal microarray testing on II.3 which revealed a 261 Kb deletion containing genes *SIX3* and *SIX2* on 2p21 (Supplementary Figure 1C). Subsequent testing showed the father I.1 and the older sister, II.1 to also have this 261

Kb deletion. Both the father and older sister had no signs of HPE and both have normal intelligence based on educational and occupational status, no formal cognitive testing was performed.

Whole exome sequencing variant analysis

WES data was only available on Family 1. After searching for variants in Family 1 that did not match the affected *SIX3* deletion carrier (II.8) compared to the two unaffected carriers (I.1 and II.1), 148,122 variants heterozygous variants were found. After including only exonic variants, this list was reduced to 7573 variants. The final filter which included a search for variants in the 16 genes in Supplementary Table 1 yielded no variants.

DISCUSSION

This is the first report of *SIX3* whole gene deletions with incomplete penetrance (Figure 1). Inherited cases of *SIX3* whole gene deletions in the families in this study allowed us to test various hypotheses of incomplete penetrance. As the first hypothesis considered, we explored whether an affected deletion carrier would have also inherited a *SIX3* hypomorph *in trans*. This is a plausible scenario given Domené et al. (2008) found that the degree of functional impairment differed amongst the *SIX3* variants. However, in family 1, both II.1 (unaffected) and II.8 (affected with HPE) inherited a deletion from I.1 and the same *SIX3* allele from I.2 suggesting that the moderator of incomplete penetrance in family 1 was not *in trans* within the *SIX3* gene. Another explanation for incomplete penetrance would be parental mosaicism; however, this too is unlikely given that in both families, the *SIX3* whole gene deletion was passed on to a healthy child, who having inherited the deletion from a parent, is unlikely to be mosaic. And finally, for family 1, we interrogated genes (Supplementary Table 1) that interact with SIX3 for variants that did not match the affected *SIX3* deletion carrier (II.8) and the two unaffected carriers (I.1 and II.1). Using this strategy, there were no exonic variants that met our criteria. Certainly, there may be other genes that interact with *SIX3* or variants in noncoding regions that we missed in the 7573 variants that segregated with the HPE phenotype in Family 1 and there may be environmental modifiers that we did not take into account for both families. Although our analysis did not give answer to the mechanism of incomplete penetrance in *SIX3* deletions, the question of incomplete penetrance warrants further research and new hypotheses.

In addition to the research applications of incomplete penetrance, this study illustrates important clinical aspects of evaluating patients with HPE. The first clinical observation is the necessity of evaluating patients with HPE for copy number variations and cytogenetic abnormalities, especially if HPE variant panels that evaluate for the more common genes (*SHH, SIX3, ZIC2*, and *TGIF*) are negative. Secondly, even with a severe variant such as a deletion in a HPE gene, further testing must include testing of the parents. As can be seen by the inheritance patterns in Figure 1, meaningful genetic counseling can only take place after molecular carrier testing of the unaffected parents.

An inherent weakness of this study was the small sample size and absence of WES from Family 2. HPE is a rare brain malformation and *SIX3* deletions that are inherited are extremely rare as these are the first two families reported in the medical literature.

In conclusion, we present the first families with deletions of the entire *SIX3* gene, allowing for testing of hypotheses of incomplete penetrance. This study stresses the importance of a complete molecular survey to include testing for copy number variants and parental testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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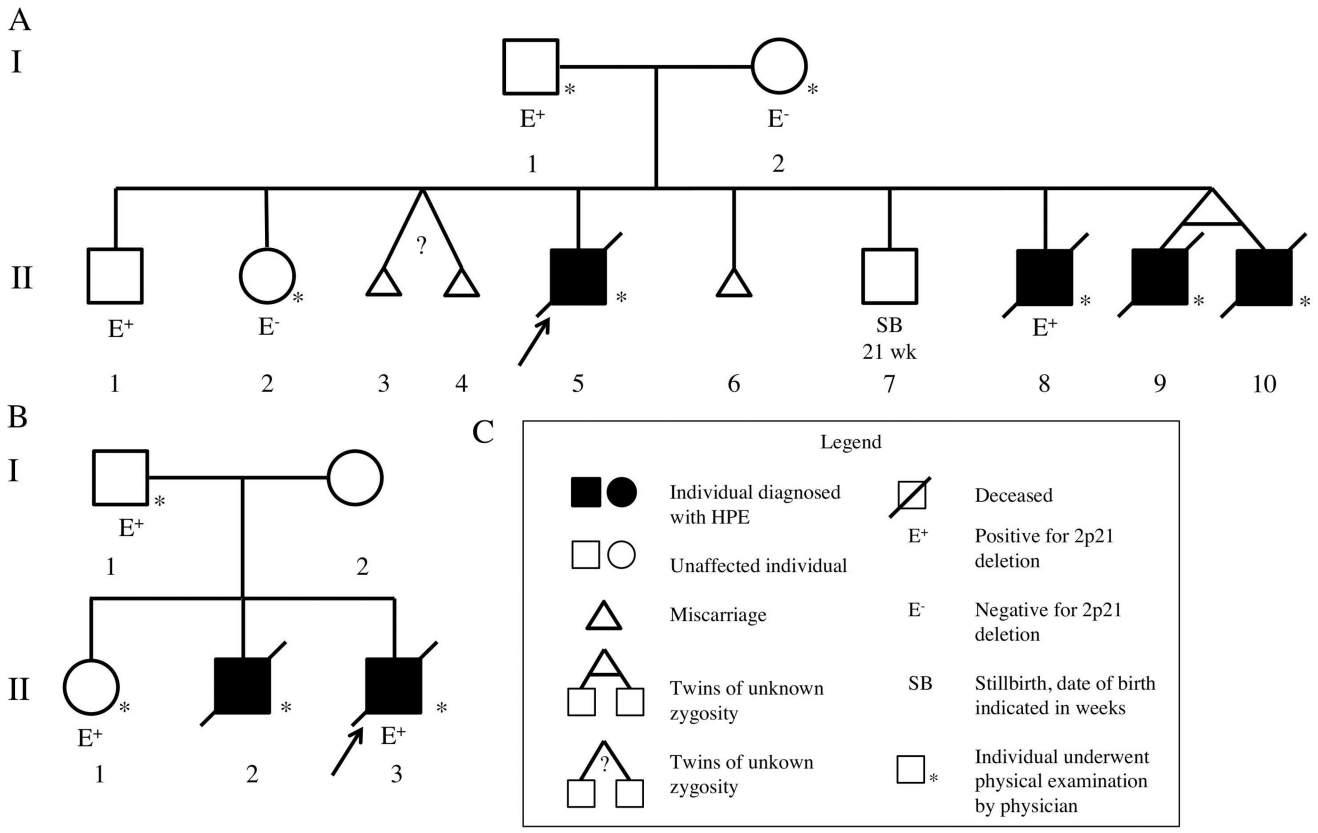

Figure 1.

(A) Pedigree of Family 1. I.1, II.1 and II.8 have an identical microdeletion containing *SIX3*. I.2 and II.2 were also tested but do not carry the deletion. (B) Pedigree of Family 2. I.1, II.1 and the proband (II.3) were found to have a microdeletion containing *SIX3*. (C) Pedigree symbol legend.

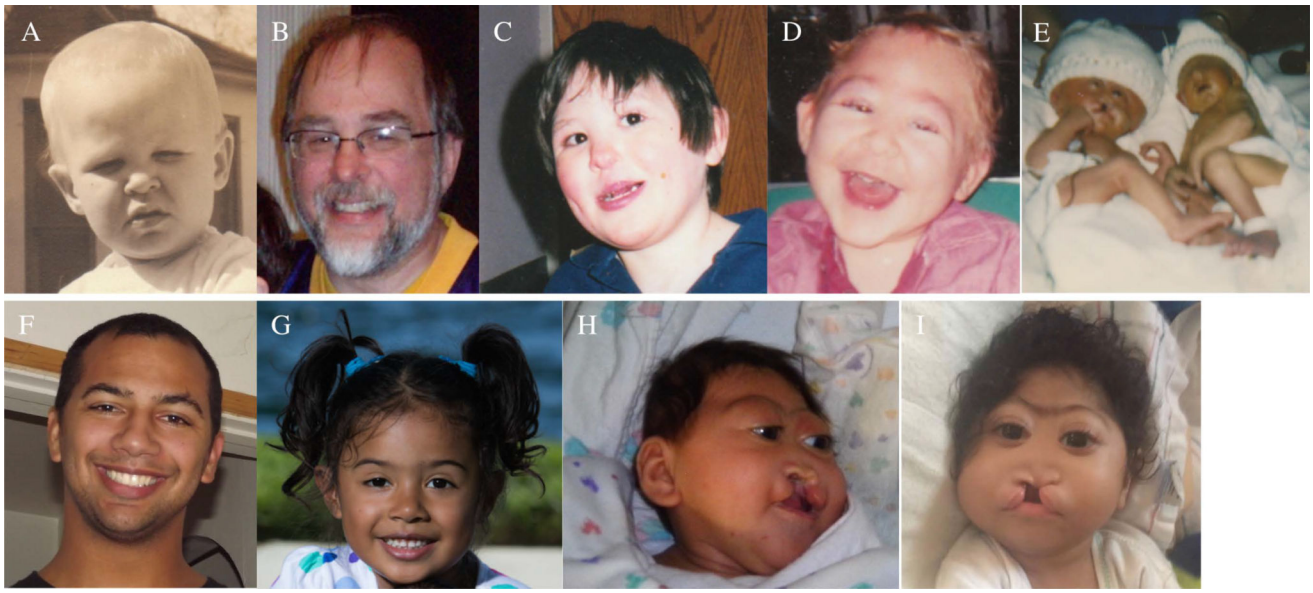

Figure 2.

Individual photos. (A–E) Family 1: (A) I.1 as a child; (B) I.1 as an adult; (C) II.5; (D) II.8; (E) II.9 and II.10. (F–I) Family 2: (F) I.1; (G) II.1; (H) II.2; (I) II.3. Permission has been obtained from the individuals and families for presentation.