Pathogenic ZIC2 polyalanine expansion detected by exome sequencing in a family with multi-generation holoprosencephaly

Nichole Owen, PhD FACMG[1,2], Liesbeth Vossaert, PhD FACMG[1,2], Lorraine Potocki, MD[2,3], Elizabeth Mizerik, MS CGC[2,3]

1. Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, USA.
2. Baylor Genetics Laboratory, Houston, TX, USA.
3. Texas Children's Hospital, Houston, TX, USA.

P703

BACKGROUND

Holoprosencephaly (HPE) is a structural brain abnormality that results from the failure of the forebrain to divide into the cerebral hemispheres during embryonic development. HPE can present as a spectrum, from the most severe alobar HPE to microform HPE. Diagnoses can be made prenatally if a severe enough structural anomaly or dysmorphisms are present, but often are made during the neonatal period when craniofacial features suggest HPE may be present. Observable phenotypes may range from mild dysmorphisms including hypotelorism, midface retrusion, microcephaly or single central maxillary incisor, to severe malformations including cyclopia, proboscis, anophthalmia/microphthalmia, or bilateral cleft lip/palate. HPE is well documented to have incomplete penetrance and wide clinical variability, even among families. Often familial cases are not identified until a there is a severe presentation of HPE, which then identifies a parentally inherited variant and other more mildly affected relatives. HPE may be isolated or syndromic, and be caused by chromosomal disorders, copy number variants or single nucleotide variants, thus broad-spectrum testing is warranted.

Isolated HPE has been linked to multiple genes; the most commonly mutated ones are *SHH, ZIC2, SIX3 and TGIF1*. *ZIC2* is unique in that relative to other HPE genes in that it has an extremely high penetrance and high rate of de novo variants; thus, familial HPE due to *ZIC2* variants is rare. Here, we present a case of familial HPE due to a pathogenic polyalanine expansion in *ZIC2*. *ZIC2* contains a polyalanine tract in exon 3 which natively contains 15 alanines. Expansion to 25 alanines is a recurrent pathogenic allele which interferes with the ability of the *ZIC2* transcription factor to bind to its DNA targets, resulting in decreased transcriptional activity.

CASE PRESENTATION

The proband presented at 19 months of age with respiratory distress due to an acute COVID-19 infection. The inpatient genetics team was consulted due to a prenatal diagnosis of semilobar holoprosencephaly (without postnatal follow-up imaging), laryngomalacia, and severe global developmental delay. No prenatal testing or screening had been performed. Physical examination revealed microcephaly, hypotelorism, and midface hypoplasia. Multiple endocrinopathies were present including diabetes insipidus and hypothyroidism raising concern for panhypopituitarism. There was no history of seizures, no polydactyly or digit anomalies, and no known laterality defects.

Family history was positive for a maternal half-sibling with semilobar holoprosencephaly. Mother denied any prior brain imaging or hormone issues; however, she did have history of learning difficulties. She also reported two first trimester miscarriages. The remaining family history was non-contributory.

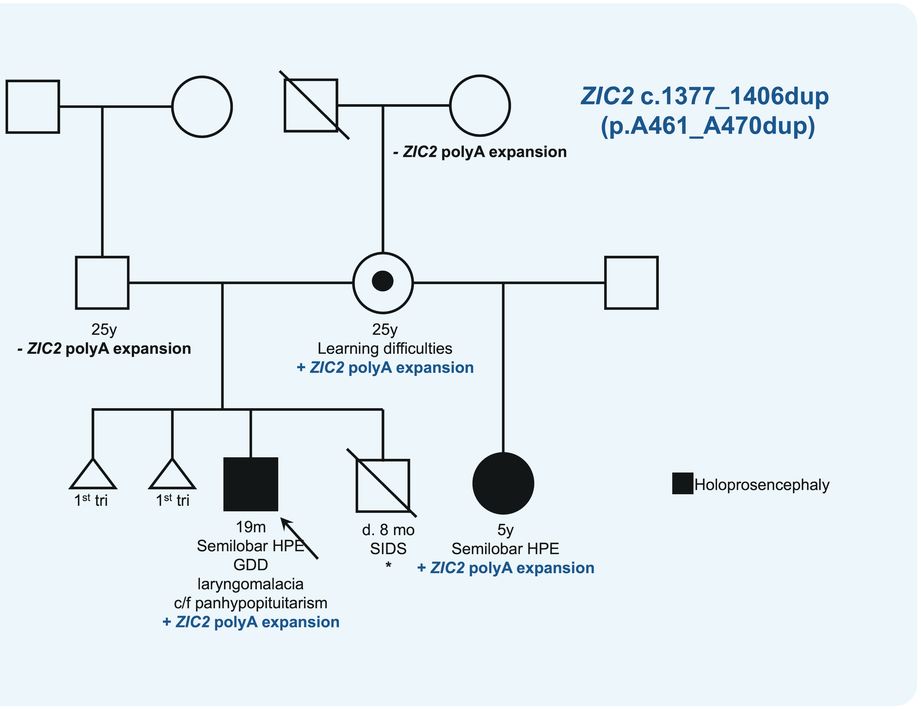

Figure 1. Pedigree of familial pathogenic ZIC2 polyalanine expansion variant c.1377_1406dup (p.A461_470dup) associated with autosomal dominant holoprosencephaly 5. The mother of the proband reported two first trimester miscarriages though it is unknown when these occurred relative to her other children. Before testing could be completed on this family, the younger brother of the proband passed away from sudden infant death syndrome. He could not be tested for the variant (*) but displayed no obvious features consistent with HPE.

GENETIC TESTING

The differential for HPE is broad and includes chromosomal disorders, CNVs, and single nucleotide variants so a broad approach was pursued for genetic testing. Chromosomal microarray was negative. Trio exome sequencing identified a maternally inherited polyalanine repeat expansion in the *ZIC2* gene (c.1377_1406dup (p.A461_A470dup)), which was subsequently confirmed by PCR. Sequencing with short read (100bp insert size) next generation sequencing identified short-clipped reads consistent with a possible insertion or duplication in the *ZIC2* gene (Figure 2) in the proband and maternal sample. Given the complexity and size of the variant, additional methods were utilized to confirm the variant including gel electrophoresis (Figure 3) and Sanger sequencing (Figure 4). Familial variant testing for the maternal half sibling and the maternal grandmother were pursued by gel electrophoresis and Sanger sequencing given the difficulties capturing this variant with short-read NGS.

Figure 2. IGV view of the sequencing files for the trio WES performed on the proband and parents. The reads are sorted by base to cluster the aberrant reads at the top. The reads appear as soft-clipped reads, with only one read having evidence of spanning the entire insertion/duplication.

POLYALANINE EXPANSION IN ZIC2

Pathogenic polyalanine repeat expansions are recurrent variants seen in many different genes and disorders. In exon 3 of *ZIC2*, this repeat expansion from 15 to 25 alanines is thought to primarily arise from a paternal mitotic recombination error. Traditionally, repeat expansions are difficult to detect on exome sequencing due to the large size and repetitive nature of the inserted sequence. However, manual review of low-confidence variants in the exome sequencing data at common HPE-associated genes allowed for the identification and confirmation of the expansion.

ZIC2 is a transcription factor and the polyalanine expansion in *ZIC2* results in 5% functionality of the ZIC2 protein, reduced DNA binding capability and reduced trans-activation activity [1,2]. Unlike other pathogenic expansions, no abnormal aggregates are formed.

ZIC2-RELATED HOLOPROSENCEPHALY

Loss of function variants in *ZIC2* cause Holoprosencephaly 5, an autosomal dominant form of non-syndromic HPE. *ZIC2* variants account for ~5% of all cases of non-syndromic HPE [3]. Unlike other HPE genes, *ZIC2* has a relatively high de novo rate and a relatively low rate of mildly affected individuals, making familial cases rare [1].

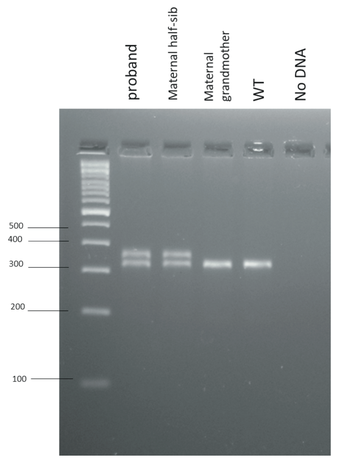

Figure 3. Gel electrophoresis confirming the duplication within exon 3 of ZIC2 in the proband and maternal half-sibling. The maternal grandmother was negative for the duplication.

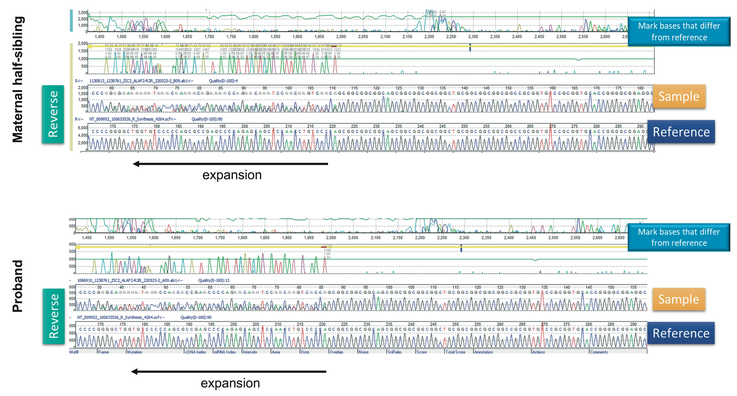

Figure 4. Sanger sequencing confirming the polyalanine expansion in the maternal half-sibling and proband.

CONCLUSIONS

Here, we present a family with holoprosencephaly due to a *ZIC2* polyalanine repeat expansion. This case is notable in that of all the monogenic holoprosencephaly genes, *ZIC2* has the highest de novo rate. Also notable is the relatively mild phenotype of the mother, as *ZIC2* has high penetrance and a relatively low incidence of mild presentations. Lastly, polyalanine expansions are difficult to detect using standard short-read next generation sequencing techniques; long-read sequencing would overcome this difficulty however, this technique is currently not yet widely adopted in clinical laboratories. Polyalanine expansions typically require specialized testing, however, they can be detected by exome sequencing when coupled with targeted manual data review.

REFERENCES

1. PMID: 19955556 2. PMID: 15590697 3. PMID: 20301702

FINANCIAL DISCLOSURE

These authors have nothing to disclose