Diagnostic Exome Sequencing Identifies Alterations in *GLI2* and *PTCH1* in a Previously Undiagnosed Patient with Holoprosencephaly, Seizures, and Hypopituitarism

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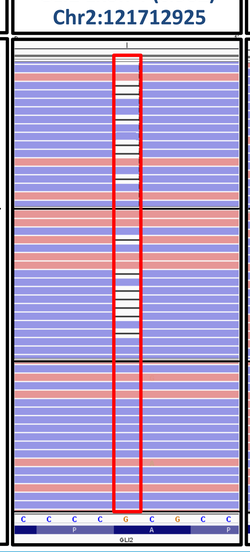
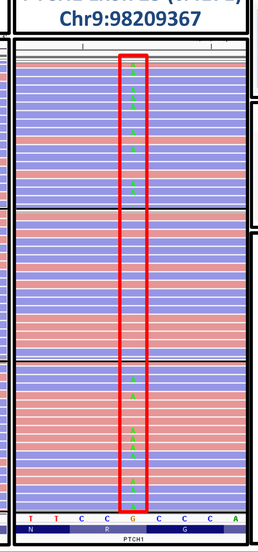
BACKGROUND

- Diagnostic Exome Sequencing (DES) has emerged as a powerful new tool for the molecular diagnosis of genetic disease in families and patients in whom traditional genetic and biochemical testing has been uninformative and/or ambiguous.
- A 20-year-old German, Irish, English, French female presenting with multiple congenital anomalies and intellectual disability was referred to Ambry Genetics for proband-parent trio DES in order to elucidate the underlying disease etiology.
- The proband's symptoms include hypopituitarism, seizures, learning difficulty, ADHD, microcephaly, congenital cataracts, hearing loss, ptosis, single central incisor, narrow high palate, micrognathia, congenital melanocytic naevi, striae, obesity, congenital subglottic stenosis, dysmorphic features, speech delay, and G-tube placement and fundoplication. The patient's family history was significant for a paternal aunt with mosaic Turner syndrome and a paternal cousin (other paternal aunt's son) with cleft lip and palate, ADHD, and learning disability.
- Prior to DES, the patient had evaded diagnosis by means of clinical evaluation and extensive genetic testing. This included negative chromosome analysis, FISH testing for Williams syndrome, and SNP array.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process.
- **DES:** Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described (Farwell et al. 2014).

FIGURE 1. Trio Exome Sequencing Identifies Heterozygous Alterations in the Proband's *GLI2* and *PTCH1* Genes

SAMPLE	GLI2 Exon 4 (c.562) Chr2:121712925	PTCH1 Exon 23 (c.4171) Chr9:98209367
PROBAND Digenic Heterozygote GLI2 c.562DELG (p.A188RFS*7) PTCH1 c.4171C>T (p.R1391W)		
FATHER Heterozygote GLI2 c.562DELG (p.A188RFS*7)		
MOTHER Heterozygote PTCH1 c.4171C>T (p.R1391W)		

GLI2 NM_005270 c.562del

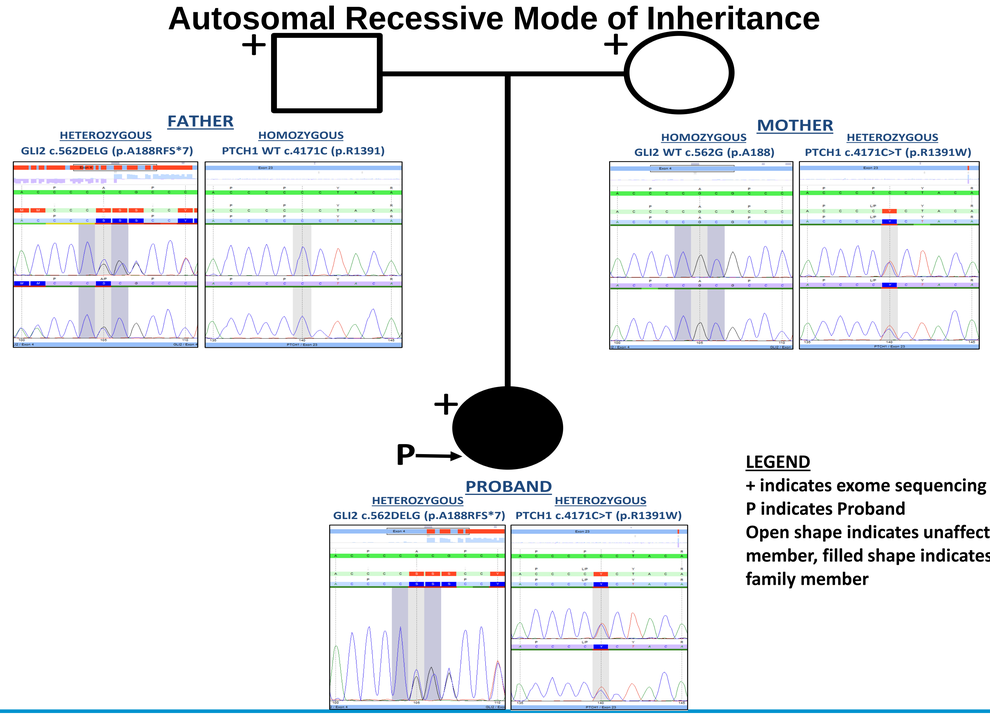
Nucleotides		Proband	Mother	Father
Reference	G	45.28% (24/53)	100.00% (86/86)	48.61% (35/72)
Mutation	-	54.72% (29/53)	0.00% (0/86)	51.39% (37/72)
Q Score		1352	0	1829

PTCH1 NM_000264 p.R1391W c.4171C>T

Nucleotides		Proband	Mother	Father
Reference	G	37.74% (20/53)	48.53% (33/68)	100.00% (52/52)
Mutation	A	62.26% (33/53)	51.47% (35/68)	0.00% (0/52)
Q Score		172	229	185

- **Red boxes (left) spanning the NGS reads indicate the presence of alterations in Exon 4 of *GLI2* and Exon 23 of *PTCH1*.**
- **Note that the father and mother are heterozygous for *GLI2* c.562DELG and *PTCH1* c.4171C>T, respectively (lower two panels, left). For *PTCH1*, the software notates the alteration as G>A because the coding sequence is on the minus strand of the chromosome.**
- **As the proband (upper panels, left) inherits *GLI2* c.562DELG from her father and *PTCH1* c.4171C>T from her mother, she is heterozygous for the alteration in each gene.**
- **No other genetic changes were considered likely to explain the clinical phenotype except for the aforementioned alterations.**

FIGURE 2. Sanger Sequencing Reveals that the Heterozygous Alterations in *GLI2* and *PTCH1* Co-Segregate with Disease According to a Digenic, Autosomal Recessive Mode of Inheritance



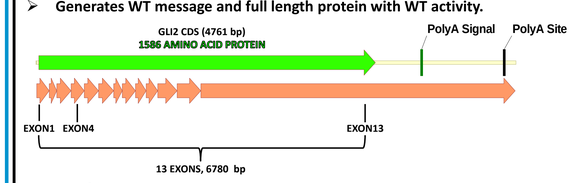
LEGEND
+ indicates exome sequencing performed
P indicates Proband
Open shape indicates unaffected family member, filled shape indicates affected family member

FIGURE 3. Amino Acid Conservation and Functional Predictions for the Alterations

***GLI2* (NM_005270) p.A188RFS*7 (c.562DELG)**

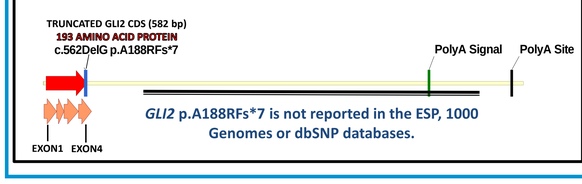
WT *GLI2* ALLELE:

- **Generates WT message and full length protein with WT activity.**



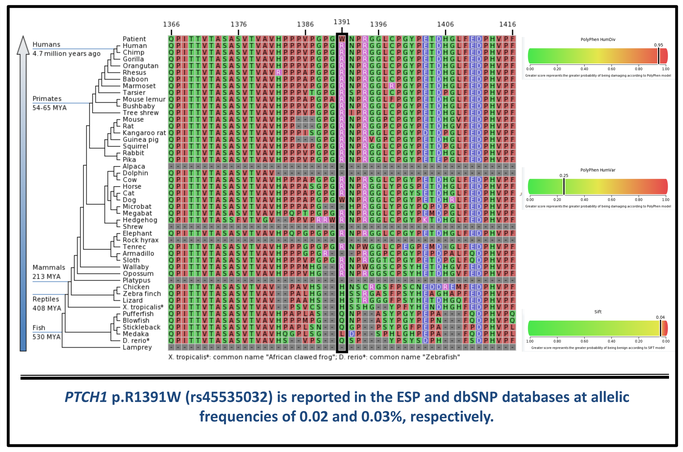
c.562DelG *GLI2* ALLELE:

- **Generates aberrant message/truncated protein subject to Nonsense Mediated Decay (NMD), effectively reducing the dose of GLI2 by one half.**



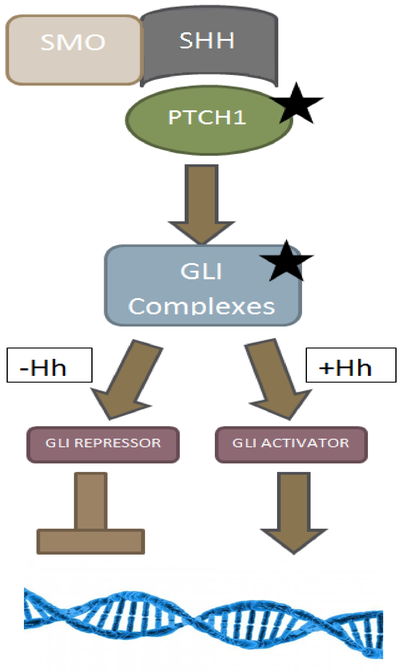
***GLI2* p.A188RFS*7 is not reported in the ESP, 1000 Genomes or dbSNP databases.**

***PTCH1* (NM_000264) p.R1391W (c.4171C>T)**



***PTCH1* p.R1391W (rs45535032) is reported in the ESP and dbSNP databases at allelic frequencies of 0.02 and 0.03%, respectively.**

SHH PATHWAY



GLI2 RESULTS

- DES analysis of the trio revealed that the proband inherited the previously unreported *GLI2* c.562delG (p.A188Rfs*7) from the unaffected father causing a translational frameshift with a predicted alternate stop codon (Fig. 3, left panel). Such alterations are typically considered deleterious in nature (Richards, 2008).
- *GLI2* is located on chromosome 2q14.2 and encodes for a vertebral transcription factor that is involved in *PTCH1* expression and SHH signal transduction. Mutations in *GLI2* are associated with autosomal dominant holoprosencephaly 9 (HPE9). HPE9 has partial penetrance and a variable phenotype, which can include seizures, hypopituitarism, and hypotelorism to full cyclopia (Roessler, 2003).

PTCH1 RESULTS

- The proband also inherited a previously unreported missense alteration, *PTCH1* c.4171C>T (p.R1391W), from the unaffected mother. This alteration is considered a Variant of Uncertain Significance (VUS).
- R1391 is **highly conserved** across higher vertebrates (Fig. 3, right panel; p.R1391W is predicted to be **damaging** by SIFT and **possibly damaging** by HumDiv *in silico* analyses (Fig. 3, right panel).
- In humans, *PTCH1* (Drosophila 'Patched', PTC or PTCH) is located on 9q22.32 and encodes a transmembrane tumor suppressor protein that is part of the hedgehog signaling pathway . Although typically associated with nevoid basal cell carcinoma syndrome (Gorlin syndrome), gain of function alterations in *PTCH1* have been described in families with holoprosencephaly type 7 (HPE7). Asymptomatic carrier parents, along with familial phenotypic variability, have been previously reported for HPE7 cases (Ming, 2002, Ribeiro, 2006).

CONCLUSIONS

- To our knowledge, this is only the second patient to be reported with double heterozygous alterations in *GLI2* and *PTCH1*. The previously reported patient was a 5-year-old female with bilateral cleft lip/palate, malocclusion, diminished frontonasal angle, hypoplastic anterior nasal spine, hypotelorism, hypoplastic premaxilla, hypoplastic nose, large ears, and poorly developed philtrum. MRI demonstrated mild gyral asymmetry in the perisylvian areas (Rahmimov, 2006).
- Our patient demonstrates overlap with HPE7 and HPE9 and possesses similarities with the previously reported patient who possessed alterations in both *GLI2* and *PTCH1*. Evidence supports the notion that the *GLI2* alteration is the most likely cause of the patient's phenotype; contribution of the *PTCH1* alteration cannot be unequivocally determined at this time. Nevertheless, due to the close association of GLI2 and PTCH1 in the SHH pathway, and because a previous patient has been reported with heterozygous alterations in both genes, we cannot rule out the possibility that the *GLI2* and *PTCH1* alterations identified herein contribute to the patient's phenotype in a digenic manner.

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