

**Bi-allelic *SMO* variants in Hypothalamic Hamartoma: a recessive cause of Pallister-Hall syndrome**

Timothy E. Green,<sup>1</sup> Mareike Schimmel,<sup>2</sup> Susanna Schubert,<sup>3</sup> Johannes R Lemke,<sup>3</sup>,  
Mark F. Bennett,<sup>1,4,5</sup> Michael S. Hildebrand,<sup>1,6\*</sup>, Samuel F. Berkovic,<sup>1\*</sup>

1. *Epilepsy Research Centre, Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria, 3084, Australia*
  2. *Children's Hospital, University Hospital Augsburg, Augsburg, Germany*
  3. *Institute of Human Genetics, University of Leipzig Medical Centre, Leipzig, Germany*
  4. *Population Health and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia*
  5. *Department of Medical Biology, University of Melbourne, Melbourne, Victoria, Australia*
  6. *Neuroscience Research Group, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia*
- \* *These authors contributed equally to this work*

**Corresponding Authors:**

Prof. Samuel Berkovic. Address: Epilepsy Research Centre, Department of Medicine, University of Melbourne, 245 Burgundy St. Heidelberg, Victoria 3084, Australia; Telephone: +61 3 9035 7121; Fax: +61 3 9496 2291; E-mail: s.berkovic@unimelb.edu.au

Associate Prof. Michael Hildebrand. Address: Epilepsy Research Centre, Department of Medicine, University of Melbourne, 245 Burgundy St. Heidelberg, Victoria 3084, Australia; Telephone: +61 3 9035 7043; Fax: +61 3 9496 2291; E-mail: michael.hildebrand@unimelb.edu.au

**Running Title:** Bi-allelic *SMO* variants in Hypothalamic Hamartoma

**Text Pages:** 11; **Word Count:** 130/150 (abstract), 1455/1500 (main body); **Figures:** 2; **Tables:** 1; **References:** 18/20; **Supplemental Material:** 1

**Abstract**

Pallister-Hall syndrome, typically caused by germline or *de novo* variants within the *GLI3* gene, has key features of hypothalamic hamartoma and polydactyly. Recently, a few similar cases have been described with bi-allelic *SMO* variants. We describe two siblings born to non-consanguineous unaffected parents presenting with hypothalamic hamartoma, post-axial polydactyly, microcephaly amongst other developmental anomalies. Previous clinical diagnostic exome analysis had excluded a pathogenic variant in *GLI3*. We performed exome sequencing re-analysis and identified bi-allelic *SMO* variants including a missense and synonymous variant in both affected siblings. We functionally characterised this synonymous variant showing it induces exon 8 skipping within the *SMO* transcript. Our results confirm bi-allelic *SMO* variants as an uncommon cause of Pallister-Hall syndrome and describe a novel exon-skipping mechanism, expanding the molecular architecture of this new clinico-molecular disorder.

**Keywords:** hypothalamic hamartoma, Pallister-Hall Syndrome, exon skipping, *SMO*

## **Introduction**

Hypothalamic hamartoma is a benign congenital lesion of the hypothalamus that is associated with a refractory gelastic epilepsy of early life. The prevalence of hypothalamic hamartoma is estimated to be between 1 in 50,000 to 100,000 people [1]. Hypothalamic hamartoma can be non-syndromic or occur as a feature of several disorders including Pallister-Hall (PHS) and Oral-Facial-Digital VI (OFD VI) syndromes [2]. PHS, clinically characterized by hypothalamic hamartoma and polydactyly [2], is predominately familial and usually arises as a consequence of germline or *de novo* variants within the *GLI3* gene [3,4]. OFD VI is a recessively inherited condition, where hypothalamic hamartomas are seen in about 25% of cases [5], that is most commonly associated with compound heterozygous variants in the cilia gene *CPLANE1* [6]. Very recently, five patients with hypothalamic hamartoma, polydactyly and other developmental anomalies have been reported with bi-allelic germline *SMO* variants [7,8].

Here we describe phenotypic and genetic analyses of two siblings with hypothalamic hamartoma, post-axial polydactyly, microcephaly and shortening of the long bones.

## **Methods**

Please refer to the **Supplementary Materials** for sample collection, bioinformatics, and molecular analyses methodologies.

## Results

### Case Histories

Two siblings from a non-consanguineous Italian family (**Fig. 1A**) were assessed because of multiple developmental anomalies. The male proband (II-1) was the product of a normal pregnancy and birth. Apgar scores were normal but height, weight and head circumference were all <3rd centile. He was dysmorphic with plagiobrachycephalus, deep-set eyes, retrognathia, hexadactyly of both feet (**Fig. 1B**) and a small skin appendage on the right hand, hypospadias with circular prepuce and penile shaft curvature. He had severe motor, speech and cognitive delay. Ultrasonography and MRI showed a large suprasellar hypothalamic lesion (Type IIb of Valdueza [9]; See **Fig. 1C**). At the age of 11 months, he developed central precocious puberty and the GnRH analogue leuprolide was given.

At age 24 months, gelastic seizures (up to 50/day) and less frequent generalized convulsive seizures developed that were refractory to anti-seizure medication. At 38 months partial resection and disconnection of the lesion was performed. Post-operatively he improved but seizures continued at 0-6/day, and he was on three anti-seizure medications.

His younger sister (II-2) was also small at birth with height, weight and head circumference all <3rd centile. MRI also revealed a suprasellar lesion (type IIb of Valdueza [9], **Fig. 1C**). She had hexadactyly of the left foot, bilateral skin appendages of the little fingers. She had short stature but no evidence of precocious puberty by age 6 years. From the age of 22 months, gelastic seizures (0-3/day) developed but convulsive seizures were not reported. She had mild motor and speech delay.

## Genomic Analysis

We analysed quad clinical exome data from the two affected siblings and both their unaffected parents. We searched for rare germline candidate variants that were shared between the two affected offspring (II-1 & II-2). Following the exclusion of variants within the known disease genes, *GLI3* or *CPLANE1*, we considered candidate variants only within the 50-known sonic-hedgehog (SHH) pathway genes, as classified by the Kyoto Encyclopedia of Genes and Genomes (<https://www.genome.jp/kegg/>) [10], given the key SHH transcription factor *GLI3* is the cause of classic PHS.

Our initial germline analysis of non-synonymous or splice site variants that alter amino acid sequence revealed an ultra-rare (gnomAD frequency:  $7.95 \times 10^{-7}$ ) [11] missense variant in exon 6 of *SMO* (NM\_005631.5 c.1199G>A; p.Arg400His). This variant was inherited from the unaffected father (I-1), was predicted to be deleterious to the SMO protein by multiple *in silico* tools, and is present only at low frequency in gnomAD consistent with recessive inheritance [11] (**Fig. 1, Table S1**).

Since *SMO* pathogenic variants were reported to be recessively inherited in two studies<sup>8,9</sup>, and there was no other obvious rare non-synonymous variant we searched for a second allele *in trans* by interrogating intronic and synonymous variants that were filtered out in our initial exome analysis. This revealed an ultra-rare (gnomAD frequency:  $1.59 \times 10^{-5}$ ) [11] synonymous variant (NM\_005631.5 c.1416C>T; p.Tyr472Tyr) in exon 8 of *SMO* in both affected offspring inherited *in trans* from their unaffected mother (I-2, **Fig. 1, Table S1**). Human Splicing Finder (<https://www.genomnis.com/access-hsf>) predicts the synonymous variant will result in

alternate splicing of the *SMO* transcript through disruption of exonic splice enhancer (ESE) and silencer (ESS) motifs within exon 8 [12].

To interrogate the effect of this synonymous variant we analysed fibroblast derived RNA samples obtained from the mother and affected son (I-2 & II-1). Sequencing of cDNA confirmed exon 8 skipping evidenced by underlying exon 9 sequence beginning at the junction of exon 7-8, and exon 7 sequence beginning at the junction of exon 8-9, shown in the forward and reverse directions (**Fig. 2B**). The exclusion of exon 8 leads to a frameshift and premature stop codon (p.Gly453AspfsTer6) which is predicted to lead to nonsense-mediated decay since exon 8 is not one of the last two exons of the *SMO* gene.

We investigated the mis-splicing further by interrogating allele specific contributions to the *SMO* transcript in the affected son (II-1). We demonstrated there is a significant reduction in normally spliced mRNA from the maternal allele that carries the synonymous variant, compared to the paternal allele housing the missense variant (**Fig. 2C**). This confirmed partial rather than complete mis-splicing of exon 8 from the maternal allele.

## **Discussion**

The two siblings we report here show phenotypic concordance with the previously described bi-allelic *SMO* cases sharing the clinical features of hypothalamic hamartoma, polydactyly, and microcephaly as well as shortening of the long bones (**Table 1**) [7,8]. The boy was more severely affected with facial dysmorphism and more severe developmental disabilities. Bi-allelic *SMO* variants were found in both siblings,

inherited in *trans* from their unaffected parents (**Fig. 1**). Both variants are ultra-rare and neither have been reported in gnomAD as homozygotes. The synonymous variant is predicted to disrupt *cis*-regulatory ESE and ESS motifs that are critical for the recruitment of spliceosome proteins to process pre-mRNA (**Fig. 2A**) [13]. We confirmed this synonymous variant leads to partial mis-splicing resulting in exon 8 skipping in patient derived RNA samples (**Fig. 2B,C**). Several previous reports have identified equivalent pathogenic synonymous variants in different genes confirmed at the RNA level to lead to exon skipping [14-17].

*SMO* is a member of the SHH signalling pathway and encodes a seven-transmembrane protein [18]. The SMO protein, known as ‘Smoothened’, is a frizzled class receptor of the SHH ligand that localises to the primary cilium along with other SHH constituents such as the *GLI1/2/3* transcription factors to facilitate SHH signalling during development [18].

Germline *SMO* variants have recently been reported in five familial cases presenting with syndromic hypothalamic hamartoma and a variety of other developmental anomalies (**Table 1**) [7,8]. Functional analysis of primary cilia within fibroblasts of these reported cases has demonstrated non-inducible *GLI1* or *PTCH1* expression, mis-localised SMO protein, and ligand-independent localisation of *GLI2* protein, which together disrupt normal SHH signalling [8].

It is important to acknowledge that hypothalamic hamartoma is not reported in all patients with bi-allelic *SMO* variants, as is the case for individuals with Greig’s

syndrome due to *GLI3* mutations [3,8]. Intriguingly, the patients with hypothalamic hamartoma and bi-allelic *SMO* variants have been shown to have more severe mislocalization of SMO protein within primary cilia than those without hypothalamic hamartoma. However, this observation was only made based on a small number of cases [8].

In summary, we report two familial hypothalamic hamartoma cases with developmental abnormalities and bi-allelic germline *SMO* variants. We characterise a rare genetic mechanism of a synonymous variant triggering exon skipping. This highlights the importance of genetic analyses extending beyond non-synonymous coding variants when routine clinical diagnostic genetic testing is unrevealing. Our findings further support that hypothalamic hamartoma is caused by dysregulation of SHH signalling during development. This recognition will provide the impetus to consider additional SHH pathway genes as candidates for other unsolved hypothalamic hamartoma syndromes, and encourage clinical trials of SMO inhibitors already under development for patients with these broad developmental disorders.

### **Acknowledgements**

We would like to thank the family for their participation in this study.

### **Author Contributions**

TEG and MFB performed bioinformatic analysis. TEG and MSH performed molecular genetics experiments. SS and JRL performed the fibroblast cell culturing. MS and SFB



conducted clinical phenotyping. TEG, SFB, and MSH drafted the paper. All authors discussed the results and commented on the final manuscript.

### **Funding**

We are grateful to the Hope for Hypothalamic Hamartoma Foundation for their generous support of our study. This work was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS.

### **Ethical Approval**

The study was approved by the Human Research Ethics Committee of Austin Health (Heidelberg, Victoria, Australia). Written informed consent for the study was obtained from both parents.

### **Data Availability**

The identified variants are publicly available in the Lieden Open Variation Database (LOVD). *SMO* NM\_005631.5 c.1199G>A; p.Arg400His: Variant #0000791113, URL: <https://databases.lovd.nl/shared/variants/0000791113#00019464> and *SMO* NM\_005631.5 c.1416C>T; p.Tyr472Tyr : Variant #0000791114, URL: <https://databases.lovd.nl/shared/variants/0000791114#00019464>.

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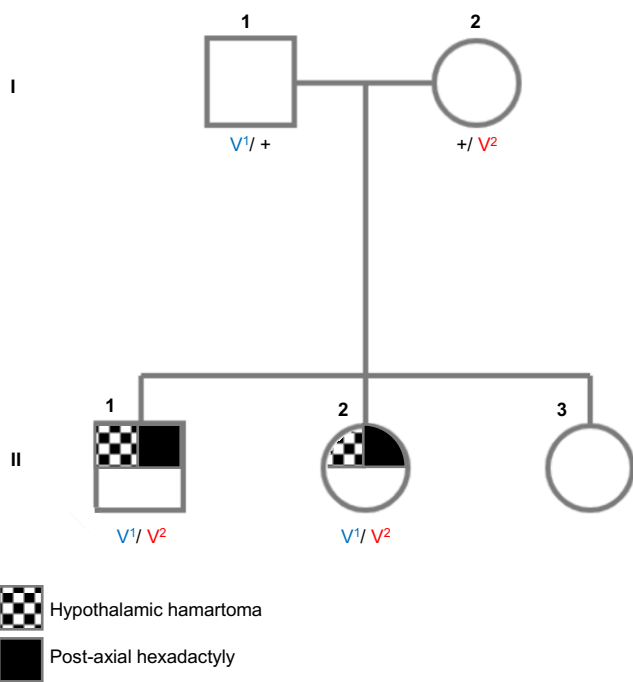
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**Figure 1. Pedigree and Clinical Imaging.** **A.** Pedigree of family. **V<sup>1</sup>:** *SMO* c.1199G>A; p.Arg400His, **V<sup>2</sup>:** *SMO* c.1416C>T; p.Tyr472Tyr **B.** Right foot X-ray of the proband (II-1) at age 12 months showing post-axial polydactyly. **C.** Axial MRI brain image of the male proband (II-1) at age 2 years showing a 2.5cm diameter hypothalamic lesion extending into the interpeduncular fossa (left). Axial MRI brain image of his younger female sibling (II-2) at age 2 months showing a similar large hypothalamic lesion (right).

**Figure 2. Molecular Analyses.** **A.** Exon 8 skipping was predicted to arise through disruption of spliceosome recruitment at the site of the *SMO* c.1416C>T synonymous variant. **B.** Sequencing of patient derived cDNA demonstrates the underlying exon 7 sequence beginning at the junction of exon 8 and 9 of the *SMO* transcript within individuals I-2 & II-1. **C.** RT-PCR demonstrates the paternal allele containing the missense variant is the most abundant normally spliced mRNA transcript, confirming a partial mis-splicing of the maternal allele housing the synonymous variant.

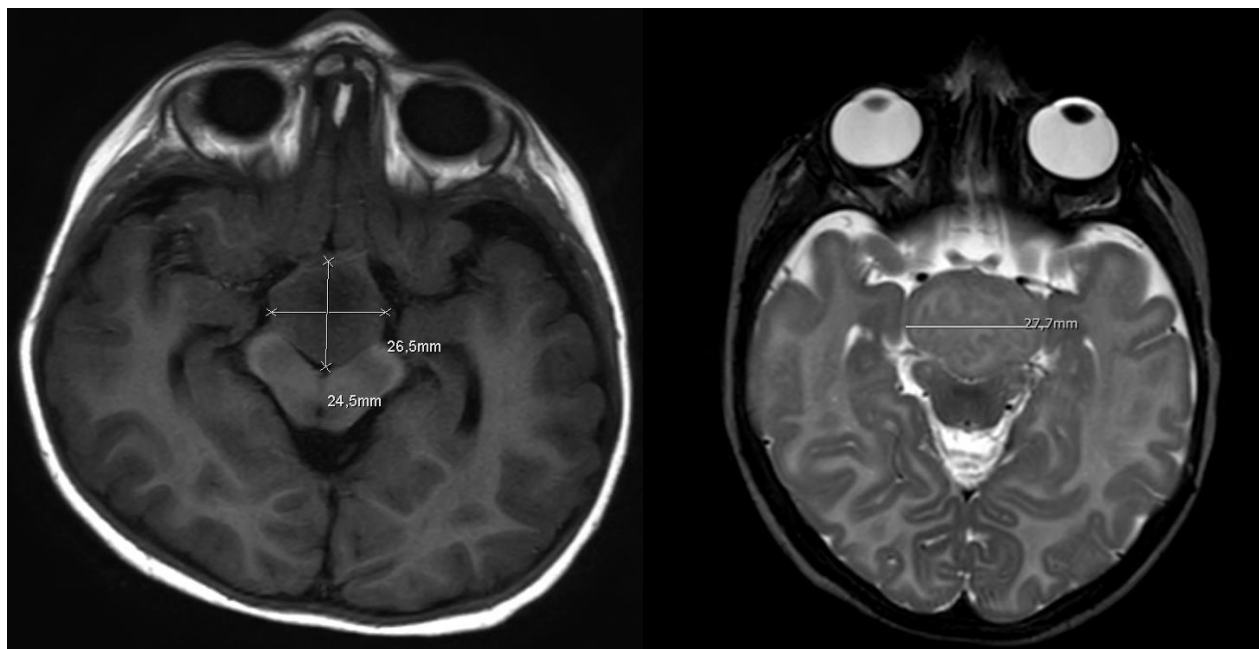
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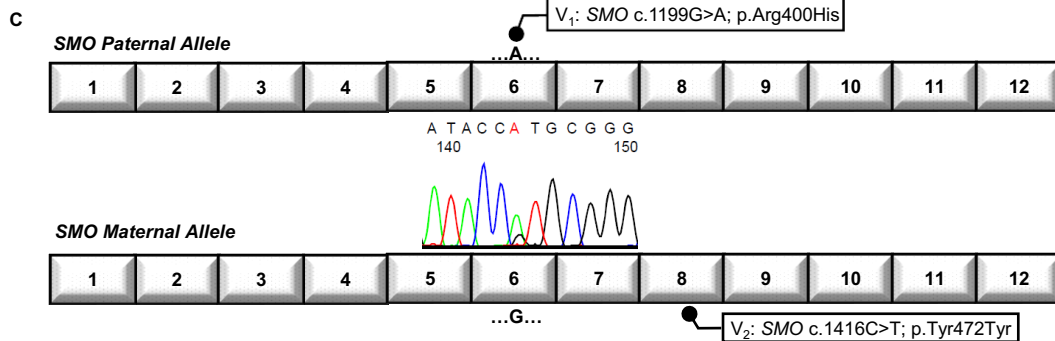
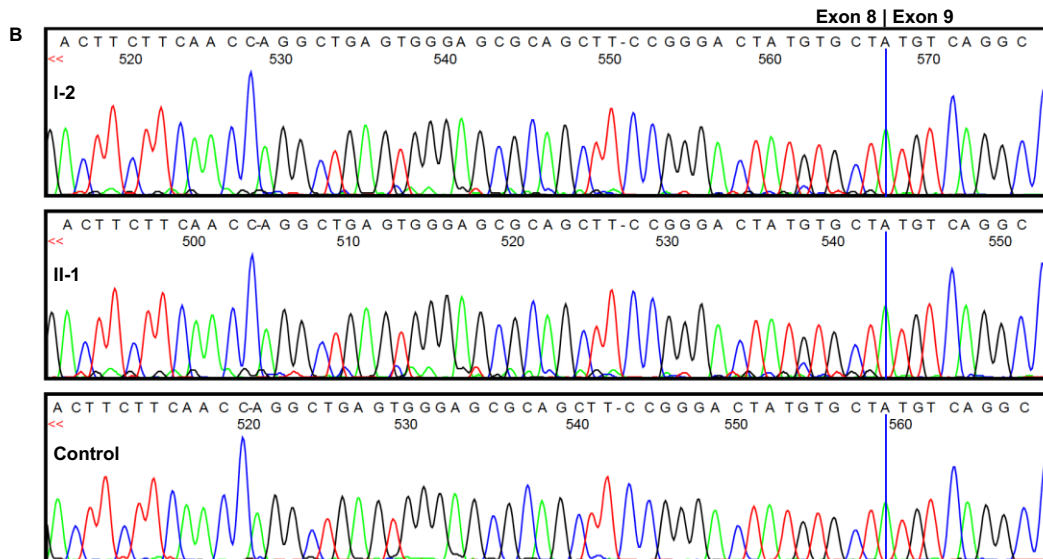
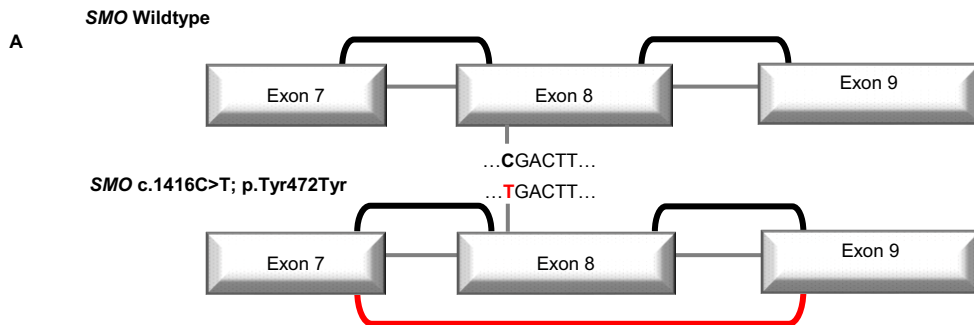


B



C





*Le et al. [8]**Rubino et al. [7]**This report*

	F1-II:1	F2-II:1	F2-II:2	F3-II:1	F4-II:4	F5-II:2	F5-II:3	Case 1	Case 2	II-1	II-2
Sex	M	M	M	M	M	M	F	M	M	M	F
Psychomotor development	normal	normal	normal	mild delay	Normal	NR	normal	normal	normal	severe delay	mild delay
Gelastic seizures	+	+	+	-	-	-	-	+	dacrystic seizures	+	+
Hypothalamic hamartoma	+	+	+	-	-	NR	NR	+	+	+	+
Microcephaly	-	+	-	-	-	-	-	+	-	+	+
Dysmorphic facial features	+	+	+	+	-	-	-	-	-	+	-
Chest and rib abnormalities	-	-	-	+	moderate	-	-	-	-	-	-
Postaxial polydactyly	+	+	+	+	+	+	+	+	+	+	+
Syndactyly	-	5/6	2/3	5/6	-	2/3	2/3	+	-	3/4	-
Cardiac defect	-	-	-	-	+	+	+	-	-	-	-
Shortening of long bones	-	-	-	+	-	-	-	-	-	+	+

**Table 1.** The reported clinical features of bi-allelic or recessive *SMO* cases [7,8]. We report two cases (II-1 & II-2) that share the clinical features of hypothalamic hamartoma, gelastic seizures, microcephaly (< 3rd centile), post-axial polydactyly and shortening of the long bones. The boy (II-1) had precocious puberty and hypospadias. NR; not reported.