

ARTICLE

Isolated hypogonadotropic hypogonadism with *SOX2* mutation and anophthalmia/microphthalmia in offspring

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Isolated hypogonadotropic hypogonadism (IHH) is a genetically heterogeneous condition in which patients frequently require assisted reproduction to achieve fertility. In patients with IHH who are otherwise well, no particular increased risk of congenital anomalies in the resultant offspring has been highlighted. Heterozygous mutations in *SOX2* are the commonest single-gene cause of anophthalmia/microphthalmia (A/M) and sometimes result in pituitary abnormalities. We report a family with a novel frameshift mutation in the *SOX2* transactivation domain, p.Gly280AlafsX91, resulting in bilateral anophthalmia and subtle endocrinological abnormalities in a male sibling, and unilateral microphthalmia in a female sibling. The mutation is present in their mother who has IHH, but has no eye disorders or other anomalies. She underwent assisted reproduction to achieve fertility. This report has important implications for the evaluation of patients with IHH, particularly in the setting of planned infertility treatment.

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INTRODUCTION

Isolated hypogonadotropic hypogonadism (IHH) is caused by impaired release of gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) in the setting of otherwise normal anterior pituitary anatomy and function. The resulting hypogonadism typically manifests as incomplete development of secondary sexual characteristics. IHH has an incidence of 1:10 000–1:86 000,¹ although the true prevalence is difficult to determine in the absence of detailed reproductive phenotyping in large unselected populations. In approximately two-thirds of patients, IHH is associated with anosmia (Kallmann syndrome). IHH is a genetically heterogeneous condition, and mutations in a number of genes have been identified to date, including *KAL1*,² *FGFR1*,³ *PROKR2*,⁴ *PROK2*,⁴ *GNRHR*,⁵ *GPR54*,⁶ *TAC3*,⁷ *TACR3*,⁷ *NELF*,⁸ *CHD7*,⁹ *FGF8*,¹⁰ *GNRH1*¹¹ and *WDR11*.¹² However, mutations in these genes only account for a small proportion of affected patients, and in the majority of cases, the aetiology is unknown.

SOX2 is a member of the sex-determining region of the Y chromosome-related high-mobility group box (SOX) family of transcription factors.¹³ Expression of *SOX2* is critical for human eye development, with heterozygous mutations in this gene found in approximately 10% of cases of anophthalmia/microphthalmia (A/M).^{14–17} *SOX2* has also been shown to have a key role in the development of other tissues, notably the pituitary, forebrain, gastrointestinal and urogenital systems.^{14,15}

We report a family with A/M in siblings, who were born to a mother with IHH and no ocular anomalies. A novel frameshift

mutation in *SOX2* was identified in the siblings, and this was also present in the mother.

PATIENTS AND METHODS

Clinical findings

The couple in this family (I.1 and I.2, Figure 1a) was reviewed after their two children (II.1 and II.2, Figure 1a) were born with A/M following assisted reproduction. The couple were Caucasian and non-consanguineous with no known eye disorders in themselves or in their families. The mother, I.2 (Figure 1a, Table 1) previously had an endocrine investigation at the age of 18 years because of primary amenorrhoea and limited development of secondary sexual characteristics. Her investigations revealed low FSH 3.5 mIU/ml (2.0–16.0), LH <3.0 mIU/ml (4.0–20.0) and oestradiol 11 pg/ml (prepubertal 10–25). Prolactin and thyroid function tests, and a computed tomography scan of the pituitary and hypothalamus were normal. Patient I.2 was normally grown, had a normal sense of smell and was not dysmorphic. Subsequent ophthalmological examination, including dilated fundal examination, was normal. She was diagnosed with IHH.

The couple's first child, II.1 (Figure 1a, Table 1), was conceived through gonadotropin ovulation induction (75 IU Humegon, NV Organon, Oss, The Netherlands, and 75 IU Puregon, Schering-Plough, Kenilworth, NJ, USA) and was delivered at 37 weeks gestation by Caesarean section for maternal pre-eclampsia. Bilateral anophthalmia was noted at birth, with deeply set orbits, and normal eyelids and eyelashes. An apnoeic episode accompanied by abnormal movements occurred at the age of 17 h in the setting of transient hypocalcaemia, and he was treated with anti-convulsants for a brief period. He was not dysmorphic, had descended testes and a normal phallus.

Birth parameters in patient II.1 were weight 3 kg, length 47 cm and head circumference 34.5 cm, all on the 50th centile. Cortisol and growth hormone

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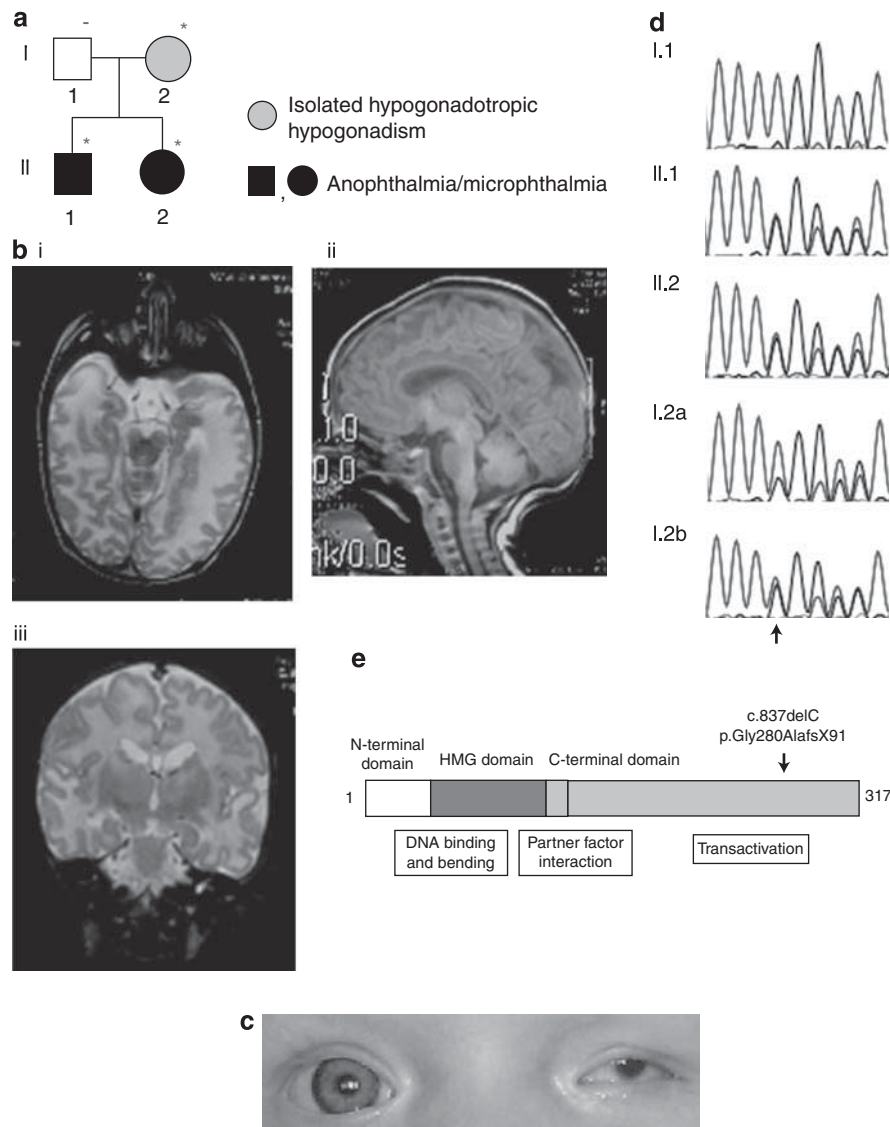


Figure 1 Pedigree, clinical features and *SOX2* mutation in family. **(a)** In this pedigree, individuals with the *SOX2* mutation, p.Gly280AlafsX91, are identified by an asterisk. The individual without the *SOX2* mutation is identified by the minus sign. Individuals identified by a black symbol (II.1 and II.2) have bilateral or unilateral anophthalmia/microphthalmia. Patient II.1 also had growth hormone deficiency. The individual identified by the grey symbol (I.2) has isolated hypogonadotropic hypogonadism with no eye disorder or other anomalies. **(b)** Magnetic resonance images of the brain and orbits in patient II.1. (i) Transverse section showing complete absence of ocular structures and optic nerves. (ii) Sagittal section demonstrating normal pituitary and corpus callosum. (iii) Coronal section demonstrating asymmetric hippocampi with the right hippocampus appearing dysplastic and under rotated. **(c)** Left microphthalmia in patient II.2. **(d)** Direct sequencing of PCR product from affected children II.1 and II.2 in the family showed heterozygous c.837delC (NM_003106). This was present in the mother, I.2, with an apparently lower level of mutant allele in her DNA sample extracted from blood (I.2a), but was clearly present in her DNA sample extracted from buccal cells (I.2b). The mutation was absent from control sequence and the father (I.1). **(e)** The c.837delC mutation results in a frameshift mutation, p.Gly280AlafsX91, in the C-terminal end of the transactivation domain of *SOX2*.

Table 1 Ocular, cerebral and pituitary phenotypes of c.837delC *SOX2* family members

	Eye morphology	Fundus	Neuroimaging results	Pituitary function
I.2	Normal	Normal	Normal (CT)	Isolated hypogonadotropic hypogonadism
II.1	Bilateral anophthalmia	N/A	Normal pituitary, Dysplastic right hippocampus (MRI)	Transient growth and GH disturbance
II.2	Left: severe microphthalmia Right: normal	Left: retinal coloboma Right: normal	Not done	Clinically normal

levels were equivocal at 3 months, and a glucagon stimulation test demonstrated a subnormal peak growth hormone response (11 mU/l) and a normal cortisol response (723 nmol/l). This was accompanied by a period of

suboptimal growth. At the age of 1 year, weight was 8.6 kg (10th centile), length was 71 cm (2nd centile) and head circumference was 43 cm (2nd centile). Growth has subsequently normalised, and at the age of 8 years, all parameters

were between 10 and 25th centiles. Pituitary function is monitored on a yearly basis, and is currently normal.

In patient II.1, MRI scan of the brain and orbits was performed at 12 months of age. No eye remnants were visible and the optic nerves were absent (Figures 1b, i). The corpus callosum was intact, and the pituitary gland was structurally normal and normally located (Figure 1b, ii), whereas the right hippocampus appeared dysplastic and under rotated (Figure 1b, iii). Auditory brainstem response testing in the newborn period and pure tone audiometry at the age of 9 years were normal. Psychomotor development is progressing normally at the age of 9 years, apart from a minor delay in the attainment of early gross motor skills consistent with visual impairment.

The couple's second child, II.2 (Figure 1a, Table 1), was conceived using *in vitro* fertilisation, and was delivered at term, following an uncomplicated pregnancy, with a birth weight of 2.7 kg (10th centile). She was noted to have left-sided moderate microphthalmia, with a large retinal coloboma, involving the central vision areas and the optic disc (Figure 1c). The other eye is structurally and functionally normal. There were no associated abnormalities or dysmorphic features. When last assessed at the age of 8 months, she was growing normally, with all parameters between 25 and 50th centile, and had normal development.

Mutation detection

Whole-blood samples were obtained from all patients, and a buccal cell sample was obtained from patient I.2. Genomic DNA was extracted for gene amplification. The coding exon and adjacent flanking regions of *SOX2* (GenBank accession number NM_003106) were amplified by PCR (primers available on request). The PCR product was purified and sequenced bidirectionally using standard techniques. Written informed consent was obtained from the patients, in accordance with ethics approval from the Children's Hospital at Westmead Ethics Committee.

RESULTS

Novel *SOX2* mutation

We identified a novel heterozygous mutation in the *SOX2* gene in child II.1 in this family. Genomic DNA analysis revealed a C nucleotide deletion at position 837 in the *SOX2* gene (c.837delC) (Figure 1d). Sequencing in other family members revealed that the affected sibling (II.2) was heterozygous for the same mutation. The mother's sequencing results from lymphocyte DNA showed presence of this mutation, but at an apparently lower level compared with her children affected with ocular anomalies in three independent sequencing reactions (Figure 1d, I.2a). A buccal sample was obtained from the mother and this showed clear presence of the mutant allele (Figure 1d, I.2b). The sequencing results in the mother suggested the possible presence of mosaicism, although they could also be due to sequencing artefacts that can occur in double-stranded sequencing. We were unable to confirm semi-quantitatively whether mosaicism might be present, as the mutation did not create a new restriction enzyme site, and attempts at capillary electrophoresis of fluorescently labelled PCR products as well as allele-specific PCR did not allow conclusive results because of the 1 bp deletion in a GC-rich region. The father's sequencing results were normal.

The one-nucleotide deletion predicts a frameshift mutation p.Gly280AlafsX91, which leads to abnormality of the protein in the C terminal end of the transactivation domain. The mutation predicts that the normal 317AA *SOX2* protein is changed to a 369AA with 90 abnormal C terminal residues (Figure 1e).

DISCUSSION

We report a family with a novel frameshift mutation in the *SOX2* transactivation domain, resulting in bilateral anophthalmia and subtle pituitary dysfunction in a male sibling and unilateral

microphthalmia in a female sibling. The mutation is also present in their mother who has IHH and underwent assisted reproduction to achieve fertility.

This is the first report of IHH occurring in the absence of eye involvement in a *SOX2* mutation patient. Previous patients reported with *SOX2* mutations and with hypogonadotropic hypogonadism have had severe ocular anomalies and anterior pituitary hypoplasia.^{18,19} One patient with unilateral anophthalmia and IHH without structural pituitary abnormality is described.²⁰ Mutations in *GNRHR*, *GNRH1*, *GPR54*, *TAC3* and *TACR3* have previously been identified in IHH patients without associated anomalies.^{5–7,11} The findings in the mother in this family add *SOX2* mutation as another possible cause of IHH in an otherwise normal individual.

The affected child in this family with bilateral anophthalmia (II.1, Figure 1a, Table 1) had transient growth hormone deficiency in the absence of pituitary hypoplasia on cerebral imaging. Anophthalmia/microphthalmia patients with heterozygous *SOX2* mutations and pituitary hormone deficiencies usually have associated anterior pituitary hypoplasia.¹⁹ *SOX2* is expressed in the developing hypothalamus and anterior pituitary in mice and humans. The functional pituitary abnormality in our patient, in the absence of anterior pituitary hypoplasia, indicates that *SOX2* could be involved independently at multiple levels in development of the hypothalamo-pituitary axis. This emphasises the need for clinical monitoring and investigation of the hypothalamo-pituitary axis in *SOX2* mutation patients, even in the absence of pituitary hypoplasia on imaging.

The ocular features of bilateral anophthalmia in one sibling and unilateral microphthalmia with retinal coloboma in the other, in this family, are consistent with the variable phenotypic ocular features that can be seen associated with *SOX2* mutations within families.^{21,22} The observed spectrum also includes normal eye development in heterozygote²³ and mosaic^{17,23–25}/queried mosaic²⁶ heterozygote *SOX2* mutation patients, as in the mother in this case. In addition to pituitary dysfunction, a substantial proportion of *SOX2* mutation patients have other cerebral abnormalities as seen in patient II.1 in this report. Other known extra-ocular features, such as oesophageal abnormalities, sensorineural hearing loss and male urogenital abnormalities^{14,17} were not present in our patients.

Children with A/M and *SOX2* mutations born to mothers with *SOX2* mutation mosaicism/queried mosaicism are reported.^{17,23–26} These mothers have all been phenotypically normal. Our case is the first report of one such mother having IHH. Despite the possibility of mosaicism in the cases reported in the literature as well as our case, the mutations have been detectable on routine sequencing of DNA extracted from a peripheral blood sample in all but one case. Management of IHH in patients without associated anomalies frequently includes gonadotropin therapy and assisted reproductive technologies to achieve fertility. In cases of IHH, which are associated with autosomal recessive inheritance and mutations in *GNRHR*, *GNRH1*, *GPR54*, *TAC3* and *TACR3*, gonadotropin therapy would not be expected to be associated with an increased risk of a child with a birth abnormality. However, our report shows that the IHH patient with a *SOX2* mutation, faces an up to 50% risk of a child with A/M and other anomalies associated with *SOX2* mutation. Most *SOX2* mutations have been ascertained through the study of patients with ocular abnormalities, and it is not known what proportion of patients with IHH may harbour *SOX2* mutations. As this family demonstrates, with the advent of assisted reproductive technology, IHH is no longer a barrier to reproduction. *SOX2* testing in IHH has important implications for risk counselling, particularly in the setting of infertility treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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