A severe case of Bosch- Boonstra-Schaaf optic atrophy syndrome with a novel description of coloboma and septo-optic dysplasia, owing to a start codon variant in the *NR2F1* gene

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## **Keywords**

### Bosch- Boonstra-Schaaf optic atrophy syndrome, coloboma, Intellectual disability, NR2F1, septo-optic dysplasia

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**Running Title**

Severe case of BBSOAS with colobomas and septooptic dysplasia.

**Abstract**

Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) is a rare congenital syndrome characterized by a range of phenotypes including optic atrophy and intellectual disability among other features. Pathogenic variants in the *NR2F1* (nuclear receptor subfamily 2 group F member 1) gene have been linked to this condition. A recent report has shown that pathogenic variants in the start codon lead to decreased expression of the NR2F1 protein and a relatively mild phenotype, similar to that seen in whole gene deletions, and due to the lack of the dominant negative effect. Here we describe a severe case of BBSOAS with an initiation codon missense variant. The developmental delay, seizures, optic atrophy are in keeping with features observed in this condition, however this is the first report to describe colobomas and septo-optic dysplaisa as associated features potentially extending the phenotype linked to BBSOAS. In addition, this is the first description of a severe phenotype linked to a *de novo* missense variant in the start codon of the *NR2F1* gene.

**Introduction**

Bosch-Boonstra optic atrophy syndrome (BBSOAS) is an autosomal dominant congenital isorder characterized by optic atrophy, intellectual disability and developmental delay. Pathogenic variants in NR2F1 (nuclear receptor subfamily 2 group F member 1 gene) (MIM 132890) have been linked to this condition (OMIM 615722) (Al-Kateb et al., 2013; Bosch et al., 2014; Brown et al., 2009; Chen et al., 2016; Kaiwar et al., 2017).

Also known as COUP TFI (chicken ovalbumin upstream promoter transcription factor 1), NR2FI belongs to the conserved family of orphan nuclear receptor proteins, a superfamily of transcriptional regulators with a diverse physiological role (M. J. Tsai & O'Malley, 1994). NR2F1 has two functional domains: the DNA-Binding domain (DBD) and Ligand-Binding domain (LBD) (S. Y. Tsai & Tsai, 1997).

Whilst variants within the DBD domain are capable of completely abolishing NR2F1 transcriptional abilities, both haploinsufficiency and a dominant negative effect have been proposed to be responsible for the phenotype (Bosch et al., 2014; Chen et al., 2016; Kaiwar et al., 2017).

Pathogenic *de novo* variants in *NR2F1* were first identified in individuals with cerebral visual impairment. All of these patients had intellectual disability and the majority had optic atrophy, leading to the description of BBSOAS (Bosch et al., 2014). Preceding this, *NR2F1* had been proposed as a candidate gene for the optic and developmental phenotype seen in 5q15 deletion (Al-Kateb et al., 2013; Brown et al., 2009; Cardoso et al., 2009). The BBSOAS phenotype has since been extended with frequent identification of oromotor dysfunction, hypotonia, thinning of corpus callosum, repetitive behaviour, seizures and attention deficit hyperactivity disorder (Bosch et al., 2014; Chen et al., 2016; Kaiwar et al., 2017).

The murine NR2F1 homolog has been extensively studied in a variety of knockout and selective deletion mouse models. It is highly expressed in the central nervous system, including the optic nerve, thalamus and pallium. The homozygous COUP TF1 knockout is perinatally lethal, a likely consequence of defective glossopharyngeal nerve development leading to starvation (Qiu et al., 1997). These mice models implicate COUP TFI and NR2F1 in neurogenesis

(Faedo et al., 2008; Yamaguchi et al., 2004; Zhou, Tsai, & Tsai, 2001), arborisation (Zhou et al., 2001), neural differentiation (Faedo et al., 2008), cortical patterning (Alfano, Magrinelli, Harb, Hevner, & Studer, 2014; Armentano et al., 2007; Faedo et al., 2008; Zhou et al., 1999), axonal guidance (Qiu et al., 1997; Zhou et al., 1999; Zhou et al., 2001), axonal myelination (Yamaguchi et al., 2004) and eye development (Satoh et al., 2009; Tang et al., 2010), whilst also providing a plausible explanation for the human neurological and ophthalmological phenotype caused by NR2F1 alteration.

More recently there have been reports of pathogenic variants within the translation start codon. Missense variations in the translation start codon have been demonstrated to reduce both NR2F1 mRNA and protein product and result in a mild phenotype (Chen et al., 2016). Here we describe a 20 year old female with severe BBSOAS phenotype caused by a non-synonymous translation initiation codon variant and further extend the clinical phenotype.

**Patients & Methods**

The patient was identified through the oculogenetics clinic in Southampton having participated in the Deciphering Developmental Disorders (DDD) study (Study, 2015).

The participant underwent whole exome sequencing of family trio. Sequencing was undertaken using Agilent Sureselect 55MB Exome Plus with Illumina HiSeq tecnology. The d*e novo* variant was identified with the DeNovoGear program and variant annotation by Ensembl’s variant effect predictor (VEP) software. This variant is publicly available in the DECIPHER database [[266126 - DECIPHER v11.4 (deciphergenomics.org)](https://www.deciphergenomics.org/patient/266126/genotype/197354/annotation/maf)]. Array CGH was performed using Oxford Gene Technology’s (OGT) (ISCA\*) 8x60K oligo array platform and dosage analysis of the 22q11.2 deletion syndrome was carried out using MLPA probe kit P250-B2 (MRC-Holland). Written consent for publication has been obtained from the family.

**Results**

The proband was born to non-consanguineous parents at 41 weeks with a birthweight of 3.856 kg (+0.97 SD). There is a history of 2 pregnancy losses. Mother had antenatal bleeding in the 1st trimester, she had a mild viral illness around 3-4 months gestation and the antenatal scans including nuchal translucency were normal. There is no history of any maternal illness or diabetes and the mother did not take any anticonvulsant drugs. The proband was born after a normal delivery with good Apgars and she was noted to have poor suck. She had difficulty in putting on weight and she was felt to have borderline failure to thrive. She was born with a right iris coloboma noted at 3 days of age and chorioretinal coloboma. In addition, she was also found to have septo-optic dysplasia (SOD) and Arnold Chiari malformation. In the first few months of age she had slow weight gain, difficulty feeding and hypotonia. When reviewed at 9 months of age, she could briefly hold her head up when prone, was hypotonic with brisk deep tendon reflexes and had a prominent Moro reflex. She developed myoclonic jerks usually occurring at the beginning of her sleep. She was able to sit unsupported at 16 months of age and she commando crawled. She had started to babble and pull to stand at 22 months, had 20 distinctive words and was making efforts to cruise at 30 months She walked independently at the age of 3 years and 6 months and she was noted to have nystagmus in association with a small optic nerve at this stage. Specialist ophthalmic assessment showed a small optic nerve and chorioretinal iris coloboma in her right eye, with a possible mild left sided iris coloboma, far more severe in the right eye than in left, very poor vision at 336 in either eye with small bilateral pale disc.

From 6 years of age she developed infrequent episodes of eye fluttering, episodic jerking movements and visual disruption lasting hours to days. A subsequent MRI showed markedly slender anterior visual pathways, almost complete absence of the septum pellucidum and possible truncation of the rostrum of the corpus callosum, highly suggestive of septo-optic dysplasia. There was a slender infundibulum but otherwise normal pituitary tissue and posterior bright spot (Figure 1). She was incidentally noted to have a chiari I malformation with no evidence of hydrocephalus. Although her initial EEG showed no diagnostic epileptiform activity, repeat EEG at 12 years of age showed possible occipital seizures with repetitive high amplitude polyspike and slow wave complexes seen bilaterally maximum in posterior and occipital regions with frequency varying between 2-4 per second.. Clobazam has been successful in seizure control.

After showing a sub-satisfactory growth hormone rise after two stimulation tests, a height below her mid-parental range (-1.55 standard deviations at 8 years of age) and borderline IGF, she was started on growth hormone replacement at 9 years of age. She had improvement in her growth velocity and attained a height on the 50th centile and within her mid-parental range. Growth hormone was stopped after reaching final height at 15 yrs. She has shown no evidence of any other pituitary deficiencies (Figure 2).

When assessed at the age of 11 years her height was 133 cm (-2.22 SD) and her head circumference was 53 cm (-1.01 SD). On examination she was found to have 2-3 toe syndactyly, thick lips and long thin fingers and hands. She was visually impaired with intermittent nystagmus and she had ongoing coordination difficulties (Table 1).

Her investigations including thyroid function tests, creatine kinase (CK), lactate, amino acids, urine amino acids and organic acids and urinary GAGs were all normal.

**Genetic test results**

Previous investigations included an array CGH which identified maternally inherited Xq13.3 duplication [ish dup(X)(q13.3q13.3)(RP11-311P8++)] the size of which was approximately 187kb and included 7 protein coding genes (NEXMIF, ABCB7, UPRT, ZDHHC15, MAGEE2, PBDC1 and MAGEE1). She was also found to have mild to moderately skewed X inactivation in DNA extracted from whole blood (Humar 74:26 mat:pat; ZNF261 80:20, mat:pat). Mother had random-moderate skewed X inactivation. The maternal X chromosome carrying the Xq13 duplication was active in the majority of cells and this duplication was felt not to be significant or explain her phenotype. There is no maternal phenotype. The proband also had MLPA for 22q11 deletion with negative result.

Whole exome sequencing as part of the DDD study revealed a *de novo* missense variant in the *NR2F1* gene ENST00000327111.8:c.2T>C, ENSP0000325819.3:p.Met1?, GRCh37 (h19) 5: 93585025 (1 M/T) resulting in loss of the 1st codon (start loss).

**Discussion**

BBSOAS is characterised by the presence of intellectual disability, developmental delay and optic atrophy. This case report describes asevere case with severe intellectual disability, septo-optic dysplasia, seizures and colobomas who was found to have a missense variant (ENST00000327111.8:c.2T>C, ENSP0000325819.3:p.Met1?) in the *NR2F1* gene.

The patient described here shares many of the BSSOAS phenotypical characteristics previously described in the literature. Of all the reported cases with NR2F1 point mutations, deletions and intragenic deletions; 35/36 (97%) have intellectual disability, 41/50 (82%) have optic nerve atrophy, 39/43 (91%) have hypotonia, 30/43 (70%) have feeding difficulties including oromotor dysfunction and 24/46 (52%) have seizures (Al-Kateb et al., 2013; Bosch et al., 2014; Brown et al., 2009; Cardoso et al., 2009; Chen et al., 2016; Dimassi et al., 2016; Hino-Fukuyo et al., 2017; Kaiwar et al., 2017; Martin-Hernandez et al., 2018; Rech et al., 2020). Chen et al (2016) reported the same coding variant of the translation initiation codon (c.2T>C) in two patients previously. Fibroblast cell lines from these individuals demonstrated reduced expression of the protein by approximately 60% when compared to control cell lines. Furthermore the study researchers demonstrated a 45% reduction of messenger RNA levels and altered transcritpion in these individuals (which is thought to be linked to a milder phenotype). Although this study has indicated possible effect on both translation and transcription, further studies are required into the effect of other translation initiation codon variants on gene expression. (Chen et al., 2016). The patient described here has significant developmental delay and intellectual disability, visual impairment with optic nerve hypoplasia, seizures, oromotor dysfunction including sucking and feeding problems. Brain Magnetic Resonance Imaging scan showed slender anterior visual pathways, possible absent septum pellucidum and Chiari I malformation. This case report therefore raises the possibility of more severe cases being associated with intiation codon variants.

The diverse BBSOAS ophthalmic phenotype includes optic atrophy, cerebral visual impairment, optic hypoplasia, strabismus, alacrima, manifest latent nystagmus, and amblyopia (Bosch et al., 2014; Chen et al., 2016; Kaiwar et al., 2017; Rech et al., 2020). Although this is the only reported case with coloboma in BBSOAS, a left iris coloboma was described in a patient with intellectual disability and a 17 Mb, 40 gene deletion encompassing *NR2F1* (Cardoso et al., 2009). Homozygous deletions of COUP TFI and COUP TFII in mice cause an open optic fissure representative of a human coloboma. There is however a notable absence of an ophthalmic phenotype when these genes are deleted individually (Tang et al., 2010). Another study has recapitulated that *Nr2f1* mice develop optic disc abnormalities, optic nerve atrophy and cerebral visual impairment (Bertacchi et al., 2019). COUP-TFI has been implicated in regulating key ocular morphogenesis genes, such as *Pax6* (Tang et al., 2010). Pathogenic *Pax6* variants in mice and humans cause a diverse ocular phenotype including; aniridia, optic nerve hypoplasia or aplasia, foveal hypoplasia (with or without nystagmus), and chorioretinal coloboma as well as corneal phenotypes including cataracts (Azuma et al., 2003; Tang et al., 2010; Yokoi et al., 2016).

Although the murine knockout and COUP TFI regulatory gene pathways provide a plausible model for human coloboma, further reports of individuals with BBSOAS and their ophthalmic phenotype will help delineate this association. Furthermore, ophthalmic examination can prove difficult in patients with intellectual disability and as a result identification of subtle colobomas may be missed.

The individual in this case report has septo-optic dysplasia, a condition characterised by the presence of two of the following signs; optic nerve hypoplasia, pituitary gland hypoplasia and midline abnormalities. The short stature in this case is presumed secondary to septo-optic dysplasia-related growth hormone deficiency.

Short stature (below 5th centile) and optic hypoplasia was described in a 14 year old male with BBSOAS. Although it is unclear whether this patient had endocrine investigation, it was hypothesised that the short stature and delayed bone maturation may be due to the interaction between NR2F1 and BMP4, a bone-morphogenetic protein known to be involved in bone mineralisation (Feng et al., 1995; Kaiwar et al., 2017). BMP4 has also been demonstrated to be important in murine pituitary development where ectopic expression of the BMP4 antagonist noggin or deletion of the BMP receptor disrupts pituitary development (Davis & Camper, 2007; Treier et al., 1998).

SOD is felt to represent an abnormality in early forebrain development where the development of structures such as the optic vesicle, telencephalon, diencephalon and pituitary gland are interlinked. COUP TFI knockout mice interestingly show anomalies in development of their forebrain with abnormal cortical arealisation, defective forebrain axonal guidance and disrupted midline crossing of callosal axons (Alfano et al., 2011; Armentano, Filosa, Andolfi, & Studer, 2006). COUP TFI has shown to interact with members of regulatory pathways that are implicated in forebrain development such as β-catenin–mediated Wnt signaling, BMP and FGF8(Faedo et al., 2008). Loss of the β-catenin pathway in the diencephalon has also been found to profoundly effect the development of the anterior pituitary gland (Camper, Daly, Stallings, & Ellsworth, 2017; Osmundsen, Keisler, Taketo, & Davis, 2017).

In addition to the optic nerve anomalies, a subset of patients with BBSOAS show radiological evidence of corpus callosum thinning. The identification of SOD in this patient and its significance in regards to BBSOAS and the forebrain patterning pathways requires further analysis, although the current report might aid with the identification of further cases. We also cannot exclude the possibility that the Xq13.3 duplication identified in this patient might work synergistically with the *NR2F1* variant and contribute to the more severe phenotype observed in this patient.

In summary, this case report further supports the pivotal role of *NR2F1* gene in neurodevelopment and raises the possibilty of a more severe phenotype (including colobomas and septo-optic dysplasia as additional features) being associated with start codon variants within this gene.

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**Conflict of Interest**

No conflicts of interest declared.

**Ethical Review**

Consent was gained for publication and photographs. The report conforms to the recognised standards of the Declaration of Helsinki.

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Figure legends

**Figure 1.** MRI brain images of the patient. a,Chiari I malfomation showing that the cerebellar tonsils extend below the formaen magnum. b, absent septum pellucidum indicated by arrow, c, hypoplasia of the optic nerves, chiasm and optic tract.

**Figure 2.** Facial and limb phenotype of patient with *NR2F1* gene variant.