Journal of Human Genetics (2018) 63:525–528
https://doi.org/10.1038/s10038-017-0398-3

BRIEF COMMUNICATION

Mitochondrial involvement in a Bosch-Boonstra-Schaaf optic atrophy syndrome patient with a novel de novo *NR2F1* gene mutation

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Received: 7 September 2017 / Revised: 27 September 2017 / Accepted: 13 November 2017 / Published online: 6 February 2018

Abstract

We report the clinical and biochemical findings from a patient who presented with Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), an autosomal-dominant disorder characterized by optic atrophy, developmental delay and intellectual disability. In addition, the patient also displays hypotonia, stroke-like episodes, and complex IV deficiency of the mitochondrial respiratory chain. Whole-exome sequencing (WES) uncovered a novel heterozygous mutation in the *NR2F1* gene (NM_005654:c.286A>G:p.Lys96Glu) that encodes for the COUP transcription factor 1 protein (COUP-TF1). Loss-of-function mutations in this protein have been associated with BBSOAS, and a luciferase reporter assay showed that this variant, in the zinc-finger DNA-binding domain (DBD) of COUP-TF1 protein, impairs its transcriptional activity. The additional features of this patient are more related with mitochondrial diseases that with BBSOAS, indicating a mitochondrial involvement. Finally, our data expand both the genetic and phenotypic spectrum associated with *NR2F1* gene mutations.

The *NR2F1* gene (nuclear receptor subfamily 2, group F, member 1; MIM #132890) encodes the Chicken Ovalbumin Upstream Promoter-Transcription Factor 1 protein (COUP-TF1) that is a nuclear hormone receptor and transcriptional regulator [1]. Mutations in this gene results in an autosomal-dominant disorder, the Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS; MIM #615722), characterized by developmental delay (DD), intellectual disability (ID), and optic atrophy (OA) with evidence of cerebral visual impairment (CVI). COUP-TF1 is involved in neurogenesis, axogenesis, neural differentiation [2], eye and optic nerve development [3], and hippocampus volume and connectivity [4].

The Spanish patient is the second daughter of non-consanguineous healthy parents. Her older brother is healthy, and her mother had two previous miscarriages of unknown cause (Fig. 1a). The patient was born at 37 weeks gestation following an uneventful pregnancy and vaginal delivery. The neonatal period and early development were normal. At 6 months of age, the patient was brought to the emergency department for vomiting, hypotonia, and impaired level of consciousness. In the physical examination she showed diminished motor activity with undetermined level of consciousness (behavioral arrest episodes), hypotonia with normal reflexes, and convergent strabismus. In complementary exams, the serum creatine kinase (CK) level was elevated (500 IU/L, normal <250 IU/L), being the rest of biochemical, hematological, and metabolic analysis (ammonia, amino acids, organic acids, and acylcarnitines) normal. The higher

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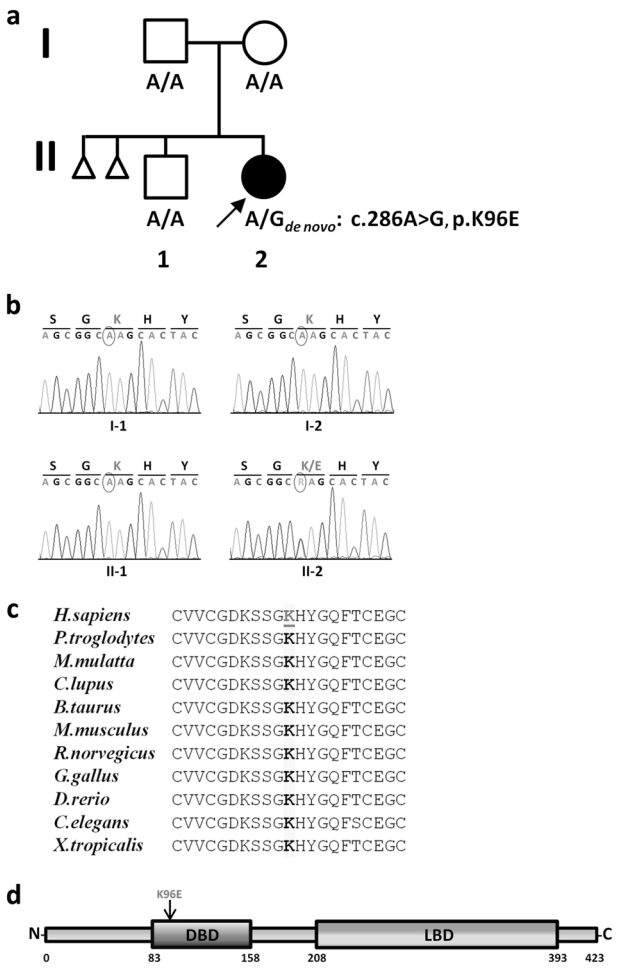

Fig. 1 Genetic analysis. **a** Family pedigree showing the genotype of the de novo c.286A>G (p.Lys96Glu or p.K96E) mutation in *NR2F1* [NM_005654]. **b** Electropherograms showing Sanger sequence validation of the *NR2F1* c.286A>G (p.K96E) mutation. **c** Multiple sequence alignment of COUP transcription factor 1 (protein encoded by *NR2F1* gene) region surrounding the p.K96 (red) mutation site in various species. **d** Schematic representation of human COUP transcription factor 1 protein showing the position of p.K96E (red) mutation, the DNA-binding domain (DBD) and the ligand-binding domain (LBD) (color online)

level of serum lactate was 2.2 mM one hour after breakfast, being 0.6 mM before breakfast (normal 0.5–2.3 mM), with an increased lactate/pyruvate ratio of 40 (normal < 25). The cerebrospinal fluid (CSF) lactate was normal (1.8 mM; normal < 2.0 mM). In electromyography (EMG) was detected myopathic potential in all explored muscle. The electroencephalogram (EEG) and video-EEG did not show epileptic activity during the acute episodes. The brain magnetic resonance imaging (MRI) was normal, except for a hypoplastic corpus callosum. In the next 48–72 h, the child became more alert and responded better to external stimuli, while hypotonia persisted along, with behavioral arrest episodes in absence of any EEG alterations. At 7 months of age, the child presented a similar episode together with right hemiparesis, while the MRI and EEG were normal. Epileptic and ischemic etiologies were reasonably ruled out, and the congenital disorders of glycosylation (CDG) studies were normal. Muscle biopsy demonstrated variations in fiber size in histological study without histochemical abnormalities. Respiratory chain analysis in skeletal muscle showed a deficiency of complex IV, with an ~33% of mean control activity, while complex I, II, III and citrate synthase activities were in the normal range (Table 1) [5]. The mtDNA analysis by Southern blot (deletions, depletion) was normal, and the whole mtDNA sequencing did not show any pathological variant. At that point in time, the symptoms of the patient were considered compatible with a mitochondrial disease (stroke-like episodes of mitochondrial etiology). She started treatment with CoQ10, carnitine, riboflavin, vitamin C and rehabilitation therapy. Ophthalmological exam at 20 months of age revealed a visual impairment (equivalent visual age of 9 months) by VAP-CAP (Visual Assessment and Programming-Capacity Attention and Processing). Fundoscopy showed bilateral partial OA. Visual-evoked potentials (VEP) showed abnormal response in two studies and brainstem evoked response audiometry (BERA) was normal. In the follow-up, the child never had any other acute neurological event and the hemiparesis was improving, but her motor and cognitive development were delayed. She started to walk when she was 4 years old, and her first words were at the age of 5. She has been attending a school for children with special needs. The last MRI/MRS (magnetic resonance spectroscopy) performed at age of 12 showed slight elevation of lactate peak in basal ganglia and hypoplastic corpus callosum. Currently, at 17 years of age, the clinical condition remains stable. Her weight is 42 kg (−1.7 SD) and her height is 151 cm (−2.0 SD). She is able to understand and respond elementary questions, and she can walk, run and climb steps with support. She has ID with an intelligence quotient (IQ) of about 30–50 (Stanford-Binet Scale). Cardiological studies have been normal. The last ophthalmologic examination showed orthophoria and latent nystagmus in the right eye. In visual field testing by confrontation, negligence was observed in low quadrants. Fundoscopy showed normal retina with bilateral partial OA.

Whole-exome sequencing (WES) was performed on genomic DNA obtained from patient blood, following a standard protocol [6]. The nuclear variants and indels were prioritized for those that may underlie a rare, severe, and recessive disease or de novo dominant disease. We found a heterozygous missense mutation (NM_005654:c.286A>G: p.Lys96Glu) in the *NR2F1* gene (Nuclear Receptor Subfamily 2 Group F Member 1) [1, 7–9]. This gene belongs to the steroid/thyroid nuclear receptor superfamily that encodes for the COUP-TF1 protein, and the mutation produces the change of lysine (Lys or K) to glutamic acid (Glu or E) in the position 96 of the protein (p.Lys96Glu).

Table 1 Mitochondrial respiratory chain activities in Skeletal Muscle

Enzyme	Values	Controls (mean ± SD)[a]
Citrate synthase (CS)	477.2[b]	535 ± 350
Complex I (NADH:ubiquinone oxidoreductase)	12.8[c]	19.0 ± 8.0
Complex II (succinate:ubiquinone oxidoreductase)	7.9[c]	17.9 ± 9.9
Complex III (ubiquinol:ferricytochrome-*c* oxidoreductase)	40.6[c]	63.0 ± 35.0
Complex IV (ferrocytochrome-*c*:oxygen oxidoreductase)	**13.1**[c]	40.0 ± 24.0

The value in bold indicates an abnormal activity value

[a]Skeletal muscle from controls: $N = 14$

[b]In SA (SA is specific activity: nmol × min^{-1} × mg $protein^{-1}$)

[c]In (SA of Complex/SA of Citrate Synthase) × 100

Sanger sequencing of patient, healthy brother, and their parents was performed. The NC_000005.10: g.92921015A>G (chr5, hg19) mutation was found to be de novo (Fig. 1a, b), and was not present in the healthy brother. The mutation was ascribed to be "pathogenic" by using well-established in silico tools of pathogenesis prediction (SIFT, PolyPhen2, MutPrep and Mutation Taster) (data not shown), and the amino acid residue was highly evolutionarily conserved (with PhastCons score of 1) from *Homo sapiens* to *Xenopus tropicalis* (Fig. 1c). At present, the missense mutation p.Lys96Glu has not been found in any genomic database (1000G, EVS, ExAC Browser or gnomAD Browser, accessed 2017-08-20).

The COUP-TF1 receptor possess two highly conserved domains: the DNA-binding domain (DBD) of 76 amino acids containing two conserved zinc-finger motifs, and the ligand-binding domain (LBD) of 186 amino acids (Fig. 1d) [10]. COUP-TF1 can repress gene expression by directly binding to the DNA-binding site of the gene, or can activate gene expression through forming a modulatory complex with the transcription factor Sp1, which binds at the Sp1 site of the gene [3].

The functional effect of this mutation in *NR2F1* gene was studied by an in vitro dual-luciferase reporter assay [9]. In this assay, HEK293T cells were cotransfected with pXP2-NGFI-A vector, containing the firefly luciferase cDNA under control of a *NR2F1*-activated promoter *NGFI-A* (−168/+33), and either wild-type or mutant FLAG-tagged mouse *Nr2f1* constructs in pcDNA5 vector. A *Renilla* luciferase cloned in pRL-TK vector was used as internal control for normalization. With the same level of protein expression of wild-type and mutant mouse COUP-TF1 (Fig. 2a), the mutation p.Lys96Glu almost abolished the ability of COUP-TF1 to fully activate the *NGFI-A* (−168/+33) reporter (Fig. 2b).

To date, several chromosome deletions that include the *NR2F1* gene [8, 11, 12], and numerous point mutation and indels in the same gene have been reported [1, 9, 13, 14]. The majority of these mutations are located in the DBD of COUP-TF1 protein, and all missense variants within the DBD abolish transcriptional activity [1, 9]. The p.Lys96Glu mutation of our patient is located in DBD (Fig. 1d) and also abolish the transcriptional activity (Fig. 2b). The mutations in the *NR2F1* gene are associated to the BBSOAS that is characterized by OA, DD, and ID, but the patients also may display symptoms of hypotonia, seizures, autism spectrum disorders, oromotor dysfunction, thin corpus callosum, or hearing defects [9]. Our patient in addition to the principal BBSOAS symptoms also displays hypotonia, stroke-like events, and a deficit of mitochondrial complex IV activity.

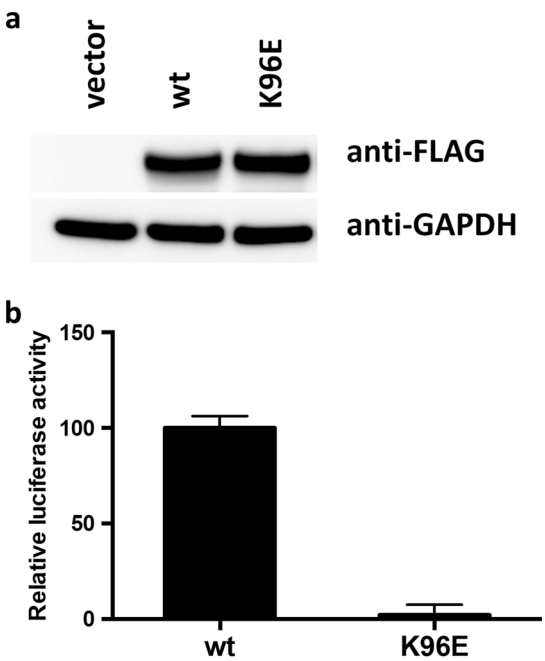

Fig. 2 In vitro luciferase reporter assay. **a** Immunoblot analysis of FLAG-tagged mouse COUP transcription factor 1 (COUP-TF1) expression in transfection. **b** Effect of the p.K96E mutation in the mouse COUP-TF1 on activation of NGFI-A promoter-driven reporter. HEK293T cells cotransfected with luciferase vector (pXP2-NGFI-A) and either wild-type (wt) or mutant (Lys96Glu or K96E) FLAG-tagged of mouse *Nr2f1* gene in pcDNA5 expression vector were analyzed. The luciferase activity of wt *Nr2f1* and the empty vector were set as 100 and 0%, respectively. Data are means ± SEM ($n = 3$) *$P < 0.001$ (unpaired two-tailed *t*-test)

The hemiparesis and the complex IV deficiency are features that have not been described before in BBSOAS patients, but they are more frequently seen in mitochondrial diseases [15]. Furthermore, the acute neurological episodes were non-epileptic events, more similar to those seen in children with mitochondrial disease due to energy deficit. A study carried out in 2010 identified 182 COUP-TF1 in vivo targets genes [16], and among them there were several mitochondrial genes (*SLC25A1*, *ECHS1*, *MRPL45*, *TIMM23*, etc). Some of these genes are involved in mitochondrial diseases that include phenotypes like encephalopathy, neurodegeneration, psychomotor development, hypotonia, seizures, etc. Therefore, we can conclude that our patient shows a mitochondrial involvement in her phenotype. However, we show data from only one patient, and further studies are needed to assess if the mitochondrial involvement is a general mechanism in BBSOAS or our patient is a particular case. Accordingly, mitochondrial parameters (biochemical and genetic) should be investigated in patients with *NR2F1* mutations, in order to clarify the relationship between BBSOAS and mitochondrial disease.

In conclusion, our findings expand both the genetic and phenotypic spectrum of *NR2F1*-associated disease.

Web resources

The URLs for the data presented here are as follows:

OMIM, http://www.omim.org/
SIFT, http://sift.jcvi.org/
Polyphen-2, http://genetics.bwh.harvard.edu/pph2/
MutPred, http://mutpred.mutdb.org/
Mutation Taster, http://www.mutationtaster.org/
1000G, http://www.1000genomes.org/
EVS, http://evs.gs.washington.edu/EVS/
ExAC Browser, http://exac.broadinstitute.org
gnomAD browser, http://gnomad.broadinstitute.org/

Acknowledgements The authors thank the patient and her family for their contribution. This work was supported by the Spanish Instituto de Salud Carlos III (ISCIII) and European Regional Development Fund (ERDF) (grant PI10/00063 and PI14/00790 to FM-A). FJC-V was supported by fellowship from the Instituto de Investigación Hospital 12 de Octubre (i+12). PC-R was supported by fellowship from Comunidad de Madrid and European Social Fund (ESF) (PEJ16/MED/TL-1655). MER-G was supported by fellowship from ISCIII and ERDF (grant PI14/00790).

Compliance with ethical standards

Conflict of interest All the authors listed have approved the content of the manuscript. All authors declare that they have no conflict of interest.

Ethics Committee Written informed consent was obtained from the patients' parents, and the Ethic Committee of the Instituto de Investigación Hospital 12 de Octubre (i+12) approved the study.

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