Extending the Phenotypic Spectrum of Bohring-Opitz Syndrome: First Mild Case Confirmed by Functional Studies

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Abstract

Bohring-Opitz syndrome (BOS) has been described as a clinically recognizable genetic syndrome since 1999. Clinical diagnostic criteria were established in 2011 and include microcephaly, trigonocephaly, distinctive craniofacial dysmorphic features, facial nevus flammeus, failure to thrive, and severe developmental delays. The same year, different *de novo* heterozygous nonsense mutations in the *ASXL1* were found in affected individuals. Since then, several cases have been reported confirming the association betweenthis chromatin remodeling gene and BOS.

Most affected individuals die in early childhood because of unexplained bradycardia, obstructive apnea or pulmonary infections. Those that survive usually cannot walk independently and are nonverbal. Some have had success using walkers and braces in late childhood. While few are able to speak, many have been able to express basic needs using communication devices as well as gestures with associated basic vocalizations.

In this paper we present the first mild case of BOS with a *de novo* pathogenic mutation c.1720-2A>G (p.I574VfsX22) in *ASXL1* detected on whole exome sequencing and confirmed by functional analysis of the mRNA splicing pattern on the patient's fibroblasts. She has typical dysmorphic features and is able to run and walk independently as well as to communicate with basic sign language.

Introduction

Bohring-Opitz syndrome (BOS), also known as Oberklaid-Danks syndrome or C-like syndrome (MIM605039), is a clinically recognizable genetic syndrome described for the first time in 1999 by Bohring et al. Twelve years later, in 2011, diagnostic criteria were established by Hastings et al. including microcephaly, trigonocephaly, distinctive craniofacial dysmorphic features, facial nevus flammeus, failure to thrive, and severe developmental delays. Craniofacial features include palatal abnormalities, prominent eyes, hypoplastic supraorbital ridges, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, and low-set posteriorly angulated ears. The same year Hoischen et al. found the association between mutations in the *ASXL1*gene and BOS after performing whole exome sequencing in combination with direct sequencing and found different *de novo* heterozygous nonsense or frameshift mutations.

Congenital anomalies like corpus callosum defects, retinal and optic nerve abnormalities are frequently reported in BOS. Seizures are common as well as truncal hypotonia with hypertonia of the extremities. Affected patients assume a typical posture of the upper limbs including ulnar deviation of the wrists and/or fingers at the metacarpophalangeal joints. In 2015, Russell et al. recommended Wilms tumor surveillance every 3 months

until age 8 given the link between *ASXL1* and myelodysplastic conditions. Severe feeding problems are common at the beginning of infancy and most affected individuals die in early childhood because of unexplained bradycardia, obstructive apnea or pulmonary infections. The ones who survive usually cannot walk independently and are nonverbal. Some have had success using walkers and braces in late childhood. While few are able to speak, many have been able to express basic needs using augmentative and alternative communication (AAC) devices as well as gestures with associated basic vocalizations (Russell et al. 2018).

Additional sex comb-like1 (*ASXL1*) is known as a chromatin modulator that plays dual functions in transcriptional regulation depending on the cell type. Recent studies using *Asxl1* knockout mice revealed its importance in proliferation and differentiation of hematopoietic progenitor cells, and in the development of organs (An et al. 2019). In this paper we present the first mild case of BOS with a *de novo* pathogenic mutation c.1720-2A>G (p.I574VfsX22) in *ASXL1* detected on whole exome sequencing and confirmed by analysis of the mRNA splicing pattern on fibroblasts. She has typical BOS dysmorphic features and is able to run and walk independently as well as to communicate with basic sign language.

Case Report

Our patient was born full term via vaginal delivery to a G1, P0, 22-year-old mother and 29-year-old father of Ethiopian descent. Prenatal and family history were unremarkable. Birth weight was 2.83 kg (13^{th} percentile) and birth length was 45.7cm (6^{th} percentile). Her newborn course was uncomplicated and she passed her newborn hearing screen. She

was noted to have glabellar nevus flammeus as an infant, which faded with age (Figures 1-3).

She sat up at 7 months, crawled at 11 months, babbled at 12 months, and stood at 14 months. At 14 months she was found to have a large cup to disc ratio on ophthalmological exam after exotropia was noted. She began to have seizures at 17 months including complex febrile and unprovoked seizures which typically occurred every few months. An EEG suggested a potential deep seizure focus from the left occipital/posterior quadrant region. Brain MRI demonstrated mild diffuse thinning of the corpus callosum, moderately small optic nerves and chiasm, mildly small pons, prominence of the left lateral ventricle likely reflecting mild left-sided periventricular white matter volume loss or hypogenesis, and no clear epileptogenic focus. An echocardiogram did not reveal cardiac disease.

She presented to our clinic at 19 months of age with global developmental delay. Her weight was in the 40th percentile, length in the 10th percentile, and HC in the 30th percentile. On physical exam, she was noted to have mild coarsening of the facial features, synophrys, upslanting palpebral fissures, prominent eyes, depressed nasal bridge, anteverted nares, narrow and high arched palate, widely spaced teeth, genu valgum, one hypopigmented macule on the chest, hirsutism, increased sandal gaps on both feet, and mild hypotonia (Figure 3-6).

She walked at 20 months of age and was able to climb the stairs with assistance at 24 months. At age 3 she was pointing and feeding herself with her hands, had balance issues, and had difficulty running. She was diagnosed with autism spectrum disorder at 4 years 3 months. At that time, she was able to respond to her name but had difficulty with eye

contact. She had repetitive behaviors including spinning in circles, clapping her hands, and slamming doors.

Growth parameters at the most recent physical exam at age 5 included weight in the 88th percentile, height in the 11th percentile, and HC in the 54th percentile. She is nonverbal but will point and sign for "more". She is hyperactive and has frequent tantrums. She can wave, and will pull a parent to what she wants. She needs assistance with dressing and brushing her teeth and is not toilet trained. She will only scribble with a crayonand does not typically play with toys but will throw them instead. She can go up and down stairs with alternating feet and runs slowly. She is seizure free on oxcarbazepine and levetiracetam and her Wilms tumor surveillance has been negative so far. She attends special education preschool and receives speech, occupational, and physical therapies.

Material & Methods

Initial genetic testing work-up included a negative SNP chromosomal microarray, comprehensive epilepsy panel, and Noonan spectrum disorder panel. Due to high suspicion for an underlying etiology of disease, trio whole exome sequencing (WES) was obtained which showed three de novo variants of unknown significance c.1720-2A>G in *ASXL1* gene (ENST00000375687), c.253G>A in the *STAG1* gene (ENST00000383202), and c.299_300del in the *BACH1* gene (ENST00000399921). Several other variants of unknown significance were reported in autosomal dominant genes on testing but all were inherited from an unaffected parent.

Patient and control's fibroblasts were obtained after signed consent and cultured in DMEM supplemented with 10% FBS (Gibco, Life Technologies) and 1% streptomycin-

penicillin (Gibco, Life Technologies) and were maintained at 37ºC and 5% of CO2. Cycloheximide (sigma-Aldrich) treatment was applied in a concentration of 1 mg/ml in DMEM during 6h. When confluence was reached, the RNA was extracted with the High Pure RNA Isolation Kit (Roche). RNA was then retrotranscribed using the High-capacity cDNA Reverse Transcription kit (Applied Biosystems). Amplification of the cDNA region containing the end of exon 12 and the beginning of exon 13 was performed by PCR using specific primers. The different isoforms obtained were cloned to a pGEM®T-easy vector (Promega) following the manufacturer's instructions. The resulting plasmids were sequenced using the Sanger method by the CCitUB genomic services (Parc Científic, Barcelona). All protocols were approved by the Ethics Committee of the Universitat de Barcelona (IRB00003099) and all methods were performed in accordance with the relevant guidelines and regulations.

Results

A de novo heterozygous intronic mutation, c.1720-2A>G, was previously identified by Whole Exome Sequencing (WES). This mutation is affecting the canonical acceptor splice site at intron 12. Fibroblasts from the patient were obtained to validate the functional implication of this change. RNA analysis and Sanger Sequencing of each band showed that the mutant allele led to the full retention of intron 12 (Figure 7). The aberrant transcript generated was not affected by the non-sense mediated decay (NMD) process, as no differences were observed when the culture was performed in the presence of cycloheximide.

Discussion

Variants previously associated with Bohring-Opitz syndrome are truncating de novo mutations mainly located in exon 13 (Hoischen et al. 2011, Urreizti et al. 2016, Russell et al. 2018), like the c.1720-A>G mutation found in our patient. The variant caused a change in the reading frame that would lead to a premature stop codon after 21 residues (p.I574VfsX22). The fact that no exon-exon junction remains downstream of the premature stop codon is consistent with the lack of NMD. The resulting protein is predicted to be truncated and will completely lack the C-terminus. For all the reasons stated above, the mutation is considered to be pathogenic.

The early diagnosis of BOS is important in order to decrease the mortality rate over time (Russell et al. 2018). However, most reported patients have a severe phenotype including the BOS posture, which was not present in our patient. The application of WES in clinically unrecognizable genetic syndromes has not only significantly helped the early diagnosis of rare and new genetic syndromes but has also broadened the clinical spectrum of several known genetic conditions like in our case.

In many cases, WES will frequently identify several variants of unknown significance including more than one *de novo* variant, therefore muddling the ability to identify a unifying diagnosis. As with the present case, our patient's medical history alone did not meet strict clinical criteria for BOS and further studies were necessary to confirm her diagnosis. These results, in addition to our patient's typical craniofacial features, allowed us to decrease our suspicion that the *STAG1 de novo* variant was the etiology of our patient's phenotype despite overlapping clinical features between *ASXL1* and *STAG1* genes related disorders. In fact our patient was reported in Yuan et al. at the beginning of

this year in a large cohort of patients with cohesinopathies as a possible Cornelia De Lange (CDL) like phenotype but the *STAG1* variant is predicted to be tolerated/benign by SIFT and Polyphen in silico analyses respectively and the patient does not have a typical CDL phenotype. The *BACH1* gene has not been associated with a disease yet.

To our knowledge, this is the first genetically confirmed patient with mild BOS who is able to communicate via sign language and walk independently whose mutation has been confirmed by functional studies. While she has typical facial dysmorphic features, she lacks the history of failure to thrive, BOS posture, severe intellectual impairment, microcephaly, and trigonocephaly.

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