Research note

A *de novo* truncating mutation in *ASXL1* associated with segmental overgrowth

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Mutations in genes involved in chromatin remodelling have been implicated in broad phenotypes of congenital abnormalities and neurodevelopment. However, limited genotype–phenotype correlations are available for some of the rarest genetic disorders that affect chromatin regulation. We hereby describe a 12-year-old girl presented at birth with severe hypotonia, developmental delay, a mid-line capillary malformation and distinctive craniofacial features. During the natural history of her disease the girl developed severe spasticity and drug-resistant seizures, leading to a diagnosis of Bohring-Opitz syndrome (BOS). We performed whole exome sequencing (WES) and identified a *de-novo* mutation in *ASXL1* (c.2033dupG) which results in the introduction of a premature stop codon (p.R678fs*6). *ASXL1* encodes a polycomb repressive complex protein implicated in chromatin regulation and *de novo* mutations are a known cause of BOS. Phenotypes with segmental craniofacial overgrowth associated to midline capillary malformations enlarge the clinical spectrum of BOS at onset and further expand the differential diagnosis in *ASXL1* mutation carriers.

Bohring-Opitz syndrome is a rare autosomal dominant (AD) syndrome, characterized by the variable combination of severe intrauterine growth restriction, feeding difficulties, hypotonia, profound intellectual disability (ID), trigonocephaly with distinctive facial features, peculiar facial *nevus flammeus* and broad MRI abnormalities (e.g., hypomyelination, hypoplastic corpus callosum, spinal cord abnormalities). BOS has been reported in literature to be caused in up to 75% of cases by *de novo* truncating variants in *ASXL1*. This gene encodes the additional sex combs-like 1 protein involved in chromatin remodelling. These epigenetic processes are mediated either by histone H2A deubiquitination 1 and/or transcriptional regulation of the polycomb group repressor complex 2 mediated homeobox (*HOX*) gene. HOX genes are critical for body patterning and segmental identity during human embryogenesis (Hoischen *et al*., 2011).

Bone marrow stromal cells (BMSCs) are an example of such homeotic genes that need to be regulated by *ASXL1* to achieve a homeostatic balance between self-renewal and differentiation. An imbalance in the cell fate or cell lineage commitment of BMSCs can lead to developmental or skeletal defects, as observed in BOS cases (Zhang *et al*., 2016). The Asxl1 protein is composed of an N-terminal DNA binding domain made up of the ASXN and ASXH sub domains, the ASXM1 and ASXM2 domains in the middle region as well as a PHD (zinc finger) domain in the C-terminal region, specifically important for mediating interactions with other proteins (Figure 1d). *ASXL1* mutations have been identified as a cause of BOS in 2011 (1) and most individuals reported so far have been found to carry *de novo* truncating mutations (Table 1) (Magini *et al.*, 2012; Dangiolo *et al.*, 2015; Arunachal *et al.*, 2016; Urreizti *et al.*, 2016; Carlston *et al.*, 2017; Bedoukian *et al.*, 2018).

Case report

Here, we report on the clinical characterization of one young girl affected with atypical BOS, whose phenotypic manifestations included global developmental delay with absent speech and severe motor impairment. She presented with failure to thrive, hirsutism, glabellar *nevus flammeus* (observed since birth) and trigonocephaly with distinctive craniofacial features including a prominent globes, widely set eyes, synophrys, and micrognathia (Figure 1).

History

Previous neurological or genetic diseases was unremarkable in the family and parents were healthy (Figure 1A). Pregnancy was complicated by threatened abortion and an intrauterine growth restriction documented by repeated ultrasound scans. The girl was born at 38 weeks with weight

<10th centile for gestational age. At birth a generalized low muscle tone as well as small hands with contractures of the fingers and a *nevus flammeus* on the face were observed. On the third day of life she suffered from an epileptic seizure characterized by peri-oral cyanosis, hypertonia and eye deviation. Axial hypotonia was observed since the neonatal age and a severe impairment of motor milestones was noticed in the first months of life. During the first year of life she started to experience frequent (almost daily) episodes of generalized hypertonic seizures with lateral eye and mouth deviation, eyelid flutter and oral automatisms. Seizures were usually followed by hypotonia and regression of motor developmental milestones. A poor visual interaction was noticed since the first months of life as well as myopia later on in life. Severe language impairment was also present, and the girl never attained an intelligible speech. She presented with microcephaly (OFC <10th) and trigonocephaly as well as distinctive facial features including prominent eyes, micro and retrognathia.

Examination

On her neurological examination at the age of 15 years old, she presented with severe spastic paraparesis and dystonic posturing of the hands, wrists, elbows as well as foot and brisk osteotendinous reflexes. Her shoulders were externally rotated and adducted, elbows and wrists flexed in ulnar deviation, and ulnar deviation of the metacarpophalangeal joints (Suppl. Video 1).

Pathological findings

The patient was treated during her first year of life with antiepileptic medication, specifically valproate, and since the second year of life with levetiracetam, with good clinical response, as she was seizure-free until the age of 8 years. Extensive diagnostic and metabolic work-up was reported to be normal. Auditory brain response showed sensorineural hearing loss only in the left ear. The

patient underwent also molecular investigation such as karyotype and array comparative genome hybridization (array-CGH) that were both reported as normal.

At the age of 5 years, electroencephalogram (EEG) activity recorded during sleep showed recurrent interictal high-voltage, bilateral, spike-wave discharges, followed by brief sequences of slow delta activities, interspersed with brief tracts of diffuse background slowing according to a fragmented hypsarrythmia pattern. A progressive organization of background rhythm occurred. At 8 years of age, EEG activity was characterized by interictal high-voltage, bilateral, spike-wave discharges, at 3 Hz, on bilateral post-central regions. Brain magnetic resonance imaging (MRI) performed at the age of 2 years, revealed brain asymmetry with reduced white matter volume in the left hemisphere, associated with enlargement of the left lateral ventricle and widening of the left hemispheric sulci; the corpus callosum is slightly thinned (Figure 1g.) These MRI features have been reported in BOS before (Dangiolo *et al*., 2015). The asymmetric appearance of the brain parenchyma appeared of mild degree, cortical abnormalities such as polymicrogyria, frequently associated with this syndrome, hydrocephalus or thickened corpus callosum were also noticed (Russell *et al*., 2015).

Materials & methods

After informed consent, we collected blood samples from the patient and her parents, and extracted DNA using standard procedures. To investigate the genetic cause of the disease, WES was performed in both the affected female and the two parents (Figure 1a: II-1, I-1, I-2). Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in an Illumina HiSeq3000 using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), and variant calling, and annotation were

performed as described elsewhere (Mencacci *et al*., 2016). In total, 55,531,100 (II-1) unique reads were generated. Sequencing data was reviewed for evidence of somatic mosaicism in both the proband and the parents' samples as the *de-novo* variant only was present in 16% of exome sequencing reads and present at an allele ratio of < 35% in the proband. After removing all synonymous changes and variants not shared by the patient and the two parents, we filtered single nucleotide variants (SNVs) and indels, only considering exonic and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants [<1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that were fitting a recessive or a *de novo* model. We have not identified rare potentially damaging recessive variants in the girl, and a *de novo* truncating mutation in *ASXL1* (c.2033dupG), resulting in the introduction of a premature stop codon six amino acids downstream (p.R678fs*6), emerged as the most likely explanation for the disease pathogenesis. This is supported by the severe impact of the *ASXL1* mutation on protein function and the existing reports linking loss of function (LoF) variants in this gene to a similar clinical phenotype (Hoischen *et al*., 2011; Magini *et al*., 2012; Dangiolo *et al*., 2015; Arunachal *et al*., 2016; Urreizti *et al*., 2016; Carlston *et al*., 2017; Bedoukian *et al*., 2018). In addition, we screened *ASXL1* in >1,000 trios exomes from individuals affected with genetically undiagnosed neurodevelopmental diseases recruited within the SYNAPS Study Group research initiative (neurogenetics.co.uk/synaptopathies-synaps), without identifying any additional *de novo* variant in this gene. Segregation analysis by traditional Sanger sequencing [primers used: forward (5'-GGTCACCACTGCCATAGAGA-3') and reverse (5'-GAGGATAAGGCGGCAGTAGT-3')] confirmed that mutation occurred *de novo* in the girl (Figure 1b). The patient has provided informed consent for publication of the case.

Most *ASXL1* mutations reported so far have been found in the last exon (exon 12) of the gene, that includes ~50% of the whole coding region of the gene, and where truncating variants are likely to generate transcripts that can escape nonsense mediated decay. The mutation that we identified occurs in exon 12, leading to a premature truncation of the downstream carboxyterminal plant homeodomain finger. Thus, the mutation may affect the binding of Asxl1 to chromatin, altering the activation or silencing of transcription factor genes involved in embryonic development (Figure 1d). During the natural history of the disease in the girl, additional progressive craniofacial and neurological features became evident, leading to a clinical diagnosis of atypical BOS.

Discussion

Previous work in mice and *Drosophila* showed a significant variability with the phenotypes observed in BOS individuals carrying *ASXL1* truncating mutations. Asxl1$^{-/-}$ mice present multiple developmental abnormalities, including anophthalmia , 30% reduction in body and skull size, resembling the microcephaly and changes in cranial shape observed in BOS individuals, as well as cleft palate and mandibular malformations (Zhang *et al*., 2016). This reinforces the role of Asxl1 as an essential protein needed for multi-lineage differentiation potential such as osteogenesis, adipogenesis and further skeletal and body development and potentially explain both the skeletal and brain asymmetries that we observed. Our patient was initially evaluated for the combination of her facial *nevus flammeus*, abnormal facial asymmetry (left > right), failure to thrive and global developmental delay and at that point a segmental overgrowth disorder associated to midline capillary malformation was also suspected as part of the differential diagnosis for her condition.

Segmental overgrowth anomalies associated to midline capillary malformation enlarge the clinical spectrum of BOS at onset and further expand the differential diagnosis in *ASXL1* mutation carriers, highlighting a possible involvement of chromatin remodeling and epigenetic regulation in the developmental and skeletal defects associated with BOS.

Mutations in other chromatin remodeling genes such as *DNMT3A* have been linked with developmental disorders associated to segmental overgrowth disorders, such as PIK3CA related phenotypes. Future studies are needed to fully assess potential epigenetic changes due to mutations in the above genes and their impact on molecular pathways associated with segmental overgrowth disorders associated with capillary anomalies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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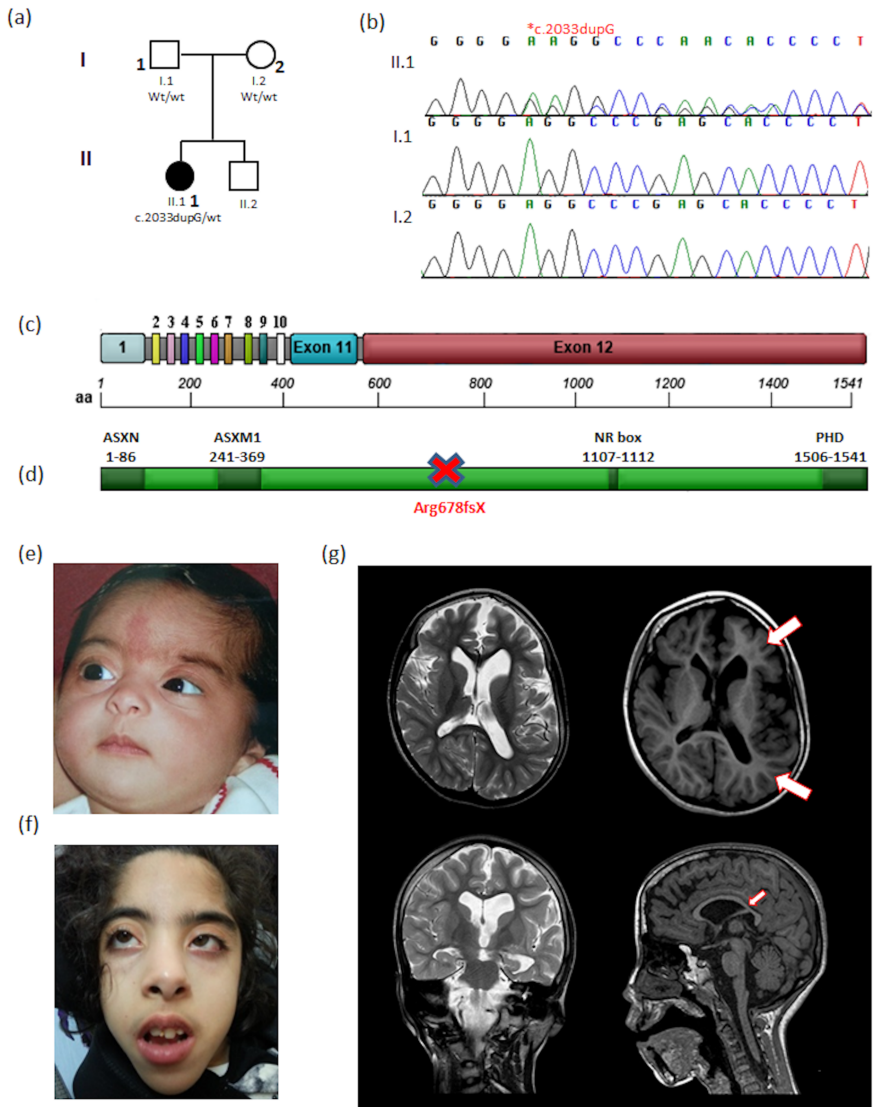

Figure 1 (a) The pedigree diagram of the family with an *ASXL1* mutation. (b) Individual results of Sanger sequencing showing the proband (II.1) carrying the heterozygous *ASXL1* truncating mutation (c.2033dupG, p.R678fs), while being absent in the two parents I.1 and I.2 (c) Representation of *ASXL1* gene showing the amino acid count. (d) representation of the protein domains; ASXN, conserved domain at the N-terminus; ASXM, conserved domain in the middle part; NR, nuclear receptor; PHD, plant homeodomain, and the position of the frameshift mutation in *ASXL1* (c.2033dupG, p.R678fs) as indicated. (e) An early and (f) late childhood portrait photo showing n*evus flammeus* on the forehead of the proband, and facial dysmorphis

typical of BOS syndrome (g) Axial and coronal T2-weighted images (wi) (top left, bottom left) and axial T1-wi (top right) show the asymmetry of the left hemisphere due to reduced white matter volume and consequent enlargement of the ipsilateral lateral ventricle and sulci. Midline sagittal T1-wi (bottom right) shows the thinned appearance of the corpus callosum and no features of hydrocephalus.

Video 1. The video shows the young girl carrying the p.R678fs*6 mutation in *ASXL1* with global developmental delay, absent speech and severe motor impairment, glabellar *nevus flammeus* and trigonocephaly with distinctive craniofacial features.

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Author	Year	Cases	Mutations
Urreizti *et al.*	2018	1 case	p.G646W*fs**12
Bedoukian *et al.*	2018	1 case	**p.R965***
Carlston *et al.*	2017	1 case	**p.R404***
Arunachal *et al.*	2016	1 case	p.G680R*fs**38
Dangiolo *et al.*	2015	1 case	p.F1373*fs*
Russell *et al.*	2015	8 cases	p.C672W*fs**4, p.G1026fs, p.G642*, p.L775*, p.I1919fs, p.Y425Q*fs**12, **p.S846*fs**5**
Urreizti *et al.*	2015	1 case	p.P701S*fs**16
Magini *et al.*	2012	2 cases	p.E803T*fs**17, **p.R965***
Hoischen *et al.*	2011	7 cases	**p. R404*,** p.Q733*, p.Q778*, p.L823*, **p.S846Q*fs**5,** p.Q925*, p.S1028*

Table 1. Previously reported cases of children which have been clinically diagnosed with BOS and also genetically confirmed with *ASXL1* mutations. The three most common mutations are shown in bold.