

RESEARCH HIGHLIGHT

# Expanding our knowledge of conditions associated with the *ASXL* gene family

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See research article: <http://genomemedicine.com/content/5/2/11>

## Abstract

Genome-wide sequencing has identified *de novo* truncating mutations in *ASXL3* in four patients with intellectual disability, feeding problems and distinctive facial features. Their presentation resembles that of Bohring-Opitz syndrome, which is associated with *de novo* nonsense mutations in *ASXL1*. This newly defined phenotype provides an important clinical resource for comparison with future cases in which mutations are found in *ASXL3*. The phenotypes for patients with mutations in each gene will undoubtedly be further delineated as more patients are reported.

## Genome-wide sequencing strikes again

Dysmorphologists and clinical geneticists have worked over the past 40 years to define clinical syndromes by their similar facial features, physical attributes and clinical course. Cytogenomic and molecular testing have helped to define the underlying genetic etiology for many of these syndromes and clarified why related genes in the same pathway sometimes lead to similar clinical phenotypes. Now, with the advent of genome-wide sequencing, dysmorphologists have a powerful new tool at their disposal. Through sequencing, clinicians and researchers are able to study a cohort of patients with mutations in the same gene and to characterize further an identifiable syndrome. Once published, other similar cases can be tested, and gene variants discovered through whole-genome or exome sequencing can be compared with the previously published phenotype to define the relevant clinical features further.

This exciting story is repeated with the new report by Bainbridge *et al.* in *Genome Medicine* [1], which describes

the discovery of *de novo* truncating mutations in *ASXL3* in four patients with similar clinical presentations. The first two patients were identified through exome sequencing of the proband and both parents. The subsequent two cases were found through sequencing of the affected child only. A custom exon-capture strategy was used, followed by high-throughput sequencing with the Illumina HiSeq platform. All coding and near-intronic differences between the patients and the human reference genome were evaluated. The unaffected family members in the first two cases were used as controls to localize only *de novo* changes in the probands. *De novo* mutations were defined as those present in a patient but absent from all other members of their family, flagged as 'high quality', and supported by at least five reads with an allele percentage of 0.4 to 0.6%. The four *de novo* mutations that were found coded for premature stop codons and probably create a truncated *ASXL3* protein. The mutations in each case were previously unreported and included insertions and deletions, which likely represent loss-of-function alleles, given that the mRNAs containing these stop codons are degraded by nonsense-mediated decay [1].

After identifying mutations in *ASXL3* in these four patients, Bainbridge *et al.* used several large datasets to look for previously reported *ASXL3* mutations. They found four other truncating mutations in *ASXL3* in phenotypically normal patients. Three of these mutations were located in the 3' end of *ASXL3* [1], bringing into question whether these *ASXL3* mutations are benign variants. The authors developed a model to determine the probability of observing multiple *de novo* mutations in *ASXL3* in their four phenotypically similar patients. The results of their analysis show that this probability is approximately  $4.0 \times 10^{-17}$ . This makes it highly unlikely that these changes occurred by chance, but functional studies are still required to confirm the pathological nature of these *ASXL3* mutations [1].

## Defining *ASXL*

The additional sex combs (*Asx*) gene has long been known to maintain homeotic gene activation and silencing in

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*Drosophila*, and three orthologs (*Asxl1*, *Asxl2*, *Asxl3*) are recognized in mice. *ASXL1* is known to act on homeobox (Hox) genes as a repressor as well as an enhancer, and is important for development. Not much is known about the developmental function of the *ASXL* gene family in humans, but in *Drosophila*, *Asx* regulation is highly variable and tightly controlled directly after fertilization [2]. In humans, *ASXL3*, like *ASXL1* and *ASXL2*, codes for a putative polycomb protein that probably forms a complex with other proteins and acts as a histone methyltransferase [3].

*De novo ASXL1* mutations have been identified in 9 out of 15 published cases of Bohring-Opitz syndrome (BOS) in which they were tested [4,5]. Somatic *ASXL1* mutations have also been linked to myeloid malignancies and myelodysplastic syndromes [6]. Following the example of other gene networks, such as the RAS signaling pathway genes and the RTK-P13K-AKT signaling pathway genes, it is plausible that somatic *ASXL1* mutations could cause cancer, while germline mutations lead to developmental disorders [4,7,8]. To date, there have been two published cases of a neoplastic condition in BOS: a child with a medulloblastoma at 5 years old [9] and bilateral nephroblastomatosis found at autopsy in a 5-month-old infant [10]. Both patients were negative for mutations in *ASXL1* and *ASXL3* [1,4]. It remains to be seen if patients with *ASXL1* or *ASXL3* mutations require tumor surveillance.

### Patients with *ASXL3* mutations versus *ASXL1* mutations

Clinically, the four patients identified by Bainbridge *et al.* with *de novo ASXL3* mutations presented with several features similar to BOS (Table 1). BOS is associated with high infant mortality, intrauterine growth retardation (89%), feeding difficulties often requiring G-tubes and leading to failure to thrive (100%), severe to profound developmental delays (100%), recurrent infections (62%), nonspecific brain abnormalities (78%), microcephaly (100%), wide prominent forehead (89%), deep palmar creases (57%) and a characteristic posturing known as BOS posture (100%) (percentages are based on the nine published *ASXL1*-mutation-positive cases). BOS posture is defined as flexion at the elbows with ulnar deviation, and flexion of the wrists and metacarpophalangeal joints. BOS patients also have distinctive facial features (Table 1) that include nevus flammeus typically over the glabella and eye lids (89%), facial hypotonia, prominent globes (100%) with high myopia (87%), hypertelorism (55%), up-slanting palpebral fissures (67%), depressed nasal bridge (50%), anteverted nares (50%), broad alveolar ridges or high narrow palate (87%), micro/retrognathia (89%), low-set posteriorly rotated ears (67%), a low posterior hairline (67%) and hypertrichosis (89%) [4,5].

**Table 1. Comparison of clinical features among patients with *ASXL1* and *ASXL3* mutations**

Features	Patients with <i>ASXL1</i> mutations (%)	Patients with <i>ASXL3</i> mutations (%)
<b>Clinical</b>		
Feeding difficulties	8/8 (100)	3/4 (75)
Severe/profound ID	8/8 (100)	4/4 (100)
IUGR	8/9 (89)	3/4 (75)
Recurrent infections	5/8 (62)	-
Seizures	5/8 (62)	-
Apnea	4/8 (50)	-
<b>Craniofacial</b>		
Prominent eyes	9/9 (100)	2/4 (50)
Myopia	7/8 (87)	-
Retinal/optic-nerve abnormalities	5/8 (62)	-
Strabismus	4/8 (50)	-
Ocular hypertelorism	5/9 (55)	2/4 (50)
Flammeus nevus	8/9 (89)	-
Arched, thin eyebrows	-	3/4 (75)
Broad, prominent forehead	8/9 (89)	-
Microcephaly	9/9 (100)	3/4 (75)
Micro/retrognathia	8/9 (89)	1/4 (25)
Depressed nasal bridge	4/8 (50)	2/4 (50)
Anteverted nares	4/8 (50)	3/4 (75)
Low-set posteriorly rotated ears	6/9 (67)	3/4 (75)
Upslanting palpebral fissures	6/9 (67)	-
Broad alveolar ridges/high narrow palate	7/8 (87)	3/4 (75)
Cleft palate	3/9 (33)	-
<b>Hair/skin</b>		
Low posterior hairline	6/9 (67)	-
Hypertrichosis	8/9 (89)	1/4 (25)
Deep palm creases	5/9 (55)	4/4 (100)
<b>Neurological/skeletal</b>		
BOS posture	9/9 (100)	-
Ulnar hand deviation	-	3/4 (75)
Brain abnormalities	7/9 (78)	1/4 (25)
Fixed contractures	8/9 (89)	-
Congenital dislocations	6/9 (67)	-
Hypotonia	7/9 (78)	2/4 (50)
<b>Other</b>		
Cardiac abnormalities	3/9 (33)	-
Genital abnormalities	3/9 (33)	1/4 (25)
Renal abnormalities	2/9 (22)	-

BOS, Bohring-Opitz syndrome; ID, intellectual disabilities; IUGR, intrauterine growth restriction. The first column includes the seven patients reported in [4] and the two reported in [5]. The second column includes the four cases reported in [1]. This table is adapted from [5]. When a specific feature is not reported, the patient is not considered in the calculation.

Although all four subjects discussed by Bainbridge *et al.* shared clinical characteristics with each other and with BOS, these features are relatively nonspecific. The patients with an *ASXL3* mutation had intrauterine

growth retardation (3/4), severe feeding difficulties present from birth and requiring intervention (3/4), severe developmental delays (4/4), microcephaly (3/4), deep palmar creases (4/4), slight ulnar deviation of the hands (3/4) and a high-arched palate (3/4). These patients with *ASXL3* mutations did not display many of the key features of BOS, including BOS posturing, myopia, nevus flammeus and a distinctive constellation of facial features. As with BOS, this novel syndrome is likely to be clinically heterogeneous. Not enough cases have been identified yet to define completely the clinical features or the facial appearance of this syndrome. Over time, as more cases are reported, this may become more evident.

### Where do we go from here?

This intriguing new finding by Bainbridge *et al.*, using genome-wide sequencing to identify a novel syndrome caused by *de novo* mutations in *ASXL3*, has added significantly to our knowledge of conditions associated with the *ASXL* gene family. As recently as 2011, the first association of mutations in the *ASXL* gene family was reported (*ASXL1* causing BOS), and previous knowledge about the association of these genes with cancers in adults and with early development in mice and *Drosophila* may shed light on their role in developmental conditions. Because developmental disorders caused by mutations in the *ASXL* gene family in humans are still quite rare and are associated with high infant mortality, it is difficult to predict cancer risks. To delineate the syndromes caused by *ASXL* mutations, it would be reasonable to test other patients with phenotypic similarities to BOS for *ASXL* gene family mutations. Patients described by Hoischen *et al.* in 2011 who were negative for *ASXL1* mutations were also found to be negative for *ASXL3* mutations [1,4]. Undoubtedly, the publication of this article will lead to further reporting of patients with mutations in the *ASXL* gene family, and the clinical features of this *ASXL3* mutation syndrome will become further defined. In the meantime, whole-genome sequencing will continue to provide insight into novel mutations causing previously undefined clinical conditions.

#### List of abbreviations used

BOS, Bohring-Opitz syndrome.

#### Competing interests

The authors declare that they have no competing interests.

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