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From Next Generation Sequence to the Phenotype: Exploring the Bainbridge-Ropers Syndrome with Loss of Function Variants in *ASXL3*

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Abstract

In 2013, Bainbridge-Ropers syndrome (MIM #615485) was described in patients with severe global developmental delay, postnatal microcephaly and feeding problems due to heterozygous loss of function variants in the *ASXL3* gene. The *ASXL3* is part of the ASXL gene family involved in gene expression during embryogenesis and they participate as epigenetic scaffolds capable of interacting with complex modifiers of chromatin and diverse transcription factors. Germline variants in *ASXL1*, *ASXL2* and *ASXL3* have been associated with neurodevelopmental disorders which clinical phenotypic presentation resembles to Bainbridge-Ropers syndrome thus elucidating these types of overlapping genetic disorders is challenging. Up to now, approximately forty patients have been confirmed with this syndrome by next generation sequencing. The implementation of whole exome sequencing allows early identification and definitive diagnosis of patients with clinically unestablished phenotypes, as seen in *AXL3* gene. This review discusses clinical and molecular features of variants in *AXL3* gene associated with Bainbridge-Ropers syndrome.

Introduction

Neurodevelopmental disorders without specific phenotype are highly undiagnosed, fortunately, next-generating sequencing (NGS) platforms are now able to confirm clinical phenotypes that in the past remained unknown[1]. These types of technologies have allowed for the discovery of new genes and new associations with diseases. In 2011, Mathew Bainbridge, a computational biologist, identified an abnormal copy of the *ASXL3* gene in a patient who, after multiple genetic tests remained without diagnosis. After collaboration between Bainbridge and other experts in NGS, three additional individuals with variants in *ASXL3* gene associated with neurodevelopment disease were found. Finally, in 2013 Bainbridge and colleagues reported *ASXL3* variants in four patients with similar clinical features to Bohring-Opitz syndrome (MIM *612990, BOS) without the classic posture. Global development delay, postnatal microcephaly and feeding difficulties with heterozygous loss of function mutations in *ASXL3* gene (MIM *615115) were detected by whole-exome sequencing (WES) and whole genome sequencing (WGS)[2]. These findings have led to the identification of nearly forty patients in large scale NGS project (Deciphering Developmental Disorders Study, www.ddduk.org)[3], cohorts of clinical exome (CES)[4,5] or isolated cases of nonspecific developmental delay[6,7] which required WES or CES as molecular approaches.

Additional Sex Combs-Like Family (ASXL)

The *ASXL3* gene is part of the ASXL (Additional Sex Combs-Like)

family in vertebrates (*ASXL1, ASXL2* and *ASXL3* genes) that encodes regulatory proteins of the trithorax and polycomb enhancers. The ASXL modulates the expression of homeotic genes during embryogenesis and functions as epigenetic scaffolds capable of interacting with complex modifiers of chromatin and diverse transcription factors[8]. *ASXL1, ASXL2* and *ASXL3* genes present common domains (ASXN, ASXH, ASXM1, ASXM2 and one PHD-finger) with thirteen exons and twelve introns[9]. The *ASXL1*, *ASXL2* and *ASXL3* genes express ubiquitously but, *ASXL3* is predominantly expressed in the brain. These genes are involved in hereditary neurological disorders and the somatic presentation is associated with different types of cancers[8]. As more patients are described, it will be possible to determine if there is an increased risk of developing cancer in constitutional carriers.

Previously, studies have shown that *ASXL1* gene is primarily involved in transcriptional activation and repression. In addition, somatic nonsense and frameshift variants have been frequently described in myelodysplastic syndrome and other hematological malignancies since, *ASXL1* is essential for erythroid development and differentiation[10]. Germline *de novo ASXL1* variants in PHD and ASXM2 domains have shown to cause BOS[9,11].

Dominant-negative effect variants in *ASXL2* gene have recently been linked to neurodevelopmental disorder (Shashi-Pena syndrome; MIM #617190, SHAPNS), as presented in six unrelated probands with *de novo* truncating variants. Reported patients demonstrated common clinical characteristics including delayed psychomotor development, variable intellectual disability, macrocephaly, prominent eyes, arched eyebrows, hypertelorism, glabellar nevus flammeus and hypotonia[12].

Furthermore, pathogenic truncating *de novo* loss of- function[4] variants in *ASXL3* gene are associated with Bainbridge-Ropers syndrome (BRS). Frameshifts included 62% of the variants described, nonsense were reported in 34% and one report a splicing variant was identified (0.03%)[6] (Table 1). These types of changes are consistent with the haploinsufficiency mechanism as proposed previously[4]. Missense variants were also reported in *ASXL3* associated within the autistic spectrum[13,14]. Three variants presented recurrence in BRS. The c.3106C>T(p. Arg1036*)[4,14,15], present in both BRS and in an individual with autism spectrum disorder[16]. The c.4330C>T(p. Arg1444*)[3,5] and the c.3039+1G>A[6,15] which were described in unrelated patients (Table 1). In 2013, Bainbridge and colleagues generated the polarity hypothesis in relation to the location of the mutations associated with BRS. This suggested that truncated variants, which occur at end 5´of the gene, are associated with a more severe phenotype. Nonetheless, this hypothesis was eliminated [5,6,7] after accumulated evidence was examined. Despite the fact that the majority of the mutations are located on the largest exon, as in *ASXL1*[17], to date, two clusters of variants have been proposed both equally affected (~43%) (Table 1). One in the N-terminal region located between the codons 404 to 659 and another in the C-terminal (functional domain of chromatin-DNA recognition) between the codons 1045 to 1444[6,17]. Additionally, transcriptomes of fibroblasts from patients with BRS demonstrated >500 genes involved in transcriptional regulation, development and proliferation of the differentially expressed genes, establishing that BRS is associated with defects in transcriptional regulation [3,5].

Of the 32 variants described in Table 1, ~90% were detected by WES. This was likely due to a higher diagnostic yield compared to CES or other NGS gene panels for neurodevelopmental disorders[21]. Based on these findings, it is necessary to include the ASXL gene family in NGS analysis in patients with subtle dysmorphism and psychomotor delay.

Recently, a compound heterozygous patient with BRS like features and primary IGF1 deficiency which presented the genotype c.(2965C> G);(3078G> C) was reported. It was proposed that the additively effect of both changes impacted in *ASXL3* gene function. Although, *in silico* analysis suggests that the variant c.2965C>G was responsible for the loss of function. Functional studies will be necessary to confirm the real pathogenicity of the c.2965C>G rare variant[22].

Clinical features

To date, fewer than 40 patients with variants in *ASXL3* with the BRS phenotype have been reported [3,4,5, 6,7,12, 13, 14, 5, 19]. Usually, BRS presents as *de novo* truncating mutations in all patients with the exception of two siblings with the same mutation, which was not present in their parents. Germline mosaicism was not ruled out in this case. BRS patients are described from the prenatal stage[18] to the age of 47[20] with consistent phenotype features such as facial dysmorphisms with a broad and prominent forehead, arched eyebrows, downslanting palpebral fissures and anteverted or hypoplastic nares. These characteristics are shown to be present in less than 50% of the patients described. The principal manifestations are those related with neurological development. Global development delay, intellectual disability, from moderate to severe, late or absent language skills and hypotonia are present in more than 80% of the affected individuals. Feeding difficulties are also prevalent among these patients and more frequent during the neonatal period. [2,3,5,] (Table 2).

Both BOS and SHAPNS show intellectual disability and neurodevelopmental delay as a main characteristic. However, development delay is mild in SHAPNS compared to the others[2,4,13]. BOS was first described in patients with feeding problems with primary or secondary microcephaly and severe neurodevelopmental delay. “BOS posture” consistent

Table 1. Loss of function variants associated with BRS phenotype.

NM_030632.2	NP_085135.1	Cluster	BRS Phenotype	Reference
Nonsense Variants n=11/32 (34%)				
c.1074T>A	p.Tyr358*	N	+	[3]
c.1210C>T	p.Gln404*	N	+	[2]
c.1369G>T	p.Glu457*	N	+	[4]
c.1396C>T	p.Gln466*	N	+	2
c.1783C>T	p.Gln595*	N	+	[3]
c.3106C>T	p.Arg1036*		+	[4,14,15]
c.3364C>T	p.Gln1122*	C	+	5
c. 3613G>T	p.Glu1205*	C	+	[4]
c.3635T>G	p.Leu1212*	C	+	[3]
c.4144C>T	p.Gln1382*	C	+	[3]
c.4330C>T	p.Arg1444*	C	+	[3,5]
Indels (Frameshift Variants) n=20/32 (62%)				
c.1082dup	p.Leu362Alafs*23		+	[3]
c.1201del	p.Ala401Glnfs*8		+	[3]
c.1219delA	p.Ser407Alafs*2	N	+	[4]
c.1314_1316delinsA	p. Ser439Argfs*7	N	+ and hyperventilation induced athetosis	[8]
c.1318dup	p.Glu440Glyfs*7	N	+ and pontocerebelar hypoplasia	[18]
c.1422dup	p.Pro475*	N	+	[2]
c.1448dupT	p.Thr484Asnfs*5	N	+	[5]
c.1484insTGAA	p.Asp497*	N	+	[3]
c.1491dup	p.Asn498*	N	+	[3]
c.1897_1898delCA	p.Gln633Valfs*13	N	+ and trigonocephaly	[19]
c.1978_1981delGACA	p.Asp660Asnfs*1		+	[2]
c.2992_2995del	p.Glu998Lysfs*26		+	[7]
c.3028delC	p.Pro1010Leufs*14	C	+ and prominence of the Sylvian Fissure	[13]
c.3127_3128dup	p.Gly1045Valfs*99	C	+	[3]
c.3178dup	p.Arg1060Profs*50	C	+	[3]
c.3313_3316delCAGA	p.Thr1106Argfs*36	C	+	[15]
c.3355dup	p.His1119Profs*7	C	+	[3]
c.3494_3495delGT	p.Cys1165*	C	+	[4]
c.4072_4073delGT	p.Val1358Leufs*8	C	+	[4]
c.6697_6710dup	p.Ser2238Thrfs*3		+	[20]
Splicing 1/32 (0.03%)				
c.3039+1G>A	?	C	+	[6,15]

to exrotation and/or adduction of the shoulders, flexion at the elbows, flexion at the wrists, and ulnar deviation of the wrists and/or fingers at the metacarpophalangeal joints, was described in those patients. This phenotype was associated to *ASXL1* gene variants and was classified as a distinct condition from BRS, based on the absence of BOS posture in patients with *ASXL3* gene variants[23,24]. SHAPNS is described as a neurological disease caused by *ASXL2* gene variants. The overlapping features are identified by global developmental delay and feeding problems. But, macrocephaly instead of microcephaly seems to be SHAPNS most distinctive clinical sign[12].

Conclusion

The relevance of the discovery of BRS and the diagnostic route lies in the reversibility of the daily routine of the patient with genetic conditions[2], given that the majority of these patients have been diagnosed through molecular findings, which subsequently allowed them to be grouped in a similar phenotype. Clinical differences within the ASXL were identified between the *ASXL1*, *ASXL2* and *AXL3* genes phenotype. Differences in epigenetic regulating function factors that contribute to phenotype and temporality of the presentation of gene variants (germinal or somatic) could play an important role[14]. Primarily, described *ASXL3* gene phenotypic variants have been classified as *de novo*. However, findings support a <1% possibility of recurrency in germline mosaicism respectively.

Five years after the initial BRS description, it has

Table 2. Clinical characteristic of 36 patients with variants in *ASXL3* compared with phenotype reported in pathological variants in *ASXL2* and *ASXL1* in the literature.

Phenotype	*ASXL3* (n=36)	*ASXL2* (n=6)[12]	*ASXL1* (n=7)[17]
Age	Prenatal-47 years	11 months – 31 years	Neonatal-14 years
Neurological			
Global developmental delay/Intellectual disability	100%	100%	100%
Late/absent language skill	92%	33%	NR
Hypotonia	83%	100%*	21%
Feeding difficulties	75%	100%	100%
Autism spectrum disorder/Autism	47%	NR	NR
Seizures	39%	83%	50%
Brain abnormalities		83%	71%
Craneofacial features.			
Microcephaly	36%	0	100%
Macrocephaly	0	100%	0
Downslanting palpebral fissures	50%	0	0
Broad/prominent forehead	42%	16% (1)	0
Glabellar nevus flammeus	0	100%	86%
Arched eyebrows	31%	100%	0
Low-set/ posteriorly rotated ears	22%	100%	43%
Anteverted nares/hypoplastic nares	22%	16% (1)	29%
Ocular hypertelorism	19%	100%	57%
Upslanting palpebral fissures	8%	0	57%
Systemic features			
Ulnar deviation	17%	0	0
Hypertrichosis	16%	16% (1)	86%
BOS posture	0	0	100%
Others			
	Neonatal macrosomy in one patient	Structural cardiopathy, hypoglicemia	Atresia choanal, atrial septal defect, hepatomegaly, thoracolumbar scoliosis, ocular abnormalities

*In one case limb hypertonia was described. NR: Not reported

been possible to provide answers to an ever-increasing number of families thanks to the significant achievement of obtaining a definite genetic diagnosis. The phenotypic definition associated with the *ASXL* gene family presents the challenge of the importance to use massive sequencing analysis in order to obtain more timely diagnosis associated with neurodevelopmental disorders.

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