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Understanding the phenotypic spectrum of *ASXL*-related disease: 10 cases and a review of the literature

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Abstract

Over the past decade, pathogenic variants in all members of the *ASXL* family of genes, *ASXL1*, *ASXL2*, and *ASXL3*, have been found to lead to clinically distinct but overlapping syndromes. Bohring-Opitz Syndrome (BOPS) was first described as a clinical syndrome and later found to be associated with pathogenic variants in *ASXL1*. This syndrome is characterized by developmental delay, microcephaly, characteristic facies, hypotonia, and feeding difficulties. Subsequently, pathogenic variants in *ASXL2* were found to lead to Shashi-Pena Syndrome (SHAPNS) and in *ASXL3* to lead to Bainbridge-Ropers Syndrome (BRPS). While SHAPNS and BRPS share many core features with BOPS, there also seem to be emerging clear differences. Here, we present 5 cases of BOPS, 1 case of SHAPNS, and 4 cases of BRPS. By adding our cohort to the limited number of previously published patients, we review the overlapping features of *ASXL*-related diseases that bind them together, while focusing on the characteristics that make

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Authorship

Each of the co-authors made substantial contributions in developing the phenotype of the described individuals, were involved in drafting or revising the manuscript, gave final approval for the publication of this manuscript, and agree to be accountable for this work.

Conflict of Interest

The authors declare that they have no competing interests.

each neurodevelopmental syndrome unique. This will assist in diagnosis of these overlapping conditions and allow clinicians to more comprehensively counsel affected families.

Keywords

ASXL; ASXL1; ASXL2; ASXL3; Bohring-Opitz Syndrome; Shashi-Pena Syndrome; Bainbridge-Ropers Syndrome

1. Introduction

Over the past 10 years, pathogenic variants in the additional sex combs-like (*ASXL)* genes have been found to lead to distinct neurodevelopmental syndromes (Bainbridge, et al., 2013; Shashi, et al., 2017; Hoischen, et al., 2011). The *ASXL* family is comprised of three genes in humans: *ASXL1*, *ASXL2*, and *ASXL3*. While these genes seem to have similar functions, and pathogenic variants cause similar clinical features, distinct features of each syndrome are starting to emerge. Here, we present 10 cases and focus on unique characteristics of each condition.

The *ASXL* family of genes are all involved in epigenetic and transcriptional regulation (reviewed in (Katoh, 2013)). ASXL1 and ASXL2 can bind to polycomb repressive complex 2 to promote histone methylation (Lai and Wang, 2013; Abdel-Wahab, et al., 2012). ASXL1, ASXL2, and ASXL3 promote histone deubiquitination through interaction with BRCA-1-associated protein 1 (Sahtoe, et al., 2016; Daou, et al., 2015; Srivastava, A., et al., 2016). In addition, ASXL2 has been found to promote histone deacetylation (Li, et al., 2017). Somatic pathogenic variants in *ASXL1* and *ASXL2* can lead to hematological cancers (Wang, et al., 2014; Li, et al., 2017), but this is less common with *ASXL3* variants (Oak and Ohgami, 2017; Duployez, et al., 2016). While there is significant interest in the oncological role of the ASXL genes, this work focuses on germ-line pathogenic variants that have been recently described to lead to distinct neurodevelopmental disorders.

Germ-line pathogenic variants in *ASXL1* are associated with Bohring-Opitz Syndrome (BOPS). BOPS was defined in 1999 as a distinct clinical syndrome characterized by microcephaly, frontal bulging/trigonocephaly, glabellar nevus flammeus, upslanted palpebral fissures, prominent eyes (exophthalmos), cleft lip and palate, thick hair, feeding difficulties, and flexion deformities of the upper limbs (Bohring, et al., 1999). The flexion deformities lead to the “Bohring-Opitz Syndrome posture” that is clinically recognizable. Neurological manifestations include completely penetrant intellectual disability, as well as epilepsy, contractures, and bulbar dysfunction to varying degrees (Bohring, et al., 2006). Individuals with BOPS may also present with agenesis of the corpus callosum, Dandy-Walker malformation, and ventriculomegaly (Bohring, et al., 2006). In 2011, BOPS was found to be due to *de novo* pathogenic variants in *ASXL1* in 7 of 13 tested individuals, suggesting that clinically-recognized BOPS is not a monogenic disease (Hoischen, et al., 2011), though other monogenic causes have yet to be identified.

The growing accessibility of whole-exome sequencing led to the discovery that pathogenic variants in *ASXL2* lead to a syndrome that manifests as macrocephaly, prominent eyes,

arched eyebrows, hypertelorism, glabellar nevus flammeus, neonatal feeding difficulties, hypotonia, variable intellectual disability, and possible hypoglycemia, now known as Shashi-Pena Syndrome (SHAPNS) (Shashi, et al., 2017). MR imaging of the brain demonstrates cerebral atrophy (Shashi, et al., 2017). While developmental delay was universal, the degree of intellectual disability later was variable, and growth parameters were not markedly affected (Shashi, et al., 2017).

Pathogenic variants in the final member of the *ASXL* family, *ASXL3*, lead to Bainbridge-Ropers Syndrome (BRPS). BRPS is characterized by severe feeding difficulties, severe developmental delay and intellectual disability, poor expressive speech development, microcephaly, arched eyebrows, prominent forehead, hypertelorism with downslanted palpebral fissures, and hypotonia (Bainbridge, et al., 2013; Balasubramanian, et al., 2017). These children commonly have autistic features with hand-flapping and rocking behaviors and can sometimes be aggressive (Balasubramanian, et al., 2017). Approximately one-third of these individuals also experience seizures (Myers, et al., 2018). In contrast to BOPS, both BRPS and SHAPNS were only recognized as clinical syndromes after identification of pathogenic variants.

Here, we present 10 individuals with pathogenic variants in the *ASXL* family of genes (9 of these individuals are previously unreported). The clinical phenotypes of these individuals are consistent with the previously described phenotypes typical of each syndrome. Finally, we delve into the phenotypic spectrum of *ASXL* mutations with a focus on unifying and distinguishing features.

2. Case Reports

This study involved a retrospective review of the electronic medical record for the 10 presented individuals (Table 1). The study was reviewed by the Institutional Review Board at the Children's Hospital of Philadelphia and granted an exemption.

2.1 ASXL1

2.1.1 Individual 1—Individual 1 is a male born full-term via vaginal delivery after a high-risk pregnancy requiring bedrest for the first 4.5 months of the pregnancy due to an enlarged ovarian cyst. He weighed between the 25-50th percentile at birth. His development was remarkable for gross motor delay in the setting of severe hypotonia, and physical therapy was initiated within the first year of life. Chromosomal microarray and Prader Willi methylation testing were negative. At 19 months of age, whole exome sequencing (WES) revealed a novel, heterozygous, *de novo* pathogenic variant in *ASXL1* (NM_015338.5:c.1867C>T; p.Q623X). By 3 years of age, he was gaining weight but being managed for sleep apnea, breath-holding spells, behavioral disturbances, and syncopal episodes of unknown etiology. EEG revealed no seizures, but bifrontal spikes were noted to be present. He was able to speak in full sentences, count to 10, and recognize colors and animals. He was receiving physical, occupational, and speech therapy.

2.1.2 Individual 2—Individual 2 is a male born at 39 weeks' gestation, during which fetal ultrasound revealed agenesis of the corpus callosum. After birth, he had feeding

difficulties and was unable to suck and swallow. A G-tube was placed at 3 months of age, and obstructive sleep apnea was identified, requiring continuous positive airway pressure during sleep. He had failure-to-thrive with weight, height, and head circumference all <5 percentile. EEG demonstrated episodes of generalized discharges and subclinical seizures, so he was started on levetiracetam. He had precocious puberty, and further clinical details related to this were not available. At 7 years of age, WES demonstrated a novel, *de novo*, pathogenic frameshift variant in *ASXL1* (NM_015338.5:c.1517_1518delGA). By 10 years of age, his respiratory status was worsening leading to a nearly continuous BiPap requirement. At 12 years of age he was not able to speak any words but was able to make choices with an eye gaze system at school. He was not able to use his hands for pointing or gestures.

2.1.3 Individual 3—Individual 3 is a female born with failure-to-thrive and severe gastroesophageal reflux disease, requiring G-tube with fundoplication by 1 year of age. She had global developmental delay. She experienced her first lifetime seizure at 6 years of age and was started on levetiracetam. She also had sleep difficulties managed with melatonin. Karyotype and chromosomal microarray were normal. At 7 years of age she was noted to have thelarche, pubarche, and body odor and diagnosed with central precocious puberty. WES by 10 years of age revealed a novel, *de novo* pathogenic variant in *ASXL1* (NM_015338.5:c.4060G>T;p.E1354X), as well as a variant of unclear significance in *TTN* associated with noncompaction type cardiomyopathy. The *TTN* variant (c.66716delT;p.L22239X) was maternally inherited, but whether the mother also has cardiac disease remains unclear. At last evaluation at 12 years of age, she continued to have breakthrough seizures requiring transition to valproic acid. She was nonverbal but able to indicate her wishes by shaking her head ‘yes’ and ‘no’. She was able to walk with leg braces. Regarding her fine motor skills, she was not able to use utensils but able to eat with her hands.

2.1.4 Individual 4—Individual 4 is a female born prematurely at 34 weeks’ gestation after a pregnancy complicated by intrauterine growth retardation (IUGR). After birth, her early course was remarkable for gross global developmental delay, respiratory insufficiency due to laryngomalacia, and frequent emesis leading to growth restriction and eventual G-tube placement with Nissen fundoplication. By 5 months of age supraglottoplasty was performed. This did not sufficiently ameliorate her respiratory insufficiency, so tracheostomy with continuous ventilatory support was necessary by 8 months of age. Her examination was remarkable for microcephaly, nevus flammeus over glabella, prominent and upslanted eyes, hypertelorism, anteverted nares, depressed nasal bridge, choanal atresia, high palate, and “BOPS posture” (Figure 1a–b). In addition, pubic hair was noted at birth, and thelarche had occurred prior to 12 months of age. At 12 months of age, she experienced febrile status epilepticus secondary to sepsis with resulting hypoxic-ischemic encephalopathy. Expedited whole exome sequencing at this time revealed a recuring (Magini, et al., 2012), pathogenic variant in *ASXL1* (NM_015338.5:c.2893 C>T;p.R965X). This variant was also noted in the mother, though in a mosaic state in blood with no overt features of BOPS. For her epilepsy, she was initially maintained on levetiracetam, but then transitioned to oxcarbazepine monotherapy for lack of efficacy. By 5 years of age, she was

non-verbal, non-mobile, and unable to use her hands. This patient was previously reported at 3 years of age as the first description of BOPS inherited from an unaffected, germline mosaic parent (Bedoukian, et al., 2018).

2.1.5 Individual 5—Individual 5 is a female born at 38 weeks' gestation after a pregnancy complicated by IUGR. After birth she was transferred to the Intensive Care Unit for management of feeding and respiratory difficulties. She was discharged home by day-of-life 10. By 2 months of age she was having significant feeding difficulty requiring nasogastric tube placement, axial and appendicular hypotonia, and purposeless eye movements. By 3 months of age, this individual was found to have a recurring (Hoischen, et al., 2011), pathogenic variant in the *ASXL1* gene (NM_015338.5:c.2332C>T; p.Gln788X) of unknown inheritance. She presented with epilepsy at 6 months of age and started on levetiracetam and phenobarbital. Seizures were subclinical and focal, originating from the right temporal lobe. She required frequent hospitalizations for recurrent emesis leading to dehydration before 18 months of age. She required close follow up with Gastroenterology due to an inability to tolerate continuous G-tube feeds. She was non-verbal with no purposeful movements. She developed severe obstructive sleep apnea requiring BiPap when sleeping.

2.2 ASXL2

2.2.1 Individual 6—Individual 6 is a male born at 32 weeks' gestation following placental abruption after maternal fever at 30 weeks' gestation. Antenatal ultrasounds were reportedly normal. He spent the first 5 weeks of life in the Intensive Care Unit for respiratory distress, apnea, and hyperbilirubinemia. By 2 months of age, he was noted to have clenched fists with cortical thumbing and episodes of leg shaking concerning for seizures, for which he was started on topiramate and subsequently changed to phenobarbital and lacosamide for treatment of partial epilepsy with secondary generalization. He had normal early growth (length and weight) with progressive fall in BMI. Dysmorphic examination at 10 years, 8 months old was remarkable for mild relative acquired macrocephaly, long face with narrow biparietal diameter, tight sublingual frenulum limiting full tongue extrusion, small vertical chin crease, as well as other details listed in Table 1 (Figure 1c–e). He had global developmental delay, and excessive sleepiness that was partially attributed to phenobarbital. He had truncal weakness and achieved independent walking by 22 months of age. He experienced repeated episodes of ketotic hypoglycemia (lowest glucose 49 mg/dL, confirmed by diagnostic fast with nadir glucose 45 mg/dL and beta-hydroxybutyrate 2.2 mM) leading to repeated hospitalizations. He eventually required gastro-jejunal feeding with total parental nutrition (TPN) supplementation for severe gastrointestinal dysmotility with chronic intestinal pseudo-obstruction. He had kyphosis, and was prescribed IV bisphosphonate therapy for multiple pathological fractures, including a hip fracture at 9 years old after which he could no longer walk (dual-energy x-ray absorptiometry scans did not disclose areal bone mineral density Z-scores <−2.0, but assessments were limited based on instrumentation) as well as a foot fracture. WES performed on a clinical diagnostic basis on his nuclear family in blood at 7 years of age demonstrated he had a *de novo*, novel, likely pathogenic variant in *ASXL2* (NM_018263.4:c.4228T>G;p.C1410G). He was also found to have

mild intellectual disability, neurodevelopmental regression with lost ability to read and write, anxiety disorder, attention deficit hyperactivity disorder, and pervasive developmental delay. Apart from these clinical problems attributable to *ASXL2*-related disease, he also exhibited symptoms of a familial progressive myopathy that also manifest in his mother and younger sister with rhabdomyolysis; progressive spasticity, clonus, progressive resting tremor, and ataxia; chronic respiratory failure (requiring nighttime BiPap); chronic pain; pigmentary retinopathy; mild left optic atrophy identified by optical coherence tomography; autonomic dysfunction characterized by tachycardia, sweating, flushing, and body temperature fluctuations; central adrenal insufficiency treated with glucocorticoid replacement; hepatic microvesicular steatosis; chronic pancreatitis; sleep disorder; immune dysfunction; sideroblastic anemia; bone marrow failure; iron deficiency anemia; and platelet dysfunction. These additional features are not typical of *ASXL2*-related disease and as many of them also occur with variable severity in his affected mother and sister who do not have the *ASXL2* variant appear likely attributable to a second, yet-to be identified, maternally-inherited genetic etiology. Further clinical information is provided in Supplementary Material.

2.3 ASXL3

2.3.1 Individual 7—Individual 7 is a female born full-term after a pregnancy remarkable for oligohydramnios. By 6 months of age, she was noted to have strabismus, axial hypotonia, and failure-to-thrive. Exotropia was later surgically corrected at 1 year of age. Development was grossly delayed; she walked at 34 months of age and was non-verbal at 4 years of age. Physical examination was remarkable for ptosis, downslanted palpebral fissures, bilateral inverted nipples, axial hypotonia, and hand flapping stereotypies (Figure 1f–h). Precocious puberty was also noted, with pubarche occurring at age 8. Thelarche was found to be normal at age 9.5. WES was sent at 8 years of age, revealing a novel, *de novo* pathogenic variant in the *ASXL3* gene (NM_030632.1:c.4322C>G;p.S1441X). At this time, patient was making slow developmental progress and able to communicate with computer software in simple sentences. Seizures began at age 11 characterized by generalized tonic-clonic movements. By this time, she knew all of her letters and numbers, was able to read at a first-grade level, but continued to be non-verbal. She had no developmental regression and was able to walk and run independently. She had ongoing physical, occupational, and speech therapy.

2.3.2 Individual 8—Individual 8 is a male born full-term after an uncomplicated pregnancy. After birth, he began to have difficulty feeding and developed failure-to-thrive, and by 4 months of age, caretakers felt that he was stiff. He then experienced global developmental delay; he started rolling at 10 months of age, and was able to sit by 16 months of age. He developed stereotypies of hand twirling in front of his face and was diagnosed with autism by 2 years of age. By 3 years of age patient was referred for WES revealing a novel, *de novo* pathogenic variant in the *ASXL3* gene (NM_030632.1:c.1895dupC;p.Q633Tfs*14). By 6 years of age, he was non-verbal with limited communication skills. He was unable to feed himself and was placed in special education at school.

2.3.3 Individual 9—Individual 9 is a female born after 39 weeks' gestation. On the first day of life, she had intermittent episodes of duskiness, found to be due to laryngotracheomalacia. By 1 year of life she was noted to have global developmental delay, microcephaly, and failure to thrive. She was able to roll, but not sit independently or vocalize. In the setting of presumed gastroenteritis at age 3, she was found to have a ketotic hypoglycemia to blood glucose of 42. Her blood glucoses were then routinely monitored without recurrent episodes. After fasting for 20 hours, she was also able to mount a normal counter-regulatory hormonal response. Patient was able to walk with the assistance of a walker and say 1 word by 4 years of age. By age 8 routine blood glucose monitoring was discontinued. By 9 years of age WES was performed revealing a recurring (Zhang, et al., 2018), *de novo* pathogenic variant in *ASXL3* (NM_030632.1:c. 3349C>T;p.R1117X). At 10 years of age psychological testing at this time revealed motors skills at 28-month level, communication skills less than 14-month level, and language at 27-month level. By age 13, she had a single unprovoked seizure and was not placed on anti-seizure medications. She continued to be non-verbal but was able to use an electronic speech device. She was able to walk independently.

2.3.4 Individual 10—Individual 10 is a male born at full-term after an uncomplicated pregnancy and delivery. He was noted to have global developmental delay with failure to thrive. Between the age of 2 to 3, patient presented to the Emergency Room twice with altered mental status and was found to have blood glucoses in the 30s, once with no ketones in the urine and the other with small ketones (15 mg/dl) in the urine. His blood glucoses were then monitored at home without recurrent episodes. Patient began walking independently at 3 years of age. By 6 years of age, he knew about 50 words and was able to create two word phrases. WES was obtained by 12 years of age revealing a recurring (Srivastava, S., et al., 2014), *de novo* pathogenic variant in *ASXL3* (NM_030632.1:c.1990C>T;p.Q664X). At age 12 he had moderate intellectual disability; he was able to speak in short phrases, but most speech was not intelligible outside of the immediate family. He was able to read 3-word sentences. His hypotonia improved over time.

3. Discussion

The patients presented here provide further confirmation of the previously described phenotypes attributed to pathogenic variants in *ASXL1*, *ASXL2*, and *ASXL3*, respectively. As the numbers of individuals identified with pathogenic variants within each of these genes grows, the degree of overlap of the clinical phenotypes has become clearer. As depicted in Figure 2, children with pathogenic variants in *ASXL1, ASXL2*, and *ASXL3* share several features including characteristic facial features (arched eyebrows and hypertelorism), feeding difficulties most prominent in early life, hypotonia, and developmental delay. Intellectual disability and epilepsy are also quite common, to variable degrees.

To better understand ASXL-related disease, we reviewed the history of these disorders and their reported clinical overlap. When *ASXL3* pathogenic variants were first described to lead to Bainbridge-Ropers Syndrome (BRPS), overlap was noted with Bohring-Opitz Syndrome (BOPS) of non-specific features including developmental delay, feeding difficulties, and arched eyebrows, but without findings of trigonocephaly and prominent eyes (Bainbridge, et

al., 2013). These similarities were attributed to overlapping regions of expression between ASXL1 and ASXL3 (Bainbridge, et al., 2013). Here, we find that in addition to these core features, patients with *ASXL1* or *ASXL3* pathogenic variants also are more likely to have microcephaly, severe growth impairment, and intellectual disability (Figure 2). Those with *ASXL1* pathogenic variants are more likely to have the "BOPS posture" (2/5 in our cohort). Those with *ASXL3* pathogenic variants tend to have a higher likelihood of having ptosis (2/4 in our cohort) and exhibiting hand flapping and aggressive behaviors (3/4 in out cohort). In our cohort, 4/5 patients with *ASXL1* pathogenic variants (individuals 2-5) had a diagnosis of epilepsy and were on anti-seizure medications as compared to 1/4 of those with *ASXL3* pathogenic variants (Individual 9). This is consistent with previous reports suggesting that epilepsy is not as common in individuals with *ASXL3* pathogenic variants as compared to *ASXL1* (Kuechler, et al., 2017; Srivastava, A., et al., 2016; Hori, et al., 2016; Myers, et al., 2018).

Children with *ASXL2* pathogenic variants leading to Shashi-Pena Syndrome (SHAPNS) share multiple core features that characterize the *ASXL*-family of diseases including arched eyebrows, prominent eyes, hypertelorism, feeding difficulties, and developmental delay (Shashi, et al., 2017). However, these patients can be distinguished by macrocephaly, essentially normal height and weight parameters, and less severe cognitive impairment (Figure 2) as observed in our patient (individual 6). Like children with BOPS, there is an increased likelihood of having seizures (5/6 of previously published patients and 1/1 in our cohort). Our patient (Individual 6) with SHAPNS was also found to have multiple endocrine imbalances including ketotic hypoglycemia, pathological fractures, and adrenal insufficiency. Hypoglycemia has been previously described in 3/6 individuals with *ASXL2* pathogenic variants, though it seemed to be persistent in only 2/6 individuals (Shashi, et al., 2017). In our patient, the etiology of the hypoglycemia was not clear, but given unexpectedly low extent of ketosis for the degree of hypoglycemia, non-specific impaired regulation of insulin signaling may contribute. His ketotic hypoglycemia responded to continuous GJ feeds that were ultimately supplemented then replaced with TPN. Additionally, 2/6 of previously reported individuals (Shashi, et al., 2017) with pathogenic variants in *ASXL2* had bone disease manifest as decreased bone density and multiple fractures, similar to our patient. However, his bone health impairment was more severe, complicated by multiple fractures and requiring ongoing IV bisphosphonate therapy (Individual 6). Mouse models support a role for *ASXL2* in bone metabolism, as mutation in *Asxl2* leads to decreased osteoclast development and lower bone mineral density (Farber, et al., 2011; Izawa, et al., 2015). Despite the known association between pathogenic variants in *ASXL2* and myeloid malignancies in mice and humans (Micol, et al., 2017; Li, et al., 2017), germ-line pathogenic variants in *ASXL2* have not been found to date to lead to cancer in humans although it is possible this may change as the cohort of children with known *ASXL2* disease grow older. Interestingly, our ASXL2 proband also demonstrated multiple clinical problems not reported in other published cases, including neurodevelopmental regression, myopathy, severe GI dysmotility with chronic pseudoobstruction, resting tremor, spasticity and clonus, contractures, progressive ataxia, chronic pain, adrenal insufficiency, pancreatic dysfunction, autonomic dysfunction, rhabdomyolysis, pigmentary retinopathy, optic atrophy, bone marrow and platelet dysfunction, iron deficiency anemia, immune dysfunction, and

microvesicular steatohepatitis. Microvesicular steatohepatitis identified at 25 month of age was attributed to his TPN-dependence. However, a separate genetic cause that has not yet been identified is strongly suspected to underlie these more complex features, which have not been reported in other individuals with SHAPNS but are seen to variable degrees in his maternal family members. In contrast, his *ASXL2* pathogenic variant is *de novo*, and explains his unique developmental disability, partial epilepsy, osteopenia, relative macrocephaly, and hypoglycemia that are not present in his family members.

While hypoglycemia does not seem to be a common feature shared by patients with BRPS (Bainbridge, et al., 2013; Kuechler, et al., 2017; Srivastava, A., et al., 2016; Hori, et al., 2016; Balasubramanian, et al., 2017), 2/4 individuals we present here (Individual 9 and 10) experienced hypoglycemia early in life. Individual 9 had a ketotic hypoglycemia in the setting of likely dehydration, which most likely was an appropriate physiological response. Individual 10 however had an inappropriate ketotic response to profound hypoglycemia early in life. The etiology of this was never confirmed and seems to have improved with age as the patient is now able to fast up to 14 hours overnight without hypoglycemia. Unlike Individual 6 with the pathogenic variant in *ASXL2*, the hypoglycemia in our two patients with pathogenic variants in *ASXL3* improved with age. There is a report of a single child with BRPS with persistent hypoglycemia who presented on day-of-life 1 with hypoglycemia after being born to an insulin-dependent diabetic mother (Dinwiddie, et al., 2013). This child had 2 family members with hypoglycemia in childhood that evolved into insulin-dependent diabetes, while the patient herself had hypoglycemic seizures early in life (Dinwiddie, et al., 2013). This child was found to have a pathogenic variant in *ABCC8* (ATP-Binding Cassette, Subfamily, Member 8) gene, thought to be consistent with a diagnosis of Familial Hyperinsulinemic Hypoglycemia Type 1 and separate from her pathogenic variant in *ASXL3* (Dinwiddie, et al., 2013). While *ASXL2* is thought to play a role in glucose regulation via insulin sensitivity (Izawa, et al., 2015), what role, if any, *ASXL3* plays in glucose metabolism remains unknown.

Further dysfunction along the neuroendocrine axis occurs in individuals with BOPS and pathogenic variants in *ASXL1*. In our newly reported cohort, 3/5 children (Individuals 2, 3, and 4) with pathogenic variants in *ASXL1* had premature onset of puberty. Individual 4 in particular was found to have pubic hair at birth and thelarche before 12 months of age. This is consistent with previous reports of 2 children with BOPS due to pathogenic variants in *ASXL1*, one of whom developed pubarche and thelarche by 10 years of age, and the other developing pubarche at seven months of age and thelarche at seven years of age (Russell, et al., 2015). In addition, 1/4 of our patients with pathogenic variants in *ASXL3* (Individual 7) had premature pubarche. Precocious puberty was not initially described to be a cardinal feature of BOPS; however, since the identification that approximately 50% of those with BOPS have pathogenic variants in *ASXL1* (Hoischen, et al., 2011), it remains possible that precocious puberty may be more likely in individuals with pathogenic variants in *ASXL1* as opposed to those clinically diagnosed with BOPS. The mechanism of premature onset of puberty in this population is unclear, but given that *ASXL1* is an important epigenetic and transcriptional regulator, mutation of this gene likely has pleotropic effects.

As summarized in Figure 2, pathogenic variants in the 3 members of the *ASXL* family of genes lead to distinct but overlapping phenotypes. While BOPS was initially described as a clinical syndrome with about 50% of cases due to a pathogenic variant in *ASXL1*, SHAPNS and BRPS are primarily defined by their underlying genetic etiologies. Over time, it is likely that further published cases of BOPS will be slanted towards those patients with known pathogenic variants in *ASXL1*. Comparing the patients with BOPS who do and do not have known pathogenic variants in *ASXL1* could lead to the identification of subtle differences in phenotype. The BOPS cohort of patients who do not have pathogenic variants in *ASXL1* may have another monogenic etiology yet to be discovered, or more simply, these patients may still have mutations in *ASXL1* that are more difficult to identify, e.g. deep intronic mutations or mutations in regulatory elements. In this era of rapidly improving genetic technologies, the *ASXL* family of disorders continues highlight the distinctions between phenotype-driven and genotype-driven diagnoses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative images of individuals with *ASXL1*, *ASXL2*, and *ASXL3* pathogenic variants. (a-b) Individual 4 with pathogenic variant in *ASXL1* demonstrating frontal prominence, glabellar nevus flammeus, upslanted eyes, hypertelorism, clenched hands with upper extremity hypertonia, and with tracheostomy. (c-e) Individual 6 with pathogenic variant in *ASXL2* demonstrating long face with narrow biparietal diameter, fullness of the superior eyelids, upslanted palpebral fissures, hypertelorism, squared superior portion helix with large lower earlobes, and low columella at 3.5 years of age (c) and 6.5 years of

age (d-e). (f-h) Individual 7 with pathogenic variant in *ASXL3* demonstrating ptosis and downslanted palpebral fissures.

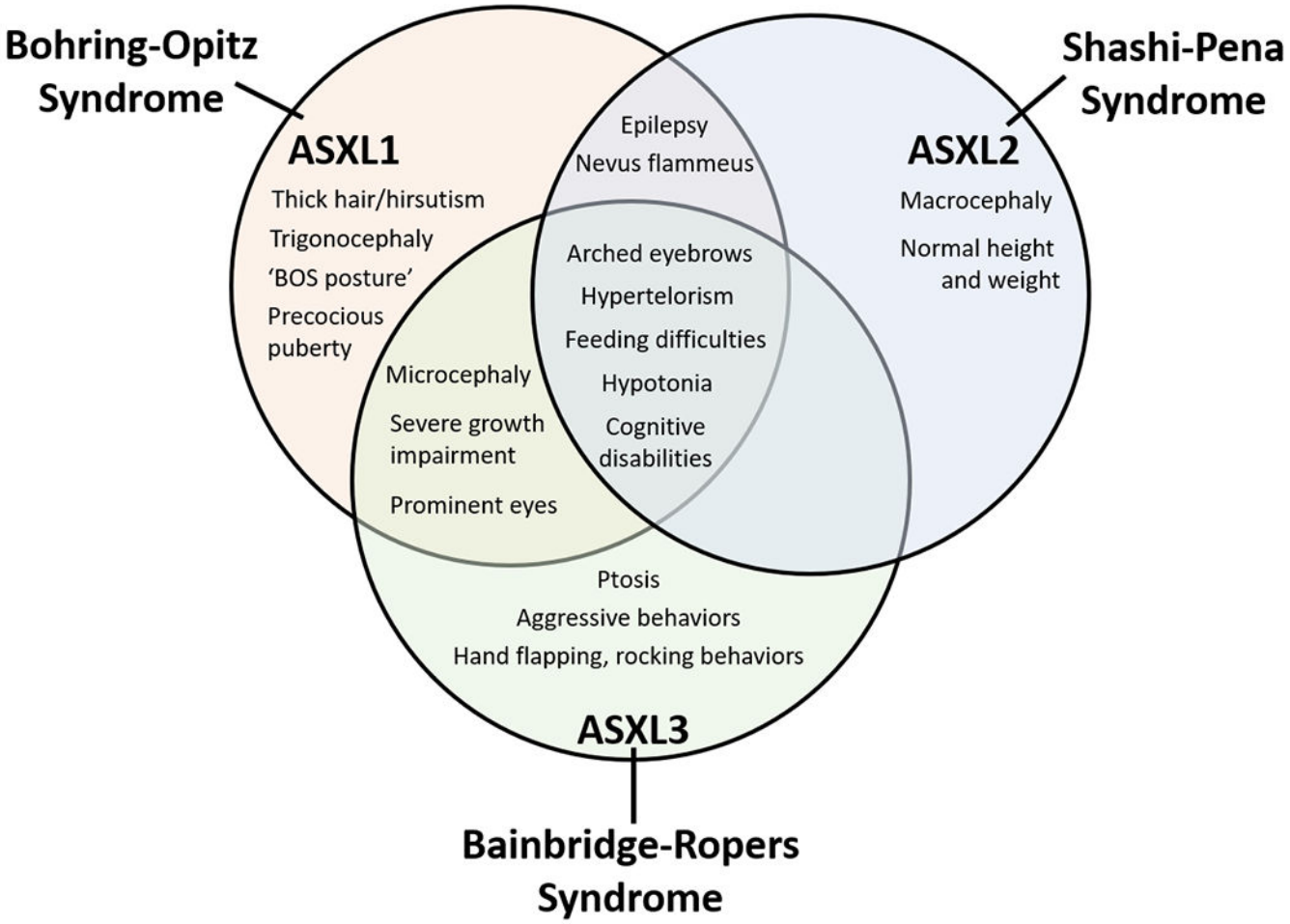

Figure 2.

Bohring-Opitz Syndrome, Shashi-Pena Syndrome, and Bainbridge-Ropers Syndrome have an overlapping phenotype but can also be distinguished by unique features.

Table 1:

Clinical features of 5 individuals with *ASXL1* pathogenic variants, 1 individual with *ASXL2* pathogenic variant, and 4 individuals with *ASXL3* pathogenic variants

Individual/ Gene	Individual 1 ASXL1	Individual 2 ASXL1	Individual 3 ASXL1	Individual 4 ASXL1	Individual 5 ASXL1	Individual 6 ASXL2	Individual 7 ASXL3	Individual 8 ASXL3	Individual 9 ASXL3	Individual 10 ASXL3
Variant	c.1867C>T (p.Q623X)	c.1517_1518del GA	c.4060G>T (p.E1354X)	c.2893 C>T (p.R965X)	c.2332C>T (p.Q788X)	c.4228T>G (p.C1410G)	c.4322C>G (p.S1441X)	c.1895dupC (p.Q633Tfs*14)	c. 3349C>T (p.R1117X)	c. 1990C>T (p.Q664X)
Inheritance	*de novo*	*de novo*	*de novo*	Mother in mosaic state	unknown	*de novo*	*de novo*	*de novo*	*de novo*	*de novo*
Age / Sex	5y / Male	13y / Male	12y / Female	5y / Female	5y / Female	13y / Male	12y / Female	6y / Male	13y / Female	16y / Male
Age at Diagnosis	19 months	7 years	6 years	12 months	3 months	7 years	8 years	3 years	9 years	12 years
Growth										
Microcephaly	− (66th %ile at 5 years)	+ (<0.01 %ile at 12 years)	− (57th %ile at 6 years)	− (3.4 %ile at 26 months)	− (1.5 %ile at 3 years)	−	− (55th %ile at 32 months)	+ (0.23 %ile at 24 months)	+ (1.6 %ile at 25 months)	− (34th %ile at 12 years)
Macrocephaly	−	−	−	−	−	+ (50th %ile at 2 months, 90th %ile at 10 years when height was at 25th %ile)	−	−	−	−
Failure-to-thrive	− (17th %ile at 5 years)	+ (<0.01 %ile at 12 years)	+ (0.14 %ile at 5 years)	+ (0.53 %ile at 26 months)	+ (<0.01 %ile at 3 years)	(90th %ile at 8 years to 7th %ile at 12 years. Improvement to 30th %ile at 13 years)	+ (0.55 %ile at 31 months)	+ (15th %ile at 24 months)	+ (<0.01 %ile at 25 months)	+ (<0.01 %ile at 12 years)
Facial Features										
Frontal prominence				+	+			+		
Nevus flammeus	−	+ (glabella)	+ (forehead)	+						
Ptosis						−	+			+
Upslanted eyes			−	+	−	+	−			−
Downslanted eyes			+	−	+	−	+			+
Eyes, other	Epicanthal folds	−	−	Prominent, hypertelorism	−	Prominent, hypertelorism, fullness of upper eyelid,	ptosis	hypertelorism	−	Ptosis, strabismus

Individual/ Gene	Individual 1 ASXL1	Individual 2 ASXL1	Individual 3 ASXL1	Individual 4 ASXL1	Individual 5 ASXL1	Individual 6 ASXL2	Individual 7 ASXL3	Individual 8 ASXL3	Individual 9 ASXL3	Individual 10 ASXL3
						prominent eyelashes				
Ear malformation		low-set ears			low set, posteriorly rotated	Squared superior portion helix, large lower lobes		posteriorly rotated ears, anteverted nares		
Nose malformation	up-turned nasal tip	depressed nasal bridge, upturned nasal tip		anteverted nares, depressed nasal bridge		Low columella				
Other facial features	High-arched palate, widely spaced teeth	Trigonocephaly, bitemporal narrowing, synophrys, high-arched palate, micrognathia	–	high palate	High-arched palate	Scaphocephaly, multiple areas of frontal upsweep hair with two posterior hair whorls, arched eyebrows, high palate, gingival overgrowth	–	Prominent forehead	Synophrys, high palate	–
Constitutional Features										
Thick hair	+			+						
"BOPS posture"			+	+						
Other constitutional features	single palmar crease, high-arched feet	Hirsutism, widely spaced nipples, 5th digit brachydactyly	Slender fingers	–	Hirsutism, single palmar crease, contracture of left 3rd proximal interphalangeal joint, and left foot metatarsus adductus	Bifid uvula, toe 2 overriding 3 bilaterally, laterally deviated distal toes	Inverted nipples	–	–	–
Muscle tone										
Hypotonia	+ (severe)	+ (severe)	+ (axial)	+ (before hypoxic event)	+	+ (axial)	+	+ (late)	+	+
Hypertonia	–	–	+ (appendicular)	+ (after hypoxic event)	–	+ (appendicular)	–	+ (early)	–	–
Seizures										
Epilepsy	–	+	+	+	+	+	+	–	–	–
Medications	–	Levetiracetam	valproic acid	oxcarbazepine	Levetiracetam, phenobarbital	Lacosamide, phenobarbital	levetiracetam	–	–	–
Neurological – Other History										

Individual/ Gene	Individual 1 ASXL1	Individual 2 ASXL1	Individual 3 ASXL1	Individual 4 ASXL1	Individual 5 ASXL1	Individual 6 ASXL2	Individual 7 ASXL3	Individual 8 ASXL3	Individual 9 ASXL3	Individual 10 ASXL3
MRI Imaging	Normal (1 year of age)	Agenesis of the corpus callosum, cerebellar vermis hypoplasia, delayed white matter development (18 months of age)	dysmorphic appearance of the craniovertebral junction (4 months of age)	Periventricular leukomalacia, paucity of white matter, findings consistent with hypoxic-ischemic injury	short corpus callosum and possible Blake's pouch cyst (fetal)	Normal (5.5 years of age)	normal	Normal (6 months of age)	thin corpus callosum, absent rostrum, and small frontal lobes	normal
Neurological - Other		cortical visual impairment	Nonverbal		Nonverbal	Optic atrophy, Pigmentary retinal dystrophy, hearing deficiency, excessive sleepiness, autonomic dysfunction	Exotropia s/p surgical correction, able to communicate with assistive device		Speech regression	diffusely decreased strength, strabismus s/p surgical correction
Developmental Delay/Intellectual Disability										
Motor delay	+	+	+	+	+	+	+	+	+	+
Speech delay	+	+	+	+	+	+	+	+	+	+
Intellectual disability		+ (profound)	+	+	+ (profound)	+	+	+	+	+
Gastrointestinal										
Feeding difficulties	+	+	+	+	+	+	+	+	+	+
GERD	+	+	+	+	+	+		+	+	
Feeding tube		G-tube with Nissen fundoplication	G-tube with Nissen fundoplication	G-tube with Nissen fundoplication	G-tube	GJ tube-dependence and TPN dependence		G-tube	G-tube with Nissen fundoplication	G-tube
Other, Gastrointestinal		hiatal hernia				chronic pancreatitis, microvesicular steatohepatitis, GI dysmotility			Alpha-1-antitrypsin deficiency	
Endocrinology										
Precocious puberty	–	+	+	+	–	–	+	–		

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Glucose	–	–	–	–	–	Ketotic hypoglycemia		–	Ketotic hypoglycemia	Hypoglycemia
Other, Endocrinology	–	ectopic pituitary								
Respiratory										
Obstructive sleep apnea	+	+ (BiPap)	+		+ (BiPap)	+ (BiPap)	–	+		–
Surgical interventions	adenoidectomy		tonsillectomy and adenoidectomy	Supraglottoplasty, tracheostomy with ventilator support			–	tonsillectomy and adenoidectomy		–
Other, Respiratory	breath-holding spells	difficult airway		Subglottic stenosis, choanal atresia, laryngomalacia			–		Laryngotracheomalacia	–
Cardiac	Stills murmur, thickened aortic root	Left superior vena cava, Left ventricular hypertrophy	Noncompaction cardiomyopathy (attributed to *TTN* mutation)	Atrial septal defect	Bradycardia	Bradycardia, ventricular ectopy	–	Murmur	–	–
Musculoskeletal	–	Scoliosis (> 45° curve), brachydactyly	Scoliosis, abnormality of the craniovertebral junction	–	contracture of left 3rd proximal interphalangeal joint, left foot metatarsus adductus	Rhabdomyolysis, hip fracture, foot fracture	–	–	–	–
Genitourinary	Penile adhesions	Hypospadias, undescended testicle	Incontinence	–	–	Neurogenic bladder requiring vesicostomy	–	–	–	Enuresis
Sleep and Behavior										
Irregular sleep/ wake cycle	+	+	+	+						
Excessive sleepiness					+	+				
Autism	+						+	+		+
Aggression						(possible)				+
Hand flapping							+	+		
Other, behavior	Behavior disturbance					ADHD, pervasive				Obsessive compulsive disorder

Individual/ Gene	Individual 1 ASXL1	Individual 2 ASXL1	Individual 3 ASXL1	Individual 4 ASXL1	Individual 5 ASXL1	Individual 6 ASXL2	Individual 7 ASXL3	Individual 8 ASXL3	Individual 9 ASXL3	Individual 10 ASXL3
						developmental delay				