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REVIEW



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ASXL3-related disorder: Molecular phenotyping and comprehensive review providing insights into disease mechanism

Emily Woods^{1,2} | Nicola Holmes³ | Shadi Albaba³ | Iwan R. Evans^{2,4} |
Meena Balasubramanian^{1,2,4}

¹Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield, UK

²Division of Clinical Medicine, School of Medicine and Population Health, University of Sheffield, Sheffield, UK

³Sheffield Diagnostic Genetics Service, Sheffield Children's Hospital, Sheffield, UK

⁴The Bateson Centre, University of Sheffield, Sheffield, UK

Correspondence

Emily Woods and Meena Balasubramanian, Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield, UK.

Email: meena.balasubramanian@nhs.net; m.balasubramanian@sheffield.ac.uk; emily.woods2@nhs.net

Abstract

ASXL3-related disorder, sometimes referred to as Bainbridge-Ropers syndrome, was first identified as a distinct neurodevelopmental disorder by Bainbridge et al. in 2013. Since then, there have been a number of case series and single case reports published worldwide. A comprehensive review of the literature was carried out. Abstracts were screened, relevant literature was analysed, and descriptions of common phenotypic features were quantified. ASXL3 variants were collated and categorised. Common phenotypic features comprised global developmental delay or intellectual disability (97%), feeding problems (76%), hypotonia (88%) and characteristic facial features (93%). The majority of genetic variants were de novo truncating variants in exon 11 or 12 of the ASXL3 gene. Several gaps in our knowledge of this disorder were identified, namely, underlying pathophysiology and disease mechanism, disease contribution of missense variants, relevance of variant location, prevalence and penetrance data. Clinical information is currently limited by patient numbers and lack of longitudinal data, which this review aims to address.

KEYWORDS

ASXL3, Bainbridge-Ropers syndrome, *Drosophila melanogaster*, neurodevelopmental disorder

1 | INTRODUCTION

ASXL3-related disorder, also known as Bainbridge-Ropers syndrome, is a neurodevelopmental disorder that was first described by Bainbridge et al.¹ It is associated with heterozygous pathogenic variants in the ASXL3 gene, located at 18q12.1 (OMIM *615115). It is associated with a non-specific neurodevelopmental phenotype; a diagnostic clinical criterion has not been established.² Diagnosis is confirmed by the identification of a pathogenic, or likely pathogenic, variant in ASXL3, as per the American College of Medical Genetics (ACMG) diagnostic standards and guidelines for interpretation of variants.^{3,4}

The Additional Sex Combs-Like (ASXL) gene family comprises ASXL1, ASXL2 and ASXL3. Germline pathogenic variants in ASXL1 cause Bohring-Opitz syndrome, a neurodevelopmental disorder characterised by feeding difficulties, eye abnormalities (exophthalmos, strabismus), dysmorphism, hypertrichosis, specific body posture and microcephaly.⁵ Germline pathogenic variants in ASXL2 cause Shashi-Pena syndrome, which is associated with a highly variable neurodevelopmental phenotype, dysmorphism, macrocephaly, hypotonia and more rarely, bone and heart abnormalities.⁶

ASXL3-related disorder is a Chromatin Modifying Disorder. The ASXL genes code for proteins that are involved in epigenetic mechanisms and transcriptional regulation, and are therefore implicated in

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embryogenesis.^{7,8} Functional studies with fibroblasts have elucidated that truncating *ASXL3* variants cause disruption in histone H2A (H2AK119Ub1) deubiquitination and therefore, have a role in transcriptional regulation and chromatin remodelling.⁹ These are relevant to developmental and proliferative molecular processes, which suggests a role in the pathophysiology of atypical neurodevelopment.⁹ However, a defined pathophysiological process of disease has not yet been identified, and further functional work is required.

Through large-scale whole exome sequencing research projects,^{10,11} and the subsequent expansion of first-line Whole Genome Sequencing, increasing numbers of individuals are being identified to have genetic heterogeneity of genes such as *ASXL3*, as a cause of underlying developmental delay or intellectual disability. Pathogenic variants in *ASXL3* are one of the 10 commonest single gene causes of developmental delay or intellectual disability, but these studies are predominantly based on people with Northwest European ancestry.^{10,12} Case report publications have since shown affected individuals from various ancestral backgrounds, which proves its relevance worldwide.^{13–19} However, the prevalence of *ASXL3*-related disorder has not yet been quantified. There is potential scope for interrogation of population-based data to bridge this gap in our knowledge.

This review summarises the current medical literature on *ASXL3*. It includes a comprehensive list of previously published variants, and a description of what is currently known of the clinical phenotypic spectrum.

2 | MOLECULAR SUMMARY

2.1 | Protein structure and function

Additional Sex Combs-Like (ASX) protein was initially discovered in *Drosophila melanogaster* as a member of the polycomb group of

proteins responsible for regulating *HOX* gene expression, which controls skeletal patterning during embryonic development.²⁰ While they are not essential for initiating homeotic gene repression, polycomb group proteins assume a critical function in maintaining repression as development advances. The human homologues of ASX were later identified as Additional Sex Combs-Like one, two and three (*ASXL1*, *ASXL2* and *ASXL3*).⁸

The *ASXL3* gene is the largest of the *ASXL* family and comprises 12 exons and encodes the *ASXL3* protein, made up of 2248 amino acids. *ASXL3* has *ASXN*, *ASXH*, *ASXM1*, *ASXM2* and PHD conserved domains⁸ (Figure 1). It is highly expressed in the cerebral cortex, ovaries, testes and smooth muscle tissues.⁸

The N terminal *ASXN* domain, also referred to as HARE-HTH (HB1, *ASXL* and restriction endonuclease helix-turn-helix), is predicted to facilitate DNA binding.²⁵ This domain is notably absent from the *Drosophila* homologue.²⁶ The *ASX* homology (*ASXH*) domain, also known as the DEUBAD (deubiquitinase adaptor) domain, spans exons 9–11. *ASXH* plays a crucial role in engaging with epigenetic regulatory proteins, such as BRCA1 Associated Protein 1 (BAP1)²⁷ (Figure 2). The *ASXM1* and *ASXM2* domains serve as protein–protein interaction modules, facilitating the association of *ASXL* proteins with nuclear hormone receptors (NHRs) and their cofactors.⁷ The C terminal plant homeodomain (PHD) is a zinc finger domain which recognises chromatin histone post-translational modifications.²⁹ In contrast with other *ASX* proteins, *ASXL3* features a proline-rich region upstream of its PHD domain.⁸

Although the role of *ASXL3* is not fully understood, there is evidence of interaction with several proteins implicated in the regulation of transcription (Figure 3). Perhaps the most well-characterised, is the interaction of *ASXL3* with BAP1, in the formation of the polycomb repressive deubiquitination (PR-DUB) complex. BAP1 is a nuclear ubiquitin carboxy-terminal hydrolase that removes mono-ubiquitin from histone H2A lysine 119 (H2AK119Ub1), maintaining transcriptional

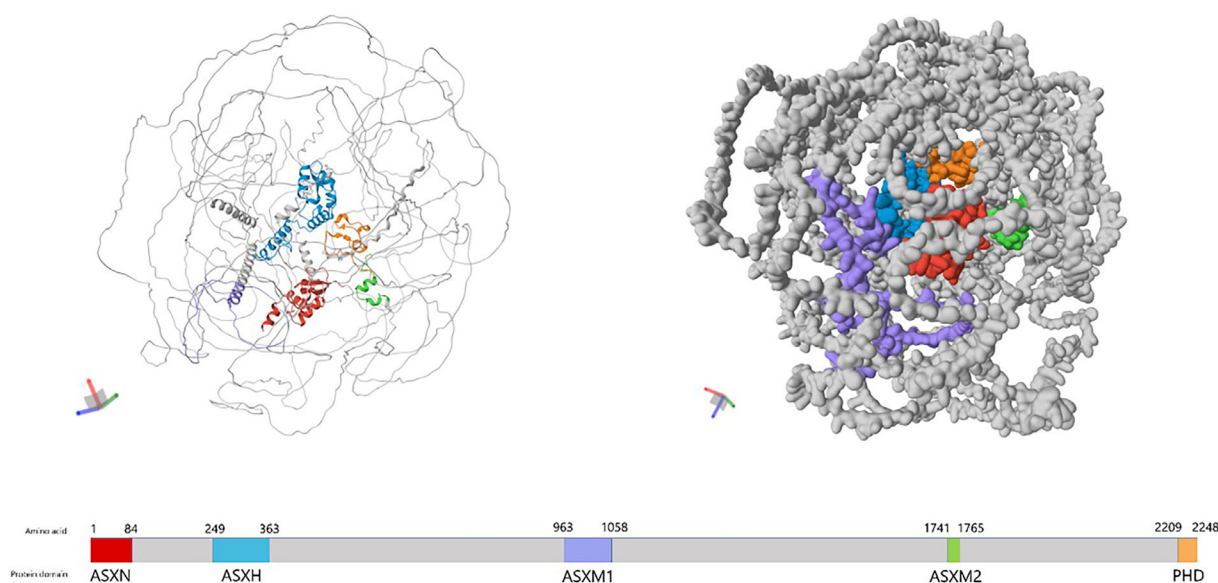


FIGURE 1 Predicted 3D structure and domains of the human *ASXL3* protein. Original illustrations created with RCSB.org²¹ based on the computed structure model AF_AFQ9C0F0F1.^{21,22,23} The domains of *ASXL3* (UniProt: Q9C0F0) are highlighted based on positional information cited by Katoh²⁴: *ASXN* (red), *ASXH* (blue), *ASXM1* (purple), *ASXM2* (green) and PHD (orange). Note that the global per residue confidence score (pLDDT) in this predicted model is very low (39.71). [Colour figure can be viewed at wileyonlinelibrary.com]

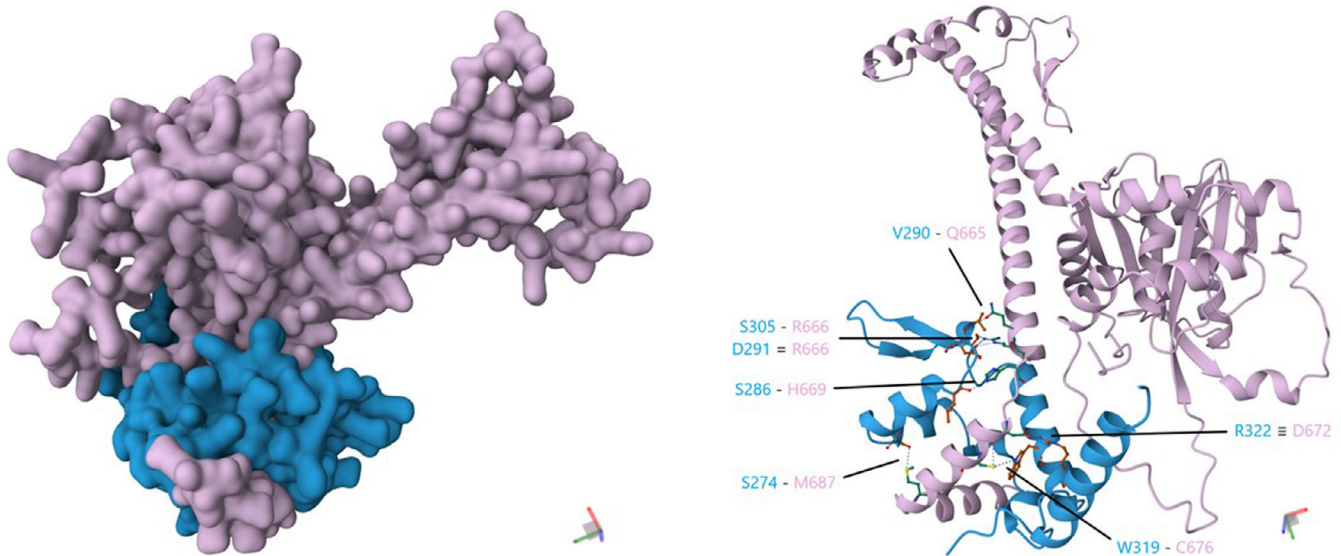


FIGURE 2 Original illustrations created with RCSB.org²¹ based on the computed structure model MA_MAT3VR3881²⁸ showing the predicted interaction between BAP1 (residue 1–699) and ASXL3 (residue 248–348). The ASXH domain of ASXL3 (UniProt: Q9CF0F; blue) is predicted to interact with the ULD domain of BAP1 (UniProt: Q92560; pink) via hydrogen bonds at the following ASXL3 residues: Ser274, Leu286, Val290, Asp291, Ser305, Trp319 and Arg322. Note that the global per residue confidence score (pLDDT) in this predicted model is low (81.747). [Colour figure can be viewed at wileyonlinelibrary.com]

repression.²⁷ The PR-DUB complex is primarily associated with gene silencing, but Kolovos et al.³¹ found that ASXL proteins, via their interaction with BAP1, facilitate gene activation associated with cell metabolism and homeostasis.

Srivastava et al.⁹ reported that fibroblasts from individuals with a pathogenic ASXL3 variant had a significant increase in H2AK119Ub1, supporting the role of ASXL3 in PR-DUB mediated deubiquitination. Furthermore, they observed differential expression of more than 500 genes in patient fibroblasts relative to controls, with a particular bias towards genes involved in transcriptional regulation, development and proliferation. Structural, biochemical and functional studies of human PR-DUB indicate that ASXL proteins are responsible for the activation of BAP1.³² The association of the ULD (ubiquitin C-terminal hydrolase L5-like domain) of BAP1 and the ASXH domain of ASXL proteins has been shown to increase the affinity of PR-DUB for ubiquitin.^{27,33,34}

Szczepanski et al.³⁵ reported that ASXL3 interacts with BRD4 (bromodomain-containing protein 4), a protein which promotes transcription and elongation by binding directly to acetylated histones at enhancers and promoters.³⁶ ASXL3 appears to function as an adaptor protein which forms a bridge between BRD4 and BAP1, maintaining chromatin occupancy of this complex to active enhancers, thus sustaining transcriptional activation.³⁵

3 | METHODS

3.1 | Literature search

A simultaneous literature search was performed on PubMed database. The initial search using keyword ‘ASXL3’ comprised 89 articles, and a filtering process yielded 54 results (Figure S1). A second similar search

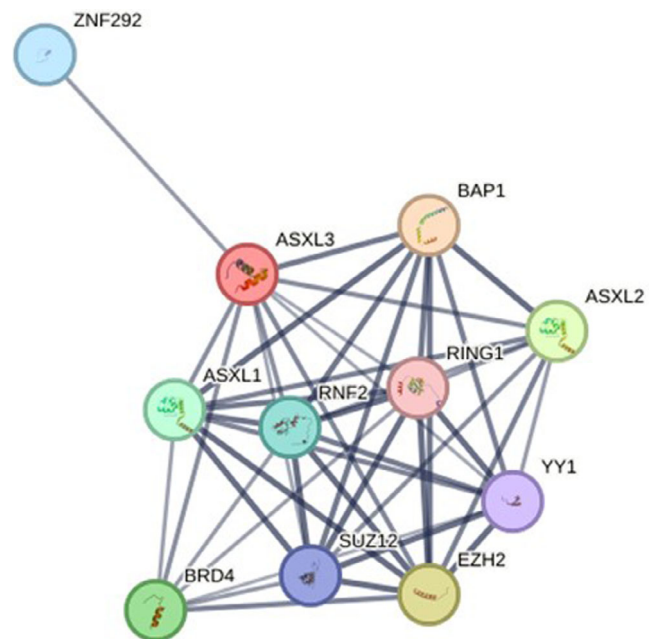


FIGURE 3 Original illustration created at STRING-db.org³⁰ depicting the interactions of the human ASXL3 protein which are predicted with medium confidence (>0.4) based on textmining, experiments, databases, co-expression, neighbourhood, gene fusion and co-occurrence evidence. In descending order of confidence, putative polycomb group protein ASXL3 is predicted to interact with ubiquitin carboxyl-terminal hydrolase BAP1, histone-lysine N-methyltransferase EZH2, putative polycomb group protein ASXL2, bromodomain-containing protein 4 (BRD4), polycomb group protein ASXL1, E3 ubiquitin-protein ligase RING2 (RNF2), zinc finger protein 292 (ZNF292), polycomb protein SUZ12, transcriptional repressor protein YY1 and E3 ubiquitin-protein ligase RING1. [Colour figure can be viewed at wileyonlinelibrary.com]

with keywords 'Bainbridge-Ropers Syndrome' yielded 32 results. Duplicate and corrigendum articles were removed, and remaining articles were screened for relevance.

3.2 | Study types

There were 11 case series published in the literature; the largest was a cohort of 45 individuals. There were 18 individual case reports describing relevant individuals internationally, including individuals from the United Kingdom, China, Japan, Korea, Sudan, Mexico and America with a variety of ancestral backgrounds. The total number of probands described in the literature with apparent heterozygous pathogenic, or likely pathogenic, ASXL3 variants was 108. Information was provided for 101/108 of these variants; the exception being seven individuals described by Ikekwere et al.³⁷ (assuming they were not a duplicated cohort). The majority of individual case reports described paediatric patients.

3.3 | Information screening, extraction and construction

A table of genetic variants was compiled including details of variant location, pathogenicity and predicted effect (Table A1 in Appendix). Cases submitted to DECIPHER database (<https://www.deciphergenomics.org/>) had their unique DECIPHER identifier number recorded.

4 | RESULTS

4.1 | Clinical features

The initial clinical phenotype described by Bainbridge et al.¹ comprised four individuals with developmental delay, feeding difficulties, neurological abnormalities and failure to thrive. With the identification and publication of more individuals, the phenotypic spectrum has expanded to include hypotonia (88%), characteristic facial features (93%), palate abnormalities (61%), musculoskeletal features (66%), behavioural issues (77%), strabismus (58%), sleep disturbance (53%) and autistic features (65%). Intellectual disability (or its precursor, global developmental delay) was almost universal, at 97% prevalence. A summary of the commonly described phenotypic features in the literature is summarised in Table 1.

4.2 | Common clinical features

4.2.1 | Developmental delay

A description of developmental delay and intellectual disability ranged from mild to profound. The majority had speech and language delay as a component of this. Some individuals have been described to have language regression in childhood, and some have been described to communicate by alternate methods, such as Makaton signing.

4.2.2 | Autistic traits and behavioural difficulties

Autistic traits included obsessions, inflexibility and incapacity for change, stereotypies such as hand-flapping and rocking, and difficulties with sensory processing. Other behavioural difficulties included self-injurious behaviours, aggression towards caregivers, emotional dysregulation with outbursts of either laughter, screaming, aggression and bruxism.

4.2.3 | Musculoskeletal

Many individuals had hypotonia, which may have explained the accounts of hypermobility. Other musculoskeletal features were accounted for by spine and thoracic-wall abnormalities (scoliosis, kyphosis, pectus excavatum), digit and joint abnormalities (contractures, arachnodactyly, ulnar deviation, camptodactyly, overlapping digits) and foot abnormalities (pes planus, narrow feet, varus deformity). A severe multi-joint phenotype of arthrogryposis multiplex congenita has been described in patients with pathogenic ASXL3 variants.⁵²

4.2.4 | Connective tissue features

There were reports of long slender hands and digits, and Marfanoid body habitus. Palate abnormalities were described in 61% of individuals; these were most often high and narrow palate. There was one description of a submucous cleft⁴⁵ and one patient with a cleft palate.⁵¹ Despite the overlapping Marfanoid features, there have been no reports of children with a dilated aorta, as is associated with Marfan syndrome and other connective tissue disorders. One child was found to have a subclavian artery aneurysm.⁵³

4.2.5 | Feeding

Several individuals with feeding difficulties presented with failure-to-thrive in infancy. Some babies required naso-gastric tube (NGT) insertion. There were reports of ongoing requirement for enteral feeding in childhood and need for percutaneous gastrostomy feeding. Some feeding difficulties in infancy were reported to improve with time.

4.2.6 | Dysmorphic features

Non-specific dysmorphism has been described, with no pathognomonic or defining facial phenotype, but a clear disposition for certain features. Common features included abnormal head shape (microcephaly, dolichocephaly), prominent forehead, highly arched eyebrows, synophrys, widely spaced and deep-set eyes, down-slanting palpebral fissures, long and tubular nose, low-hanging columella, prominent nasal bridge, wide mouth, high arched palate, everted vermilion of the lower lip, micrognathia and crowded teeth, but these may not be recognised until after diagnosis.² Additional dental

TABLE 1 A summary of the clinical phenotype of individuals with ASXL3-related disorder in the literature.

Paper	Feeding difficulties	Hypotonia	Characteristic facial features	Down-slanting palpebral fissures	Palate abnormality	Speech delay/absent speech	Global delay/Intellectual disability	Behavioural issues	Seizure	Musculoskeletal features	Strabismus	Sleep disturbance	Autism diagnosis/autistic traits
Schirwani et al. ³⁸ <i>n</i> = 45	28/40	33/40	31/31	21/33	21/30	43/43	29/29	29/36	11/39	27/35	Unknown	Unknown	Unknown
Balasubramanian et al. ³⁹ <i>n</i> = 12	9/12	12/12	9/12	6/12	9/12	12/12	12/12	9/12	3/12	7/12	7/12	2/12	9/12
Wang et al. ¹⁷ <i>n</i> = 1	1/1	1/1	1/1	0/1	0/1	1/1	1/1	0/1	0/1	1/1	0/1	1/1	0/1
Wu and Cong ¹⁸ <i>n</i> = 1	1/1	1/1	1/1	Unknown	1/1	1/1	1/1	1/1	0/1	0/1	1/1	Unknown	1/1
Srivastava et al. ⁹ <i>n</i> = 3	3/3	3/3	2/3	2/3	Unknown	3/3	2/3	1/1	1/3	1/2	Unknown	Unknown	Unknown
Dad et al. ⁴⁰ <i>n</i> = 1	0/1	0/1	1/1	1/1	Unknown	1/1	1/1	Unknown	0/1	Unknown	Unknown	1/1	1/1
Qiao et al. ⁴¹ <i>n</i> = 1	1/1	1/1	1/1	0/1	1/1	Unknown	Unknown	Unknown	0/1	1/1	Unknown	1/1	Unknown
Li et al. ⁴² <i>n</i> = 1	1/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	0/1	0/1	1/1	Unknown	1/1
Yang et al. ¹⁹ <i>n</i> = 1	1/1	1/1	1/1	0/1	0/1	Unknown	1/1	Unknown	0/1	1/1	1/1	Unknown	Unknown
Cuddapah et al. ⁴³ <i>n</i> = 4	4/4	4/4	2/4	1/4	1/4	3/3	4/4	3/4	Unknown	0/4	1/4	1/4	3/4
Bainbridge et al. ¹ <i>n</i> = 4	3/4	4/4	4/4	3/3	1/1	3/3	4/4	Unknown	Unknown	3/4	Unknown	Unknown	Unknown
Koboldt et al. ⁴⁴ <i>n</i> = 2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	Unknown	Unknown	Unknown	2/2
Kuechler et al. ⁴⁵ <i>n</i> = 6	6/6	6/6	6/6	6/6	5/6	6/6	6/6	Unknown	2/6	6/6	5/6	2/2	Unknown
Chinen et al. ¹³ <i>n</i> = 1	0/1	1/1	1/1	0/1	0/1	1/1	1/1	1/1	0/1	1/1	0/1	0/1	0/1
Contreras-Capetillo et al. ¹⁴ <i>n</i> = 1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	Unknown	1/1	0/1	0/1	Unknown	Unknown
Dinwiddie et al. ¹⁵ <i>n</i> = 1	1/1	1/1	1/1	0/1	0/1	1/1	1/1	1/1	0/1	1/1	0/1	1/1	1/1
Wayhelova et al. ⁴⁶ <i>n</i> = 1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	Unknown	Unknown	0/1	1/1	Unknown	1/1
Khan et al. ⁴⁷ <i>n</i> = 1	1/1	1/1	Unknown	Unknown	1/1	Unknown	1/1	Unknown	1/1	1/1	Unknown	1/1	1/1
Myers et al. ⁴⁸ <i>n</i> = 3	1/3	1/3	Unknown	Unknown	Unknown	1/1	3/3	Unknown	3/3	1/1	Unknown	Unknown	0/1
Yu et al. ⁴⁹ <i>n</i> = 3	1/4	4/4	4/4	1/4	0/2	4/4	4/4	Unknown	0/4	1/4	Unknown	1/4	1/4

(Continues)

TABLE 1 (Continued)

Paper	Feeding difficulties	Hypotonia	Characteristic facial features	Down-slanting palpebral fissures	Palate abnormality	Speech delay/absent speech	Global delay/intellectual disability	Behavioural issues	Seizure	Musculoskeletal features	Strabismus	Sleep disturbance	Autism diagnosis/autistic traits
Hori et al. ¹⁶ n = 1	1/1	0/1	1/1	1/1	Unknown	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
Bacrot et al. ⁵⁰ n = 1	N/A	N/A	1/1	0/1	1/1	N/A	N/A	N/A	N/A	1/1	N/A	N/A	N/A
Schirwani et al. ⁵¹ n = 5	5/5	5/5	5/5	4/5	3/5	5/5	4/5	3/5	1/5	2/5	3/5	4/5	3/4
Ikekwe et al. ³⁷ n = 7	Unknown	Unknown	Unknown	2/7	1/7	2/7	6/7	4/7	3/7	Unknown	Unknown	5/6	3/7
Total	72/95 = 76%	84/95 = 88%	77/83 = 93%	51/90 = 57%	49/80 = 61%	93/98 = 95%	87/90 = 97%	56/73 = 77%	28/92 = 30%	56/85 = 66%	21/36 = 58%	21/40 = 53%	28/43 = 65%

Note: Percentages are rounded to the nearest whole number. 'Unknown' means there was no mention of this feature in the clinical description, or that clinical description was insufficient to infer otherwise. 'N/A' means not applicable, in the case of foetal death. Denominators may vary per study due to the inclusion only of individuals described in sufficient detail to infer if feature is present or not.

abnormalities have also been reported, including large teeth, missing dentition and enamel hypoplasia.⁴²

4.2.7 | Sleep abnormalities

Sleep disturbance included reports of obstructive sleep apnoea, poor sleep and easy waking. A report of hyperventilation athetosis was reportedly associated with the sleep-wake cycle, immediately prior to falling asleep.⁴⁰

4.2.8 | Brain abnormalities

Structural brain abnormalities have been described, but are not as easily quantified, as MRI brain imaging is not always carried out unless there is a clinical indication.

Normal brain imaging was reported in most individuals, however there were some non-specific abnormalities such as white-matter loss.^{1,9,13,38,45} One individual was reported to have normal early MRI scans but saw a clinical decline in correlation with subsequent cerebral and cerebellar atrophic changes.⁴⁷ Cerebellar vermis hypoplasia has been described in some individuals.^{1,9,45} There has been one reported case of pontocerebellar hypoplasia, a severe and progressive neurodegenerative disorder, which resulted in foetal termination.⁵⁰ Prominence of the sylvian fissure has been described in two individuals,^{13,49} ventricular dilatation in three individuals,^{18,38,45} and thinning of the corpus callosum in eight individuals.^{9,13,38,44,45} Other abnormalities described included abnormal brainstem and decreased myelination.^{1,38}

4.2.9 | Seizures and EEG phenotype

A variety of seizure types have been reported, including generalised (tonic-clonic and absence), focal and complex focal seizures. Variations of focal seizures have been described including atonic seizures (associated with other behaviours such as head-bobbing or spontaneous outbursts of laughter), myotonic seizures (with partial-body stiffness or shaking) and subclinical seizures.^{43,47} A typical epilepsy phenotype was described consisting of generalised epilepsy that starts in childhood, with absence seizures and tonic-clonic seizures.⁴⁸ However, there have been reports of later-onset epilepsy starting during adulthood.⁴⁷ Seizure frequency has been variably reported, with some individuals having sporadic and self-limiting epilepsy, some that resolved with treatment, and some individuals having up to 140 seizures a day with intractable treatment resistant epilepsy.^{47,48}

Electroencephalogram (EEG) testing has been variably reported, with some children having normal EEGs, and some having severely abnormal EEGs. Abnormal EEG reports included multifocal epileptiform activity including sharp-waves, variable background slowing, posterior rhythmic delta activity, diffusely increased beta waves.^{38,47,48} No consistent recognisable EEG pattern has been currently identified.

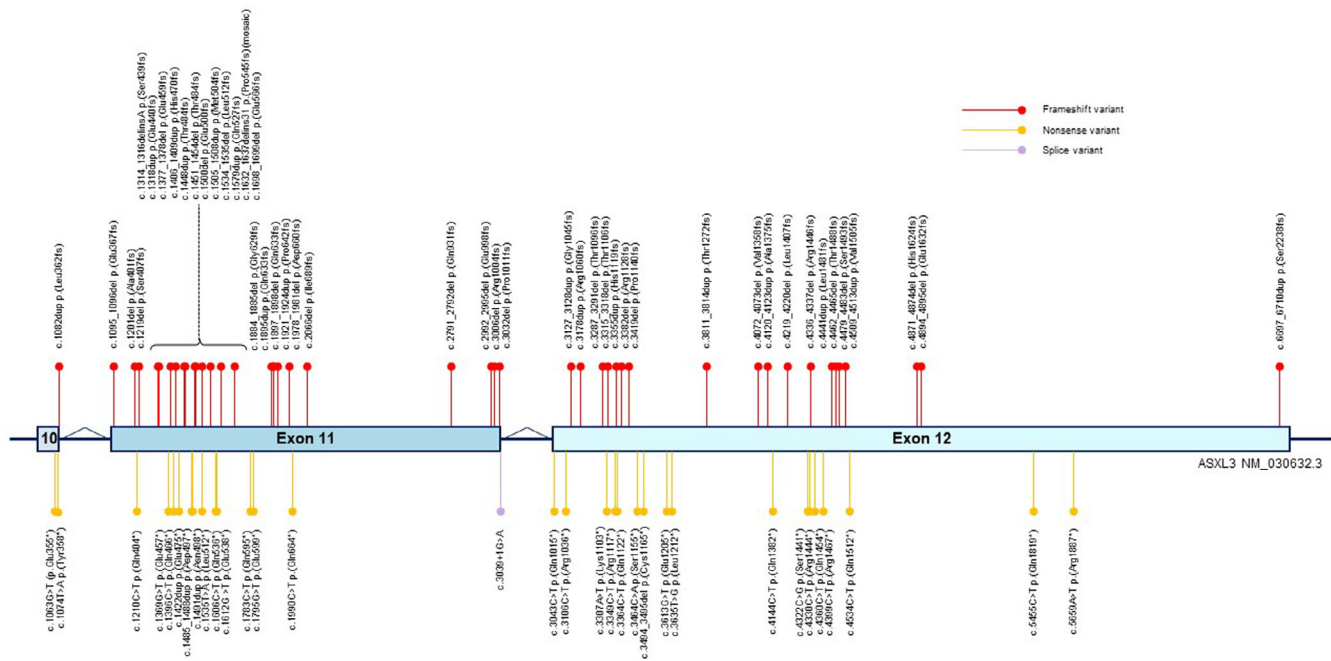


FIGURE 4 Original illustration detailing location of previously published pathogenic, or likely pathogenic, heterozygous variants in exons 10, 11 and 12. [Colour figure can be viewed at wileyonlinelibrary.com]

Different treatments for epilepsy have been tried with variable success including valproate, clobazam, ethosuximide, lamotrigine, levetiracetam, lorazepam, oxcarbazepine, phenobarbital, topiramate, lacosamide, eslicarbazepine, brivaracetam and perampnel. One patient with a severe phenotype was on oxcarbazepine, rufinamide, clobazam and cannabidiol, after previously unsuccessful treatments with zonisamide, clonazepam, topiramate, lacosamide and valproic acid.⁴⁷ Consideration of vagal nerve stimulator (VNS), ketogenic diet or focal resection has been suggested for patients with intractable medication-resistant epilepsy. One individual described by Schirwani et al.⁵³ was non-responsive to VNS insertion.

5 | VARIANTS

5.1 | Location of variants

The location of ASXL3 variants have been primarily identified within exon 11 and exon 12, which may be in keeping with their large size at 83% of the total ASXL3 gene coding sequence (Figure 4). However, as represented in Figure 4, there is propensity for variant clustering within these two exons. There have been two de novo splice-site variants published.^{16,48} There has been one publication of a variant in exon 3 (c.187C>T p.(Arg63*)),³⁸ There have been three published variants in exon 10 (c.1074T>A p.(Tyr358*), c.1082dup p.(Leu362fs), c.1063G>T p.(Glu355*)).^{17,39} An individual with an intragenic deletion at 18q12.1 spanning exons 2–8 was also described by Schirwani et al.³⁸ Intragenic deletions have not been described elsewhere, but the loss-of-function mechanism fits with what is seen in the eligible variants.

Some variants have been reported in more than one individual. The clinical details and DECIPHER identifying numbers were compared to ensure these were not duplicate reports of the same individual. Variant c.3106C>T p.(Arg1036*) has been reported five times^{38,44,45,48}; c.4330C>T p.(Arg1444*) has been reported five times^{9,38,39}; c.4399C>T p.(Arg1467*) has been reported four times^{38,49}; c.4534C>T p.(Gln1512*) and c.1534_1535del p.(Leu512fs) have both been reported twice³⁸; c.3464C>A p.(Ser1155*) has been reported twice^{38,41}; c.1990C>T p.(Gln664*) has been reported twice.^{38,43}

Yu et al.⁴⁹ compared the clinical phenotypes of previously reported loss-of-function variants at the 5' mutational cluster region in exon 11 ($n = 15$), with the 3' mutational cluster region in exon 12 ($n = 20$) and did not demonstrate any statistically significant differences in phenotype. The only exception was the clinical feature of hypertelorism, which was more common in the 3' mutational cluster region.⁴⁹ Currently, there are insufficient patient numbers with the same variant to draw any meaningful conclusions about specific location genotype–phenotype correlations. The location of known mutational clusters are highlighted in Figure 5.

The majority of variants were described as de novo. The majority of these were confirmed by parental genetic testing. In a few individuals, inheritance was not genetically confirmed (Table A1 in Appendix). Individuals with variants c.4534C>T p.(Gln1512*) and c.2791_2792del p.(Gln931fs) were presumed to be inherited, as parents had a consistent ASXL3-related disorder phenotype.⁵³ There have been genetically confirmed inherited cases. Of these, a c.4441dup p.(Leu1481fs) variant was seen in a clinically severely affected daughter and clinically unaffected mother.⁵³ This intrafamilial heterogeneity poses the possibility of either a very mild, or asymptomatic phenotype, or elucidates the possibility of reduced penetrance.

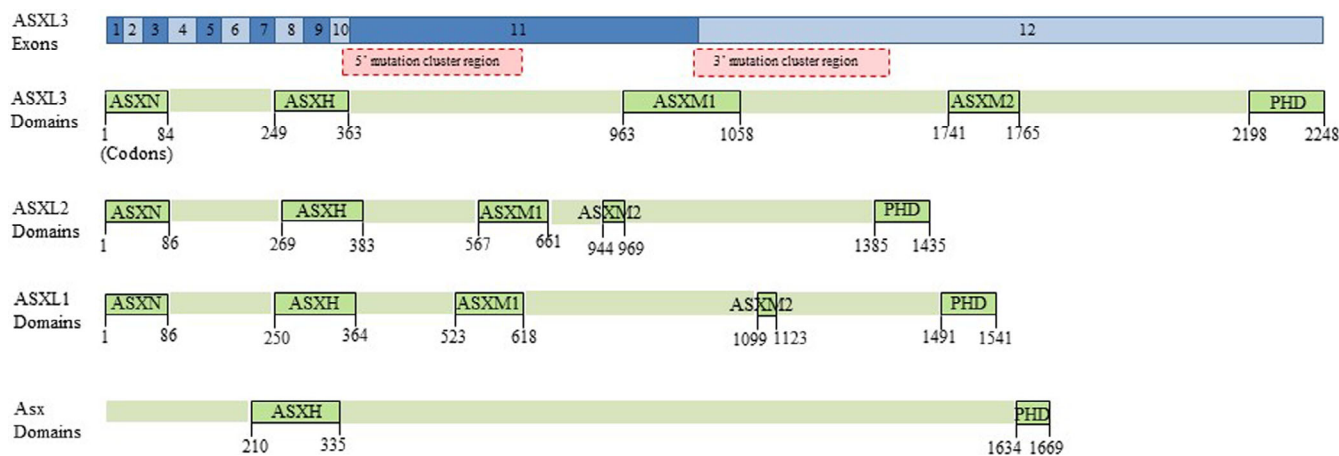


FIGURE 5 Original illustration showing the position of ASXL3 exons, domains, and codons, highlighting the location of 5' and 3' mutational cluster regions. [Colour figure can be viewed at wileyonlinelibrary.com]

In order to help with the classification of variants of uncertain clinical significance and to understand the role of epigenetics, one study has looked for a methylation signature for ASXL disorders, but did not elucidate a methylation signature for ASXL3-related disorder.⁵⁵ However, this was the first published study of this nature, and was limited to three ASXL3 variants.

5.2 | Missense variants

Missense variants in ASXL3 have been associated with Autism Spectrum Disorder without the additional phenotype of developmental delay and dysmorphism, as seen in ASXL3-related disorder.⁵⁶ An individual with homozygous c.3136G>A p.(Gly1046Arg) missense variant in exon 12 had developmental delay and seizures, this could be explained by an alternative confirmed genetic diagnosis, and so this ASXL3 variant was reported as likely benign.⁵⁷ However, the individual also had cortical atrophy, gastroesophageal reflux and microcephaly that may not have been attributable to this alternative diagnosis. A second individual with homozygous c.2471C>T p.(Pro824Leu) missense variant in exon 11 was reported as benign by Alfares et al.,⁵⁸ but this lacked detailed phenotyping. An additional individual with compound heterozygous missense variants c.2965C>G p.(Arg989Gly) and c.3078G>C p.(Lys1026Asn) had severe short stature and IGF1 deficiency, developmental delay and craniofacial abnormalities.⁵⁹ This was described as the first report of compound heterozygous variants causing a 'typical' ASXL3 phenotype and hypothesised a molecular interaction between ASXL3 and IGF1. However, the ASXL3 variants were not classified as pathogenic, and Schirwani et al.³⁸ later reported that an alternative diagnosis had been established.

Lack of functional assays for missense variants limits the use of further evidence for the assignment of pathogenic/likely pathogenic classification. Previously reported missense variants were classified as likely benign or variants of unknown clinical significance by Schirwani et al.³⁸ in accordance with the ACMG criteria.^{3,4} Missense constraint scores of 0.61 on gnomAD v2.1.1⁶⁰ and 1.08 on DECIPHER⁶¹ suggest that ASXL3 is fairly tolerant of missense variants, in contrast with its

intolerance to loss of function variants. This may be consistent with a possible recessive mode of inheritance for missense variants.

5.3 | Mosaic variants

A severely developmentally delayed individual with typical features of ASXL3-related disorder was found to have a mosaic c.1632_1637delins31 p.(Pro545Leufs) variant present at 35% level in saliva (but unknown Central Nervous System contribution).⁵¹ There is evidence that mosaicism in more than 20% of cells indicates an early embryonic event, and therefore a more likely clinically significant phenotype.⁶²

Two pairs of siblings have been reported with c.3284_3288del p.(Thr1096Asnfs) and c.4509_4513dup p.(Val1505Aspfs) variants, with assumed gonadal mosaicism in parents.⁵¹

5.4 | Genetic aetiology

The majority of variants are de novo truncating variants with a predicted loss-of-function (Table A1 in Appendix). Haploinsufficiency is a proven mechanism of pathogenicity, as a result of RNA mediated decay, truncated or null protein (Table A1 in Appendix). A dominant-negative effect was also proposed by Bainbridge et al.,¹ but there is evidence against this, whereby frog assays result in rescue of the ASXL3 knockdown phenotype by both full-length and truncated proteins.⁶³

Preliminary mouse studies have found that compound heterozygous ASXL3 variants show significantly reduced mRNA levels in the cerebrum and cerebellum compared to controls, which conforms with the neurodevelopmental phenotype.⁶⁴ In a study of *Xenopus laevis*, ASXL3 knockdown caused abnormal neuronal embryological development, which further elucidates the underlying mechanism of disease in early development.⁶³ However, this study presented some contradictory results with ectopic human ASXL3 protein.⁶³

Further molecular and functional work is required to further understand the genetic aetiology and disease mechanisms.

6 | DISCUSSION

6.1 | Description of literature

The current literature consists primarily of individual case reports. There are relatively few case series, the largest detailing 45 individuals. There is relatively little literature on the mechanisms of disease and functional studies.

There is literature on somatic *ASXL3* variants, its role in the oncogenic axis, and association with cancers. This is not relevant to *ASXL3*-related disorder, which describes germline *ASXL3* variants. There has been one report of cancer (precursor B-cell acute lymphoblastic leukaemia) in a child with *ASXL3*-related disorder.⁶⁵ Individuals with *ASXL3*-related disorder are not thought to be at any increased risk of cancers. Comparatively, somatic gene variants in *ASXL1* are associated with myeloid malignancies, and *ASXL1*-related disorder (Bohring-Opitz syndrome) is associated with an increased risk of Wilms tumour.⁵

Compound heterozygous *ASXL3* missense variants have been identified in two families with congenital heart disease.⁶⁶ Studies showed that homozygous or compound heterozygous *ASXL3* expression caused reduced cell viability, induced cell apoptosis and resulted in abnormal cardiac structure and function in mice.^{66,67} There has been one individual published with compound *ASXL3* missense variants (although these variants were not classified for pathogenicity); this individual had a normal echocardiogram.⁵⁹ Schirwani et al.³⁸ found that nine individuals of the 45-patient cohort, had an echocardiogram for an alternate reason. All of these were reported as normal. Recommendation for routine echocardiography is not described, with the exception of one publication, where an individual with subclavian artery aneurysm and connective-tissue features was described.⁵³

6.2 | Gaps in the literature

In some instances, there was assumption of inheritance given a similar parental phenotype, and in some instances, inheritance status was not discussed (Table A1 in Appendix). De novo status was never given in the absence of confirmatory parental genetic testing. However, inheritance of *ASXL3* variants, from a normal or mildly affected parent, has been reported.⁵³ Therefore, it cannot be presumed that all cases are de novo in the absence of a clinically affected parent.

Similarly, less frequently reported features are also difficult to quantify or associate causality. Details of specific dysmorphology (and other examination findings) were variably reported, and description of dysmorphology can be subjective. Other features, such as hypospadias and naevus flammeus, were rarely reported.

6.3 | Clinical relevance

Confirming the genetic status of the parents of an affected child is important in determining inheritability, and therefore the risk-estimate of a further child with the genetic variant. More accurate data for penetrance is required to attribute disease-recurrence estimates. Even in

the absence of the variant in the blood of parents, there is the small theoretical chance of gonadal mosaicism.

If a pathogenic *ASXL3* variant is identified, there are options for an individual in pregnancy including pre-natal diagnosis (PND) and pre-implantation genetic diagnosis (PGD). There has been one published case of pregnancy termination as a result of significant foetal abnormalities, presenting as pontocerebellar hypoplasia type 1.⁵⁰ A pathogenic *ASXL3* variant was detected on post-mortem whole exome testing.

6.4 | *ASXL3* fly models

The founder and sole member of the *ASXL* family, *Asx* (*Additional sex combs*),¹ was first discovered in fruit flies by Gerd Jurgens.⁶⁸ It was also identified in the ground-breaking *D. melanogaster* screen for developmental genes,⁶⁹ for which Wieschaus and Nusslein-Volhard later received the Nobel Prize ('discoveries concerning the genetic control of early development'), along with Ed Lewis, in 1995. In a series of screens, ethyl methanesulfonate (EMS) was used to induce mutations that perturbed embryonic development, with mutants identified via defects in larval cuticles. This approach enabled the detection of abnormal body patterning phenotypes, for example, segment polarity and denticle morphogenesis (denticles are small bristles that can be seen in cuticle preparation of *Drosophila* larvae). Fly genes are typically named according to phenotype observed when that gene is mutated: correspondingly, *Asx* is named due to the supernumerary sex comb bristles or 'teeth' that enlarge in males.⁶⁸ Sex combs, which are found on the forelegs of male flies, are clusters of thick bristles that male fruit flies use during copulation⁷⁰ and are familiar structures that can be used to determine the sex of *D. melanogaster* when setting up genetic crosses.

Larval cuticle preparations revealed a visible embryonic phenotype characterised as 'head broad, homoeotic, head and thorax partially transformed into abdomen'.⁶⁹ *Asx* was subsequently revealed as a negative regulator of the Bithorax complex,^{71,72} which regulates segment identity in the posterior developing embryo. Once cloned, *Asx* was shown to bind chromatin in regions that overlapped substantially with other polycomb proteins but also at distinct sites.⁷³ Subsequently the PR-DUB complex was defined in flies as consisting of Calypso (BAP1 homologue) and *Asx*, and this complex's deubiquitinating activity is key to balancing H2Aub1 levels and maintaining repression of *HOX* genes and other loci during development.^{27,74}

While *Asx* lacks the N-terminal HARE-HTH domain of vertebrate *ASXL* family members and also contains poly-glutamine and poly-alanine regions unique to the fly gene, the PR-DUB complex plays critical and conserved roles across evolution, though there are differences in regulation of the PRC1 complexes that generate its ubiquitinated substrate.³² Furthermore, the molecular interactions between *Asx* and Calypso have strongly informed our structural understanding of the PR-DUB complex.^{75,76}

While *Asx* is expressed ubiquitously in the fly embryo, specific expression changes are observed within the developing CNS, which may potentially be useful in understanding pathophysiological changes linked to neuronal function (e.g., Reference 69). Model organisms such as *D. melanogaster* are incredibly useful in understanding genetic

mechanisms. Highly genetically tractable with reduced genetic redundancy, yet with a high degree of conservation of key human disease genes, flies are inexpensive, small, have a rapid life cycle, and are amenable to imaging approaches. Their use in research for over 100 years has enabled a generation of functional genetic tools.⁷⁷ With the advent of next generation sequencing of patients, clinical researchers are faced with vast numbers of potentially pathogenic variants in key genes. Flies represent an *in vivo* tool in which the contributions of such allelic variants may be investigated, by coupling the conservation of genes critical to human health with genetic means to introduce human disease gene variants. For example, re-expression of *Asx*—with or without patient-derived polymorphisms—in *Asx* null backgrounds, or direct gene-replacement strategies, enable testing of whether such variants have the potential to be pathogenic, when coupled with observable phenotypes. Such an approach has been taken to study a range of human disease genes, with a particular emphasis on genes involved in neurodevelopmental and neurological conditions^{78,79}; in some cases, the human disease gene is sufficiently related and is able to rescue loss of the *Drosophila* gene's function. Complementary approaches using patient data, human cell-line work and model organisms such as *Drosophila* and zebrafish, have the potential to uncover the underlying mechanisms in disorders such as Bainbridge-Ropers syndrome. There is, therefore, potential to define the pathogenicity of human genetic variants of otherwise uncertain significance.

7 | CONCLUSION

ASXL3-related disorder is a neurodevelopmental disorder caused by pathogenic, or likely pathogenic, heterozygous variants in the ASXL3 gene. To date there have been 108 individuals published in the literature with ASXL3-related neurodevelopmental disorder, in case series or individual case reports. The majority of reported variants are loss-of-function variants in exon 11 and exon 12. Common clinically described features of the condition include feeding difficulties, hypotonia, characteristic facial features, global delay (particularly speech delay), behavioural difficulties and musculoskeletal features.

With increasing patient numbers through our group's ongoing Natural History Study (and ASXL3-registry), in time, we will establish the wide clinical spectrum of the condition, elucidate penetrance data and establish any potential genotype–phenotype correlation (including the role of splice site variants). As there is evidence of inheritability and variability of ASXL3 within families, this will likely unveil a potential mild, and even unaffected, clinical phenotype.

AUTHOR CONTRIBUTIONS

EW: Carried out the literature search, literature analysis and wrote the majority of the article. **NH:** Contributed to the article with molecular insights, including original molecular diagrams and variant formatting. **SA:** Provided expertise and oversight as senior laboratory scientist, and ensured scientific and molecular accuracy. **IR:** Contributed expertise with insights into fly models. **MB:** Supervised the project throughout development and writing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Emily Woods  <https://orcid.org/0000-0003-2349-2688>

Iwan R. Evans  <https://orcid.org/0000-0002-3485-4456>

Meena Balasubramanian  <https://orcid.org/0000-0003-1488-3695>

ENDNOTE

¹ N.B., not to be confused with *asx* (*ascutex*), which remains an unmapped genetic locus.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

TABLE A1 Variants in the literature.

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Schirwani et al. ³⁸	1	258 284	c.4479_4483del p.(Ser1493fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸ Schirwani et al. ⁵³	2	286 691	c.4534C>T p.(Gln1512*)	12	Maternal*	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	3	300 784	c.2066del p.(Ile689fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	4	362 009	c.1534_1535del p.(Leu512fs)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Schirwani et al. ³⁸	5	307 301	c.5659A>T p.(Arg1887*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	6	305 747	c.3811_3814dup p.(Thr1272fs)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	7	275 275	c.1606C>T p.(Gln536*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	8	274 520	c.1921_1924dup p.(Pro642fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	9	286 921	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸ Schirwani et al. ⁵³	10	/	c.2791_2792del p.(Gln931fs)	11	Paternal*	PVS1, PM2	Likely pathogenic	NMD likely
Schirwani et al. ³⁸	11	/	c.2791_2792del p.(Gln931fs)	11	Unknown	PVS1, PM2	Likely pathogenic	NMD likely
Schirwani et al. ³⁸	12	/	c.3382del p.(Arg1128fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	13	/	c.1095_1096del p.(Glu367fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	14	/	c.1884_1885del p.(Gly629fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	15	/	c.55_879del p.(Ala19_Gln293del)	2 to 8	De novo	PM4, PM2, PS2_mod	Likely pathogenic	Arr[hg19] 18q12.1 (31172484_31311379)x1 dn. Size of intragenic deletion is 139 kb
Schirwani et al. ³⁸	16	/	c.1534_1535del p.(Leu512fs)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Schirwani et al. ³⁸	17	/	c.1579dup p.(Gln527fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely

(Continues)

TABLE A1 (Continued)

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Schirwani et al. ³⁸	18	/	c.4894_4895del p.(Glu1632fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	19	/	c.1451_1454del p.(Thr484fs)	11	De novo	PVS1, PM2	Likely pathogenic	NMD likely
Schirwani et al. ³⁸	20	/	c.1990C>T p.(Gln664*)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Schirwani et al. ³⁸	21	/	c.1698_1699del p.(Glu566fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸ Schirwani et al. ⁵³	22	/	c.4441dup p.(Leu1481fs)	12	Maternal	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	23	/	c.4462_4465del p.(Thr1488fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	24	/	c.1579dup p.(Gln527fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	25	/	c.1535T>A p.(Leu512*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	26	/	c.4399C>T p.(Arg1467*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	27	/	c.4399C>T p.(Arg1467*)	12	Unknown	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	28	/	c.1783C>T p.(Gln595*)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Schirwani et al. ³⁸	29	/	c.4871_4874del p.(His1624fs)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	30	/	c.3178dup p.(Arg1060fs)	12	De novo	PVS1_str, PM2, PS4_sup, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	31	/	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	32	/	c.1500del p.(Glu500fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	33	/	c.3464C>A p.(Ser1155*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	34	262 694	c.3106C>T p.(Arg1036*)	12	Unknown	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	35	/	c.4360C>T p.(Gln1454*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost

TABLE A1 (Continued)

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Schirwani et al. ³⁸	36	/	c.4399C>T p.(Arg1467*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	37	305 643	c.4534C>T p.(Gln1512*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	38	/	c.1505_1508dup p.(Met504fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ³⁸	39	/	c.4219_4220del p.(Leu1407fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	40	/	c.4120_4123dup p.(Ala1375fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	41	/	c.4336_4337del p.(Arg1446fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	42	274 637	c.3419del p.(Pro1140fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ³⁸	43	264 183	c.1406_1409dup p.(His470fs)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Schirwani et al. ³⁸	44	263 416	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Schirwani et al. ³⁸	45	/	c.187C>T p.(Arg63*)	3	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	46	274 629	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Balasubramanian et al. ³⁹	47	275 029	c.1201del p.(Ala401fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	48	274 593	c.1074T>A p.(Tyr358*)	10	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	49	278 695	c.4144C>T p.(Gln1382*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Balasubramanian et al. ³⁹	50	261 513	c.1783C>T p.(Gln595*)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	51	265 854	c.3355dup p.(His1119fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Balasubramanian et al. ³⁹	52	271 912	c.1082dup p.(Leu362fs)	10	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	53	275 860	c.3635T>G p.(Leu1212*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost

(Continues)

TABLE A1 (Continued)

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Balasubramanian et al. ³⁹	54	265 908	c.3127_3128dup p.(Gly1045fs)	12	De novo	PVS1_str, PM2, PS2_mod	Pathogenic	>10% protein lost
Balasubramanian et al. ³⁹	55	259 240	c.3178dup p.(Arg1060fs)	12	De novo	PVS1_str, PM2, PS4_sup, PS2_mod	Likely pathogenic	>10% protein lost
Balasubramanian et al. ³⁹	56	272 591	c.1485_1488dup, p.(Asp497*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Balasubramanian et al. ³⁹	57	208 772	c.1491dup p.(Asn498*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Wang et al. ¹⁷	58	/	c.1063G>T (p.Glu355*)	10	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Wu and Cong ¹⁸	59	/	c.3043C>T p.(Gln1015*)	12	De novo	PVS1, PM2, PS2_mod	Pathogenic	>10% protein lost
Srivastava et al. ⁹	60	/	c.4330C>T p.(Arg1444*)	12	Unknown	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Srivastava et al. ⁹	61	/	c.3364C>T p.(Gln1122*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Srivastava et al. ⁹	62	/	c.1448dup p.(Thr484fs)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Dad et al. ⁴⁰	63	/	c.1314_1316delinsA p.(Ser439fs)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Qiao et al. ⁴¹	64	/	c.3464C>A p.(Ser1155*)	12	De novo	PVS1_str, PM2, PS2_mod, PS2_sup	Likely pathogenic	>10% of protein lost
Li et al. ⁴²	65	/	c.1795G>T p.(Glu599*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Yang et al. ¹⁹	66	/	c.3494_3495del p.(Cys1165*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Pathogenic	>10% of protein lost
Giri et al. ⁵⁹	67	/	c.2965C>G, p.(Arg989Gly) and c.3078G>C, p.(Lys1026Asn)	11 and 12	Maternal and paternal	c.2965C>G BS1, BS2, BP1, BP4 c.3078G>C BP1, BP4	c.2965C>G Benign c.3078G>C Likely benign	-
Cuddapah et al. ⁴³	68	/	c.4322C>G p.(Ser1441*)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Cuddapah et al. ⁴³	69	/	c.1895dup p.(Gln633fs)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Cuddapah et al. ⁴³	70	/	c.3349C>T p.(Arg1117*)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Cuddapah et al. ⁴³	71	/	c.1990C>T p.(Gln664*)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely
Bainbridge et al. ¹	72	/	c.1210C>T p.(Gln404*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely

TABLE A1 (Continued)

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Bainbridge et al. ¹	73	/	c.1396C>T p.(Gln466*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Bainbridge et al. ¹	74	/	c.1422dup p.(Glu475*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Bainbridge et al. ¹	75	/	c.1978_1981del p.(Asp660fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Koboldt et al. ⁴⁴	76	/	c.3106C>T, p.(Arg1036*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% of protein lost
Koboldt et al. ⁴⁴	77	/	c.3106C>T, p.(Arg1036*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% of protein lost
Kuechler et al. ⁴⁵	78	327 923	c.1219del p.(Ser407fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Kuechler et al. ⁴⁵	79	327 924	c.1369G>T p.(Glu457*)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Kuechler et al. ⁴⁵	80	327 925	c.3106C>T p.(Arg1036*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Kuechler et al. ⁴⁵	81	327 926	c.3494_3495del p.(Cys1165*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Pathogenic	>10% protein lost
Kuechler et al. ⁴⁵	82	327 927	c.3613G>T p.(Glu1205*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Kuechler et al. ⁴⁵	83	327 928	c.4072_4073del p.(Val1358fs)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Chinen et al. ¹³	84	/	c.3032del p.(Pro1011fs)	11	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Contreras- Capetillo et al. ¹⁴	85	/	c.2992_2995del p.(Glu998fs)	11	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Dinwiddie et al. ¹⁵	86	/	c.1897_1898del p.(Gln633fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Wayhelova et al. ⁴⁶	87	/	c.3006del p.(Arg1004fs)	11	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Verhoeven et al. ⁵⁴	88	/	c.6697_6710dup p.(Ser2238fs)	12	Unknown	PVS1_mod, PM2	Variant of unknown significance	<10% of protein lost
Khan et al. ⁴⁷	89	/	c.1612G>T, p.(Glu538*)	11	De novo	PVS1, PM2	Pathogenic	NMD likely
Myers et al. ⁴⁸	90	/	c.3106C>T p.(Arg1036*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost

(Continues)

TABLE A1 (Continued)

Source/ Reference	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect
Myers et al. ⁴⁸	91	271 709	c.3039+1G>A	Splice site	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	Predicted skipping of exon 11 >10% protein lost
Myers et al. ⁴⁸	92	295 117	c.3315_3318del p.(Thr1106fs)	12	De novo	PVS1_str, PM2	Likely pathogenic	>10% protein lost
Yu et al. ⁴⁹	93	421 415	c.3307A>T p.(Lys1103*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Yu et al. ⁴⁹	94	421 418	c.5455C>T p.(Gln1819*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Yu et al. ⁴⁹	95	421 420	c.1377_1378del p.(Glu459fs*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Yu et al. ⁴⁹	96	/	c.4399C>T p.(Arg1467*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	>10% protein lost
Hori et al. ¹⁶	97	/	c.3039+1G>A	Splice site	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	Predicted skipping of exon 11 >10% protein lost
Bacrot et al. ⁵⁰	98	/	c.1318dup p.(Glu440fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Schirwani et al. ⁵¹	99	292 512	c.3287_3291del p.(Thr1096fs)	12	De novo (likely parent mosaic)	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ⁵¹	100	292 513	c.3287_3291del p.(Thr1096fs)	12	De novo (likely parent mosaic)	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ⁵¹	101	/	c.4509_4513dup p.(Val1505fs)	12	De novo (likely parent mosaic)	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ⁵¹	102	/	c.4509_4513dup p.(Val1505fs)	12	De novo (likely parent mosaic)	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost
Schirwani et al. ⁵¹	103	/	c.1632_1637delins31 p.(Pro545fs) (mosaic)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely
Ikekwe et al. ³⁷	Possible 104–110	/	No variant data provided Unable to determine if otherwise previously reported	/	/	/	/	/

Note: De novo—confirmed with parental genetic testing. ACMG Criterion applied: PS2_mod: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history, used at moderate level since the phenotype in our cohort of patients was consistent but not highly specific to the ASXL3 gene. PVS1: null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease, used at very strong level. We downgraded this evidence too strong for truncating variants within the last exon but predicted to result in >10% loss of protein size (PVS1_str) or <10% loss of protein size (PVS1_mod). PM2: Absent from controls in gnomAD database, used at moderate level. PM4: Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants, used at moderate level. PS4_sup: Variant previously identified in one unrelated individual and not seen in gnomAD. PS4_mod: Variant previously identified in two or more unrelated individuals.

*Presumed inheritance due to parental phenotype consistent with ASXL3-related disorder.