

Expanding the phenotype of *ASXL3*-related syndrome: a comprehensive description of 45 unpublished individuals with inherited and *de novo* pathogenic variants in *ASXL3*

Schaida Schirwani MBChB, MRCP, Shadi Albaba PhD, Deanna Alexis Carere ScD, CGC, Maria J. Guillen Sacoto MD, Francisca Milan Zamora MD, Yue Si MD, PhD, Rachel Rabin MS, John Pappas MD, Deborah L. Renaud MD, Natalie Hauser, Evan Reid MBChB, PhD, Patricia Blanchet, Nichola Foulds, Abhijit Dixit, Richard Fisher, Ruth Armstrong, Isidor Bertrand, Benjamin Cogne, Samantha A. Vergano, MD, FACMG, Serwet Demirdas, Natalie Dykzeul MS, LCGC, Julie S. Cohen ScM, CGC, Katheryn Grand MS, LCGC, Dayna Morel, Anne Slavotinek, Hessa F. Albassam, Swati Naik, John Dean, Nicola Ragge, Costa Cinzia MD, PhD, Tedesco Maria Giovanna MD, Rachel Harrison BMBS, PhD, Arjan Bouman, Emily Palen, Thomas D. Challman, Marjolein H. Willemsen MD, PhD, Julie Vogt MBBS, MD, Christopher Cunniff, Katherine Bergstrom, Jagdeep S Walia MBBS, FCCMG, Ange-line Bruel, Usha Kini, Fowzan Alkuraya, Valerie Slegesky MS, Naomi Meeks, Paula Giroto MD, Diana Johnson Bm, BSc, MD, DDD study, Ruth Newbury-Ecob MBChB, MD, FRCPCH, Charlotte Ockeloen, Paolo Prontera MD, PhD, Sally Ann Lynch, Dong Li PhD, John M Graham, Jr. MD, ScD, Meena Balasubramanian MBBS, MD

Author affiliation

Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, UK

Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield, Sheffield, UK

Schaida Schirwani & Meena Balasubramanian

Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield

Shadi Albaba

Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Dong Li

Division of Medical Genetics, Department of Pediatrics, Weill Cornell Medical College, New York, NY, USA

Katherine Bergstrom & Christopher Cunniff

Clinical Genetics Service, Nottingham University Hospitals NHS Trust, Nottingham, UK

Rachel Harrison & Abhijit Dixit

**Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust,
EDS National Diagnostic service, UK**
Diana Johnson

**Teesside Genetics Unit, The James Cook University Hospital, Middlesbrough,
United Kingdom**
Richard Fisher

**Inova Health System, Department of Pediatrics, Division of Medical Genomics,
Falls Church, VA USA**
Natalie Houser

Autism & Developmental Medicine Institute, Geisinger, Danville, Pennsylvania
Emily Palen & Thomas D. Challman

Welcome Trust Sanger Institute, Hinxton, Cambridge, UK
DDD Study

**Department of Clinical Genetics, Oxford University Hospitals NHS Trust,
Oxford, United Kingdom**
Usha Kini

Care National Hospital, Department of Pediatrics, Riyadh, Saudi Arabia
Hessa F. Albassam

**Department of Pediatrics, Medical Genetics, Cedars-Sinai Medical Center, Los
Angeles CA**
Katheryn Grand

**Cedars-Sinai Medical Center, Harbor-UCLA Medical Center, David Geffen
School of Medicine at UCLA, Los Angeles CA**
John M Graham, Jr.

**Division of Neurogenetics, Kennedy Krieger Institute, Baltimore, Maryland
21205, USA. Department of Neurology, Johns Hopkins University School of
Medicine, Baltimore, Maryland 21287, USA**
Julie S. Cohen

**West Midlands Regional Genetics Service, Birmingham Women's and
Children's Hospital, Birmingham, UK**
Swati Naik, Julie Vogt & Nicola Ragge

**Department of Clinical Genetics, Erasmus MC University Medical Center,
Rotterdam, The Netherlands**
Arjan Bouman & Serwet Demirdas

**Radboud university medical center, Department of Human Genetics, Nijmegen,
The Netherlands**
Charlotte Ockeloen & Marjolein H. Willemsen

**Div. of Medical Genetics, Departments of Pediatrics, Queen's University,
Kingston, ON, K7L 2V7**
Jagdeep S Walia

**Department of Clinical Genetics, Temple Street Children's Hospital, Dublin,
Ireland**
Sally Ann Lynch

**Division Director, Medical Genetics and Metabolism, Children's Hospital of The
King's Daughters, Eastern Virginia Medical School, Norfolk, VA**
Samantha A. Vergano

**Bristol Regional Genetics Service, St Michael's Hospital, Southwell Street,
Bristol, UK**
Ruth Newbury-Ecob

University of Colorado & Children's Hospital Colorado; Denver, CO, USA.
Valerie Slegesky & Naomi Meeks

**Department of Pediatrics, Division of Genetics, University of California, San
Francisco, San Francisco, CA 94115, USA.**
Anne Slavotinek

Département de Génétique Médicale, CHU de Montpellier, Montpellier, France
Patricia Blanchet

**UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR1231 GAD
Génétique des Anomalies du Développement, FHU-TRANSLAD, Dijon, France.**
Ange-Line Bruel

**Cambridge Institute for Medical Research, Department of Medical Genetics
University of Cambridge, Cambridge, UK.**
Evan Reid

Division of Child Neurology, Department of Pediatrics, Santa Casa de São Paulo School of Medical Sciences, São Paulo, Brazil
Paula Girotto

Service de génétique médicale, CHU Nantes, Nantes, France
Isidor Bernard & Benjamin Cogne

Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. College of Medicine, Alfaisal University, Riyadh, Saudi Arabia
Fowzan Alkuraya

Division of Child and Adolescent Neurology, Departments of Neurology and Pediatrics, Mayo Clinic, Rochester, MN, USA
Deborah L. Renaud

GeneDx, Inc., Gaithersburg, MD, USA
Deanna Alexis Carere
Maria J. Guillen Sacoto
Yue Si
Francisca Milan Zamora

Lucile Packard Children's Hospital, Stanford Children's Health, 725 Welch Road, Palo Alto, CA 94304, USA
Natalie Dykzeul,

Medical Genetics Unit, Santa Maria della Misericordia Hospital, University of Perugia, Perugia, Italy
Paolo Prontera,

Medical Genetics Unit, Santa Maria della Misericordia Hospital, University of Perugia, Perugia, Italy
Genetics Unit, "Mauro Baschirotto" Institute for Rare Diseases (B.I.R.D.), Costozza di Longare, Vicenza, Italy
Maria Giovanna Tedesco

Neurology Clinic, Department of Medicine, Santa Maria della Misericordia Hospital, University of Perugia, Perugia, Italy
Costa Cinzia

Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK; Departments of Medical Genetics and Paediatric Neurology, Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK

Ruth Armstrong

Department of Pediatrics, New York University School of Medicine, New York, NY 10016, USA

Rachel Rabin & John Pappas

Wessex Clinical Genetics Services, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom.

Faculty of Medicine, University of Southampton, Southampton, United Kingdom

Nicola Foulds

University of Miami, Miller School of Medicine

Dayna Morel

Department of Clinical Genetics, Oxford University Hospitals NHS Trust, Oxford, United Kingdom

Usha Kini

Clinical Genetics Service, NHS Grampian, Aberdeen Royal Infirmary, Aberdeen AB25 2ZA, UK

John Dean

Corresponding authors:

Schaida Schirwani, Sheffield Children's Hospital, Sheffield, United Kingdom, mobile no: (0044) 7856638949, email: schaida.schirwani@nhs.net

Meena Balasubramanian, Sheffield Children's Hospital, Sheffield, United Kingdom, phone: (0044) 1142717025, e-mail: meena.balasubramanian@nhs.net

CONFLICT OF INTEREST

Deanna Alexis Carere, Maria J. Guillen Sacoto, Yue Si and Francisca Milan Zamora are employees of GeneDx, Inc. No other conflict of interest to declare for the rest of the authors.

ABSTRACT

Purpose

The study aimed at widening the clinical and genetic spectrum of *ASXL3*-related syndrome, a neurodevelopmental disorder, caused by truncating variants in the *ASXL3* gene.

Methods

In this international collaborative study, we have undertaken a detailed clinical and molecular analysis of 45 previously unpublished individuals with *ASXL3*-related syndrome, as well as a review of all previously published individuals. We have reviewed the rather limited functional characterisation of pathogenic variants in *ASXL3* and discuss current understanding of the consequences of the different *ASXL3* variants.

Results

In this comprehensive analysis of *ASXL3*-related syndrome, we define its natural history and clinical evolution occurring with age. We report familial *ASXL3* pathogenic variants, characterise the phenotype in mildly affected individuals and discuss non-penetrance. We also discuss the role of missense variants in *ASXL3*. We delineate a variable but consistent phenotype. The most characteristic features are neurodevelopmental delay with consistently limited speech, significant neuro-behavioural issues, hypotonia and feeding difficulties. Distinctive features include down-slanting palpebral fissures, hypertelorism, tubular nose with a prominent nasal bridge and low-hanging columella.

Conclusion

The presented data will inform clinical management of individuals with *ASXL3*-related syndrome and improve interpretation of new *ASXL3* sequence variants.

Key words: *ASXL3*, *ASXL3*-related syndrome, Bainbridge-Ropers syndrome, BRPS, Intellectual disability, ID, Speech impairment

INTRODUCTION

The additional sex combs-like 3 (*ASXL3* gene, MIM# 615115) was first identified as a disease-causing gene in 2013 with a report of four patients with *de novo* truncating pathogenic variants in the gene and a syndromal neurodevelopmental phenotype (Bainbridge et al., 2013). We have previously made several contributions to the literature with publication of three papers delineating the phenotype of *ASXL3*-related syndrome (Balasubramanian et al., 2017; Myers et al., 2018; Schirwani et al., 2020). So far, clinical details from a total of 45 patients with *ASXL3*-related syndrome have been reported in literature (Bainbridge et al., 2013; Balasubramanian et al., 2017; Contreras-Capetillo, Vilchis-Zapata, Ribbon-Conde, & Pinto-Escalante, 2018; Dad et al., 2017; Dinwiddie et al., 2013; Hori et al., 2016; Koboldt et al., 2018; Kuechler et al., 2017; Myers et al., 2018; Neeta Lakhani, 2017; Qiao, Liu, Ge, & Li, 2019; Schirwani et al., 2020; Srivastava et al., 2016; Verhoeven et al., 2018; Wayhelova et al., 2019). Clinically, *ASXL3*-related syndrome, also known as Bainbridge-Ropers Syndrome (BRPS, MIM# 615485), is characterised by a variable degree of intellectual disability (ID), developmental delay (DD) with absent or very limited speech development, hypotonia, feeding difficulties, behavioural problems and characteristic craniofacial features. Other findings like seizures, skeletal, palatal and dental abnormalities are common but not universal. Abnormal brain scans and Marfanoid habitus have also been described.

The *ASXL* family of genes consist of three members *ASXL1*, *ASXL2* and *ASXL3*, all of which share domains architecture, biological functions and have been implicated in human disease. The conserved domains in *ASXL* family consist of the *ASXN*, *ASXH*, *ASXM1*, *ASXM2* and *PHDs*. The *ASXH*, *ASXM1* and *ASXM2* domains facilitate interaction of epigenetic regulators and nuclear hormone receptors. While *ASXN* and *PHD* are zinc finger domains, that play a role in regulating gene transcription (Kato, 2015; Kato & Kato, 2004). The human *ASXL3* gene with its 12 exons maps to the 18q12.1 chromosome region. *ASXL3* protein has 2248 amino acids (NM_030632.2; NP_085135.1), which is significantly larger than the *ASXL1* and *ASXL2* encoded proteins, highly expressed in cerebral cortex, particularly occipital cortex, ovaries, testicles and smooth muscle (Kato, 2015; Kato & Kato, 2004). Studies on skin fibroblasts from individuals with truncating *ASXL3* variants have shown that *ASXL3* protein is an epigenetic regulator that controls and regulates gene expression through chromatin remodeling (Kato & Kato, 2004; Srivastava et al., 2016). In *ASXL3* knockdown frog embryos, the early stages of neural cell fate specification including early nervous system induction and anteroposterior patterning are disturbed. These embryos also express a lower level of genes required for normal brain, primary neurons and neural crest formation (Licht et al., 2020).

In this report we have undertaken detailed clinical evaluation of 45 individuals with pathogenic or likely pathogenic *ASXL3* variants. We have expanded and clarified the *ASXL3*-related phenotype, delineating important and recurrent clinical associations, which will inform clinical management of individuals with pathogenic variants in this gene and allow better decision-making in the context of a prenatal setting but also better health care provision for children identified early in their clinical presentation.

METHODS

Patients were identified through the Deciphering Developmental Disorders (DDD) research study (Firth, Wright, & Study, 2011) (n=14), the testing lab (GeneDx) which facilitated clinician contact (n=13), GeneMatcher (n=12), or through personal contact following publication of (Balasubramanian et al., 2017) (n=6). The numbers refer to patients we successfully obtained adequate clinical and molecular information from, which was a subset of the total number identified by each source. Individuals with variants classified as likely pathogenic or pathogenic according to ACMG classification recommendations (Richards et al., 2015), and phenotypes consistent with *ASXL3*-related syndrome were included in this study. Data was contributed by paediatric neurologists, paediatricians and, in most instances, clinical geneticists. Detailed phenotypic, molecular, cognitive and behavioural data were collected through a standardized *ASXL3* specific clinical proforma and clinical review by one of the authors. Variants were interpreted using ACMG classification recommendations (Richards et al., 2015). Genomic testing was performed in several laboratories (see supplementary information for further detail).

Ethics statement

Written informed consent for publishing clinical information was obtained from all individuals and/or families. Patients or their legal representatives have provided written consent for using images.

RESULTS

We identified 111 individuals with a clinical diagnosis of *ASXL3*-related syndrome and pathogenic or likely pathogenic variants in *ASXL3*, of which 66 were previously unpublished. Out of the 66 patients, adequate clinical information was obtained from 45, including 24 males and 21 females. The age during last assessment ranged from one month to 37 years. Patients originated from the United States, United Kingdom, Republic of Ireland, Netherlands, Spain, Italy, France, Brazil and Saudi Arabia. Cumulative prevalence of clinical features, growth parameters and milestones within this cohort is summarised in Figure 1. All numbers and percentages are related to the total number of participants for a given feature. Figure 2 A-F shows photographs of patients at various ages. Tables 1, S1 and S2 provide detailed clinical data reported in total of 90 individuals (45 previously unpublished and additional 45 reported in literature). Table S3 is an overview of features reported in one or two individuals.

Clinical data

Perinatal history

In line with previous reports, prenatal history was uneventful in most cases. The average gestational age was 39.2 weeks, with a range between 35 and 42 weeks.

Nutrition and growth

Feeding difficulty was found in 62% of individuals. Out of the seven individuals who needed long-term enteral feeding, one patient continued to require gastrostomy tube at five years of age. Feeding difficulties seem to be worst during infancy. As children got older, feeding difficulties improved, and the reflux and vomiting resolved. However, a small number of individuals continued to have recurrent vomiting,

gastroesophageal reflux disease (GERD) and sensory issues related to food texture that contributed to poor nutrition. Feeding difficulties included poor suck and latch, slow feeding, recurrent vomiting, severe GERD, inability to transition to age-appropriate textured purees and solid foods, feeding refusal behaviours and a self-limiting variety of foods accepted. In eight individuals, failure to thrive (FTT) was the reason for referral.

Generally, height and weight were normal at birth, but often showed significant decline over time. At birth, only two individuals had weight below -2SD, however, measurements later in life show that 16 individuals had weight below -2SD. Seven individuals had a height of below -2SD. Occipitofrontal circumference (OFC) of below -2SD was observed in two individuals at birth. Measurements later in life showed microcephaly in 4 individuals (Figure 1). One individual's OFC dropped from -1.2SD at birth to -4 SD at 26 years of age, with MRI brain being normal. Most individuals were of slender build with normal body proportions.

Craniofacial and dental anomalies

Individuals with *ASXL3*-related syndrome share characteristic facial features comprising down-slanting palpebral fissures, ocular hypertelorism, tubular nose with a prominent nasal bridge and low hanging columella. High-arched and narrow palate, micrognathia, synophrys, deep-set eyes, long face and abnormal head shape were reported in many individuals (Figure 2A-D). Dental abnormalities were a common finding including dental over-crowding, malocclusion, large teeth to severe hypodontia, with either primary or secondary dentition missing (Figure 2E).

Musculoskeletal abnormality

The majority of individuals with *ASXL3*-related syndrome had hypotonia. Hypotonia was usually central and associated with increased tone in the upper and lower limbs. It was more prominent during the neonatal period and infancy, however, in some individuals it continued to be an issue and impacted motor development. Hypermobility was present in some individuals; one individual had a Beighton score 8/9 and another was able to put his legs behind his ears. Digital abnormalities included arachnodactyly, syndactyly, clinodactyly, contractures and tapering fingers (Figure 2E). Pectus excavatum and spinal deformity including scoliosis, kyphoscoliosis, lumbar hyperlordosis and pes planus were noted. An unusual posture and contractures were present with elbow, wrist and fingers held in flexion position (Figure 2C).

Neurodevelopment

Intellectual disability (ID)

ID of variable severity was present in all individuals who could be assessed. Severity of ID ranged from mild with one individual able to hold a part-time job, while others had profound learning difficulties and needed significant support (Figure 1).

Motor development

Gross motor skills were generally delayed in individuals with *ASXL3*-related syndrome. Motor skills ranged from normal to severely delayed, 22% sat independently at or before the age of nine months, while only 11% walked at or before 18 months of age (Figure 1). The average walking age in individuals who started walking after 18 months of age was three years old. One individual had

started taking few steps using a frame at the age of six years; two individuals had not yet achieved walking at seven years and another was only able to walk with assistance at 10 years.

Speech and language development

All individuals with *ASXL3*-related syndrome had significantly delayed speech and language development. In this cohort, 48% were completely non-verbal, the age range in this group was 2-32 years with an average age of 6.6 years. In 52% of individuals, speech acquisition was achieved at an average age of 39 months; the majority had less than 10 words, while three individuals were able to speak in short sentences (Figure 1). In older non-verbal individuals, there was history of regression after acquisition of few words as toddlers. Despite absent/limited expressive language, many individuals had better receptive language skills and communicated successfully using alternative methods, such as augmentative and alternative communication (AAC).

Behavioural phenotype

A diagnosis of autism spectrum disorder (ASD) was made in 30% of individuals (Figure 1). Autistic traits including stereotypies, poor eye contact, hand flapping, rocking and head shaking, which were present in the majority of individuals. Some individuals were assessed for ASD but did not fulfil the criteria. Physical aggression was commonly reported in individuals in association with frustration and inability to express and communicate feelings. Self-injurious behaviour, including self-biting, face scratching and head banging was common too. Onset of self-injurious behaviour was as early as age two years; in other individuals, this did not start until later in life. Other behaviours included fascination with water, bruxism, inappropriate laughter, screaming, grunting and smearing faeces.

Seizures and structural brain abnormalities

Seizures were reported in 25% of individuals in this cohort. The age of onset varied from infancy to teenage years. Seizures ranged from generalized tonic-clonic seizures to absence seizures with no predominant type. Individuals with later onset epilepsy had more severe epilepsy that continued into adult life and tended to be intractable.

The electroencephalogram (EEG) findings were obtained in 12/45 individuals with reportedly normal or equivocal findings in seven individuals and abnormal findings such as multifocal generalised seizure, theta rhythmic activities, diffusely increased beta waves in five individuals. Generally, seizures responded well to standard anti-epileptic medications.

There were no characteristic findings on brain MRI, a majority of the 21% who had abnormal brain MRIs had white matter abnormalities, cerebellar vermis hypoplasia and corpus callosal abnormality (Figure 1) (Table S4 summarises the structural brain abnormalities reported in *ASXL3*-related syndrome in the current and previously published cohorts).

Other phenotypes

A total of 10 individuals were described as having abnormal breathing patterns including apnoea, breath-holding episodes and irregular breathing patterns

particularly at night, which coincided with sleep disturbances. Polysomnography results were available in three individuals, one of whom was diagnosed with moderate obstructive sleep apnoea. Recurrent ear and upper respiratory tract infections were common. Echocardiography was performed in nine individuals for various reasons and showed normal heart structure. Mild conductive hearing loss was reported in three individuals in this cohort. Cancers were not reported in our cohort.

Updated clinical information

P5 was 3.4 years and non-verbal at the time of recruitment. At six year and 10 months he has acquired 50-100 words. P11 was 10 months at the time of recruitment. At the age of four year and six months, she continues to be gastrostomy fed and unable to feed orally. She is unable to walk, non-verbal and has severe behavioural issues with self-harm and persistent crying.

Variants

In this cohort we identified 38 novel variants in 45 previously unpublished individuals. One variant was a gross deletion affecting exons 2-8, identified through chromosomal microarray (CMA). With the exception of one variant on exon three, all the other sequence variants were located in exons 11 and 12. Table 2, Table S5 and Figure 3 detail all *ASXL3* variants reported in this study as well as variants previously published in the international peer-reviewed literature (PubMed database) and Human Gene Mutation Database (HGMD professional 2019.4) (Stenson et al., 2017).

Interestingly, we observed recurrent variants in the published and our current cohort. The c.3106C>T p.(Arg1036*)(De Rubeis et al., 2014; Koboldt et al., 2018; Kosmicki et al., 2017; Kuechler et al., 2017; Myers et al., 2018) variant has been observed in six individuals, c.4330C>T p.(Arg1444*)(Balasubramanian et al., 2017; Srivastava et al., 2016) in five, c.4399C>T p.(Arg1467*) in three, and c.4534C>T p.(Gln1512*) and c.1534_1535del p.(Leu512fs) in two individuals each. Variants c.4534C>T p.(Gln1512*), c.2791_2792del p.(Gln931fs) and c.4441dup p.(Leu1481fs) were inherited, and the rest were *de novo*.

DISCUSSION

Clinical and diagnostic relevance

In the probands recruited to the DDD study, a study aimed to identify the cause of ID using large-scale exome sequencing, the prevalence of *de novo* variants in *ASXL3* was estimated at approximately 1/193 (50/9,625). This puts *ASXL3* among the top 10 neurodevelopmental genes for the frequency of *de novo* variants (Wright et al., 2015). Therefore, it is expected that a larger number of individuals with *ASXL3*-related syndrome will be identified with the growing use of genome/exome sequencing for diagnosis of neurodevelopmental disorders. With rare exceptions, diagnosis of *ASXL3*-related syndrome was made through exome/genome sequencing methods rather than clinical evaluation. This could be attributed to various reasons including lack of diagnostic clues, distinctive facial features, lack of familiarity of clinicians with this specific condition and/or expanding use of genome/exome sequencing as a first-line molecular diagnostic test.

Although the clinical diagnosis of *ASXL3*-related syndrome relies on molecular confirmation of the pathogenic variant in *ASXL3*, a distinctive phenotype is emerging and can be identified. It may not be an instantly recognisable syndrome based on facial appearance alone, but the facial features along with the history of ID, hypotonia, feeding difficulties and speech delay should prompt the clinician to consider this diagnosis. With a significant number of the more recently identified genes associated with developmental delay, one may speculate that phenotypes associated with one gene may be attributable to variants in other genes. However, facial features, albeit subtle, in addition to other features could be helpful in characterizing pathogenicity of variants and assigning causality when a variant is identified on gene panels, whole exome or whole genome sequencing. As these next generation sequencing technologies are utilized throughout the world, many additional individuals with *ASXL3*-related syndrome are likely to be identified.

This study changes the previous perception regarding *ASXL3*-related syndrome as a profound-severe neurodevelopmental disorder and highlights the prevalence of milder ID in some individuals with *ASXL3*-related syndrome. Previously, we reported an individual with apparently normal intelligence at age five years (Schirwani et al., 2020). However, when it comes to assessing cognition and behaviour, reliability is a major challenge. Most data on cognition and behaviour in the currently published individuals is based on subjective information from clinicians rather than formal testing results. Furthermore, neurobehavioral abnormalities, which are a common feature of *ASXL3*-related syndrome, may complicate the picture and impact cognitive function.

Natural history

Prenatal period and birth tend to progress normally. Feeding difficulties are present in the majority of individuals and tend to be more problematic during infancy leading to FTT in some. Development is generally delayed, particularly speech and language, with nearly half of individuals being non-verbal. Although motor delay is present in a majority of individuals, it is usually mild and tends to be made more pronounced due to a combination of hypotonia, hypermobility and FTT. The majority of individuals were referred for evaluation due to FTT, DD and hypotonia. Some individuals were not diagnosed until they were assessed later in life for behavioural problems and autism. Skeletal abnormalities, particularly digital abnormalities were common, but no major limb defects were reported. Seizure occurs in around quarter of individuals, it can be a single episode seizure, or it may continue into adulthood, and is well controlled in most individuals. Structural brain abnormalities were seen in 5/12 of brain MRIs, but other congenital anomalies (cardiac anomalies, renal anomalies and cleft palate) were very rare.

Truncating variants in *ASXL3*

The exact function of the *ASXL3* protein is not well understood which resulted in some uncertainty over the mechanism through which *ASXL3* variants impacted neurodevelopment. Both dominant negative (Bainbridge et al., 2013) and a loss of function mechanism (Srivastava et al., 2016) have been proposed in this gene. Evidence from animal models suggests that truncated protein can be functional depending on the location of the truncating variants (Lichtig et al., 2020). *ASXL3* is

predicted to be intolerant to protein-truncating variants with the probability of loss of function intolerance (pLI) of 1 and observed / expected (o/e) metric of 0.1. We also know that *ASXL3* knock-down frog embryos have a reduced number of primary neurons with disturbed neuronal arrangement (Lichtig et al., 2020). The last two exons of *ASXL3* (exons 11 and 12) harbour almost 98% of the variants found in this gene, consistent with their large size encompassing 83% of its coding sequence. In our study, one variant was located in exon 3 and previously a pathogenic variant was reported in exon 10 in an individual with *ASXL3*-related syndrome. (Balasubramanian et al., 2017) An *ASXL3* allele with a truncating variant in exon 12 (the last and largest exon) is expected to escape NMD, while alleles with variants within the penultimate exon (exon 11) are expected to be associated with NMD if they are located upstream of base c.2985 or codon p.(995), which is 55 bps away from the last exon/intron junction. We have decided to use the PVS1 evidence (see variant table for details) at a very strong level for all truncating variants which are expected to be associated with NMD, i.e. Located upstream of base c.2985. The PVS1 strength has been used with a one level downgrade to a strong level for truncating variants within the last exon of the gene and expecting to result in the loss of equal or larger than 10% of the protein size (Abou Tayoun et al., 2018). The cut off for a 10% loss in protein size is estimated to be at base c.6072 and codon p.(2024). Therefore, all truncating variants within the last exon and which are upstream of base c.6072 has been given a PVS1 pathogenicity evidence at a strong level (see Table 2 for more details).

Missense variants in *ASXL3*

As genomic studies become the gold standard for the identification of causative gene changes in individuals with neurodevelopmental disorders, an increasing number of missense variants are being identified. Assigning likely pathogenic/pathogenic status to missense variants continues to be challenging especially when no functional assessment of the variants is available. Genomic studies have identified missense variants in *ASXL3* in individuals with ASD in the absence of other characteristics of *ASXL3*-related syndrome (De Rubeis et al., 2014; Dinwiddie et al., 2013). Homozygous missense variants in *ASXL3* have also been implicated in *ASXL3*-related syndrome (Table S5). However, these variants classify as likely benign or variants of uncertain significance (VUS) according to ACMG guidelines, and in at least one report the patient has an alternative molecular finding, in keeping with their phenotype (Table S6).

Population data obtained from apparently typical individuals (gnomAD v2.1.1) shows constraint z score of 0.61, which indicates lower constraint and more tolerance to missense variation. The missense variants seem to be distributed throughout the gene, while the truncating variants are clustered in the last two exons of the gene. This is an area that requires further work; as more information is gathered on missense variants, our predictive ability to interpret the clinical significance of missense changes will improve.

Penetrance

Direct estimation of penetrance of rare variants is challenging, however, the inheritance mechanism and prevalence of apparently pathogenic variants in population databases can provide some guidance. Previously published pathogenic

variants in *ASXL3* were apparently *de novo* in all cases where biological parents could be tested. Although the vast majority of *ASXL3* variants identified in this study were *de novo*, three inherited variants were identified. Variants p.(Gln1512*) in P2 and p.(Gln931fs) in P10, have been inherited from affected parents and p.(Leu1481fs) in P22, has been inherited from an apparently asymptomatic mother who had a higher education qualification; there was no evidence of mosaicism in the tested blood samples. This, however, does not rule out mosaicism in these individuals as other tissues could be mosaic for the variants. Analyses of gnomAD database revealed eighteen nonsense/frameshift variants (Table S7). In three families, three non-twin siblings were found to have *de novo* pathogenic variants indicating gonadal mosaicism (Koboldt et al., 2018; Schirwani et al., 2020). We also reported an 11 year old girl with hypotonia, feeding difficulties, ID, DD, and behavioural issues who had a *de novo* pathogenic variant in *ASXL3* in 30-35% of both blood and saliva samples on trio-exome sequencing. This may indicate that only 30% mosaicism may be sufficient to cause the phenotype (Schirwani et al., 2020). Taken together, these data suggest that while the penetrance of *ASXL3* pathogenic variants appears to be high and the variants are more likely to be *de novo*; reduced- or even non-penetrance should be considered. When counselling asymptomatic parents of an affected child, *de novo* status of the variant should be confirmed prior to counselling on recurrence risk in future pregnancies.

Limitations and future prospects

Although we have undertaken a comprehensive literature review of all previously reported cases and present the largest cohort of previously unpublished individuals so far, the numbers are still small. We did not include variants that were classified as VUS according to the current ACMG guidelines (Richards et al., 2015), but we expect that some of these classifications may change as we learn more about the condition. Moreover, many variants are reported with very limited clinical data preventing the effort to delineate the phenotype associated with *ASXL3*-related syndrome. More information on larger groups of individuals with *ASXL3*-related syndrome is needed to determine the complete phenotypic spectrum. We have tried to account for different sources of bias when estimating various clinical features, however, it is possible that existence of some features is over or underestimated. Detailed studies of cognition, behavioral and psychiatric phenotypes through neuropsychiatric screening is crucial for all individuals with *ASXL3*-related syndrome to enable early recognition of such concerns and access to relevant support systems. Our observations suggest that children, who have not produced spoken words by the age of 6 years, are unlikely to acquire expressive language. Therefore, early introduction of intervention strategies alternative to speech is essential to improve communication.

Molecular and cellular mechanisms underlying *ASXL3* protein function and the pathways through which neurodevelopment is impacted remains unclear. Studying epi-signatures in individuals with variants in *ASXL3* may elucidate the effect of these variants on various biological pathways, cellular function and genotype-phenotype association. Identifying unique signatures associated with specific types of variants may help differentiate between benign and pathogenic variants. This will aid interpretation of the increasing number of *ASXL3* variants recognized through exome or genome sequencing, particularly in the prenatal setting (Bacrot et al., 2018).

Furthermore, all confirmed pathogenic *ASXL3* variants reported to date have been assumed or confirmed *de novo*, limiting the study of intra-familial phenotypic variability, penetrance, expressivity and recurrence risk. Until now the recurrence risk of *ASXL3*-related syndrome has been predicted to be low, albeit higher than the general population due to the possible higher risk of germline mosaicism (Koboldt et al., 2018; Schirwani et al., 2020). We emphasize the importance of parental testing prior to providing clinical recurrence risk estimates given the potential inheritance from a mildly affected or apparently asymptomatic parents.

Elucidating the role of *ASXL3* in normal development will help better understand the mechanisms through which variants in the gene may lead to neurodevelopmental disorder. It is also important to understand the variability in phenotype in individuals with *ASXL3*-related syndrome and interaction of the underlying pathogenesis in *ASXL3* with the resulting phenotype. This variability may be due to the type of variation in *ASXL3*, genetic background of the individual or environmental factors. Further research is needed to explore the function of both normal and variant *ASXL3* protein. This may pave the way for exploring potential therapies that directly target *ASXL3* to replace the current symptom-guided approach for individual management.

The identification of a large number of individuals (n=45) for a rare genetic condition emphasizes the importance of data sharing, allowing delineation of clinical phenotype of *ASXL3*-related syndrome.

CONCLUSION

By presenting and analysing data from a total of 90 individuals with *ASXL3*-related syndrome, we show that *de novo* and inherited variants in *ASXL3* cause a distinctive neurodevelopmental phenotype, hallmarked by a variable degree of ID and DD with significant speech delay as a consistent feature, a variety of behavioural disorders, severe feeding difficulties, hypotonia and distinctive facial features.

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CONTRIBUTIONS

SS established collaborations, performed data collection, curation and analysis, and wrote the manuscript, SA analysed variants, MB supervised the project; all authors contributed to data collection and manuscript review

REFERENCES

- Abou Tayoun, A. N., Pesaran, T., DiStefano, M. T., Oza, A., Rehm, H. L., Biesecker, L. G., . . . ClinGen Sequence Variant Interpretation Working, G. (2018). Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat*, 39(11), 1517-1524. doi:10.1002/humu.23626
- Bacrot, S., Mechler, C., Talhi, N., Martin-Coignard, D., Roth, P., Michot, C., . . . Attie-Bitach, T. (2018). Whole exome sequencing diagnoses the first fetal case of Bainbridge-Ropers syndrome presenting as pontocerebellar hypoplasia type 1. *Birth Defects Res*, 110(6), 538-542. doi:10.1002/bdr2.1191
- Bainbridge, M. N., Hu, H., Muzny, D. M., Musante, L., Lupski, J. R., Graham, B. H., . . . Ropers, H. H. (2013). De novo truncating mutations in ASXL3 are associated with a novel clinical phenotype with similarities to Bohring-Opitz syndrome. *Genome Med*, 5(2), 11. doi:10.1186/gm415
- Balasubramanian, M., Willoughby, J., Fry, A. E., Weber, A., Firth, H. V., Deshpande, C., . . . Tomkins, S. (2017). Delineating the phenotypic spectrum of Bainbridge-Ropers syndrome: 12 new patients with de novo, heterozygous, loss-of-function mutations in ASXL3 and review of published literature. *J Med Genet*, 54(8), 537-543. doi:10.1136/jmedgenet-2016-104360
- Contreras-Capetillo, S. N., Vilchis-Zapata, Z. H., Ribbon-Conde, J., & Pinto-Escalante, D. (2018). Global developmental delay and postnatal microcephaly: Bainbridge-Ropers syndrome with a new mutation in ASXL3. *Neurologia*, 33(7), 484-486. doi:10.1016/j.nrl.2017.01.022
- Dad, R., Walker, S., Scherer, S. W., Hassan, M. J., Kang, S. Y., & Minassian, B. A. (2017). Hyperventilation-athetosis in ASXL3 deficiency (Bainbridge-Ropers) syndrome. *Neurol Genet*, 3(5), e189. doi:10.1212/NXG.0000000000000189
- De Rubeis, S., He, X., Goldberg, A. P., Poultney, C. S., Samocha, K., Cicek, A. E., . . . Buxbaum, J. D. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*, 515(7526), 209-215. doi:10.1038/nature13772
- Dinwiddie, D. L., Soden, S. E., Saunders, C. J., Miller, N. A., Farrow, E. G., Smith, L. D., & Kingsmore, S. F. (2013). De novo frameshift mutation in ASXL3 in a patient with global developmental delay, microcephaly, and craniofacial anomalies. *BMC Med Genomics*, 6, 32. doi:10.1186/1755-8794-6-32
- Firth, H. V., Wright, C. F., & Study, D. D. D. (2011). The Deciphering Developmental Disorders (DDD) study. *Dev Med Child Neurol*, 53(8), 702-703. doi:10.1111/j.1469-8749.2011.04032.x
- Hori, I., Miya, F., Ohashi, K., Negishi, Y., Hattori, A., Ando, N., . . . Saitoh, S. (2016). Novel splicing mutation in the ASXL3 gene causing Bainbridge-Ropers syndrome. *Am J Med Genet A*, 170(7), 1863-1867. doi:10.1002/ajmg.a.37653
- Katoh, M. (2015). Functional proteomics of the epigenetic regulators ASXL1, ASXL2 and ASXL3: a convergence of proteomics and epigenetics for translational medicine. *Expert Rev Proteomics*, 12(3), 317-328. doi:10.1586/14789450.2015.1033409
- Katoh, M., & Katoh, M. (2004). Identification and characterization of ASXL3 gene in silico. *Int J Oncol*, 24(6), 1617-1622. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/15138607>
- Koboldt, D. C., Mihalic Mosher, T., Kelly, B. J., Sites, E., Bartholomew, D., Hickey, S. E., . . . White, P. (2018). A de novo nonsense mutation in ASXL3 shared by siblings with Bainbridge-Ropers syndrome. *Cold Spring Harb Mol Case Stud*, 4(3). doi:10.1101/mcs.a002410

- Kosmicki, J. A., Samocha, K. E., Howrigan, D. P., Sanders, S. J., Slowikowski, K., Lek, M., . . . Daly, M. J. (2017). Refining the role of de novo protein-truncating variants in neurodevelopmental disorders by using population reference samples. *Nat Genet*, *49*(4), 504-510. doi:10.1038/ng.3789
- Kuechler, A., Czeschik, J. C., Graf, E., Grasshoff, U., Huffmeier, U., Busa, T., . . . Wieczorek, D. (2017). Bainbridge-Ropers syndrome caused by loss-of-function variants in ASXL3: a recognizable condition. *Eur J Hum Genet*, *25*(2), 183-191. doi:10.1038/ejhg.2016.165
- Lichtig, H., Artamonov, A., Polevoy, H., Reid, C. D., Bielas, S. L., & Frank, D. (2020). Modeling Bainbridge-Ropers Syndrome in *Xenopus laevis* Embryos. *Front Physiol*, *11*, 75. doi:10.3389/fphys.2020.00075
- Myers, K. A., White, S. M., Mohammed, S., Metcalfe, K. A., Fry, A. E., Wraige, E., . . . Scheffer, I. E. (2018). Childhood-onset generalized epilepsy in Bainbridge-Ropers syndrome. *Epilepsy Res*, *140*, 166-170. doi:10.1016/j.eplepsyres.2018.01.014
- Neeta Lakhani, P. V., Chaya Kapugama and Deciphering Developmental Disorders Study DDD Study. (2017). *Milder phenotype in a boy with Bainbridge Ropers Syndrome and ASXL3 mutation. Expanding the phenotypic spectrum*. Retrieved from
- Qiao, L., Liu, Y., Ge, J., & Li, T. (2019). Novel Nonsense Mutation in ASXL3 causing Bainbridge-Ropers Syndrome. *Indian Pediatr*, *56*(9), 792-794.
- Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., . . . Committee, A. L. Q. A. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, *17*(5), 405-424. doi:10.1038/gim.2015.30
- Schirwani, S., Hauser, N., Platt, A., Punj, S., Prescott, K., Canham, N., . . . Balasubramanian, M. (2020). Mosaicism in ASXL3-related syndrome: Description of five patients from three families. *Eur J Med Genet*, 103925. doi:10.1016/j.ejmg.2020.103925
- Srivastava, A., Ritesh, K. C., Tsan, Y. C., Liao, R., Su, F., Cao, X., . . . Bielas, S. L. (2016). De novo dominant ASXL3 mutations alter H2A deubiquitination and transcription in Bainbridge-Ropers syndrome. *Hum Mol Genet*, *25*(3), 597-608. doi:10.1093/hmg/ddv499
- Stenson, P. D., Mort, M., Ball, E. V., Evans, K., Hayden, M., Heywood, S., . . . Cooper, D. N. (2017). The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet*, *136*(6), 665-677. doi:10.1007/s00439-017-1779-6
- Verhoeven, W., Egger, J., Rakers, E., van Erkelens, A., Pfundt, R., & Willemsen, M. H. (2018). Phenotypic characterization of an older adult male with late-onset epilepsy and a novel mutation in ASXL3 shows overlap with the associated Bainbridge-Ropers syndrome. *Neuropsychiatr Dis Treat*, *14*, 867-870. doi:10.2147/NDT.S153511
- Wayhelova, M., Oppelt, J., Smetana, J., Hladilkova, E., Filkova, H., Makaturova, E., . . . Kuglik, P. (2019). Novel de novo frameshift variant in the ASXL3 gene in a child with microcephaly and global developmental delay. *Mol Med Rep*, *20*(1), 505-512. doi:10.3892/mmr.2019.10303
- Wright, C. F., Fitzgerald, T. W., Jones, W. D., Clayton, S., McRae, J. F., van Kogelenberg, M., . . . study, D. D. D. (2015). Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. *Lancet*, *385*(9975), 1305-1314. doi:10.1016/S0140-6736(14)61705-0

FIGURE LEGENDS

Figure 1. Overview of clinical features in individuals with *ASXL3*-related syndrome.

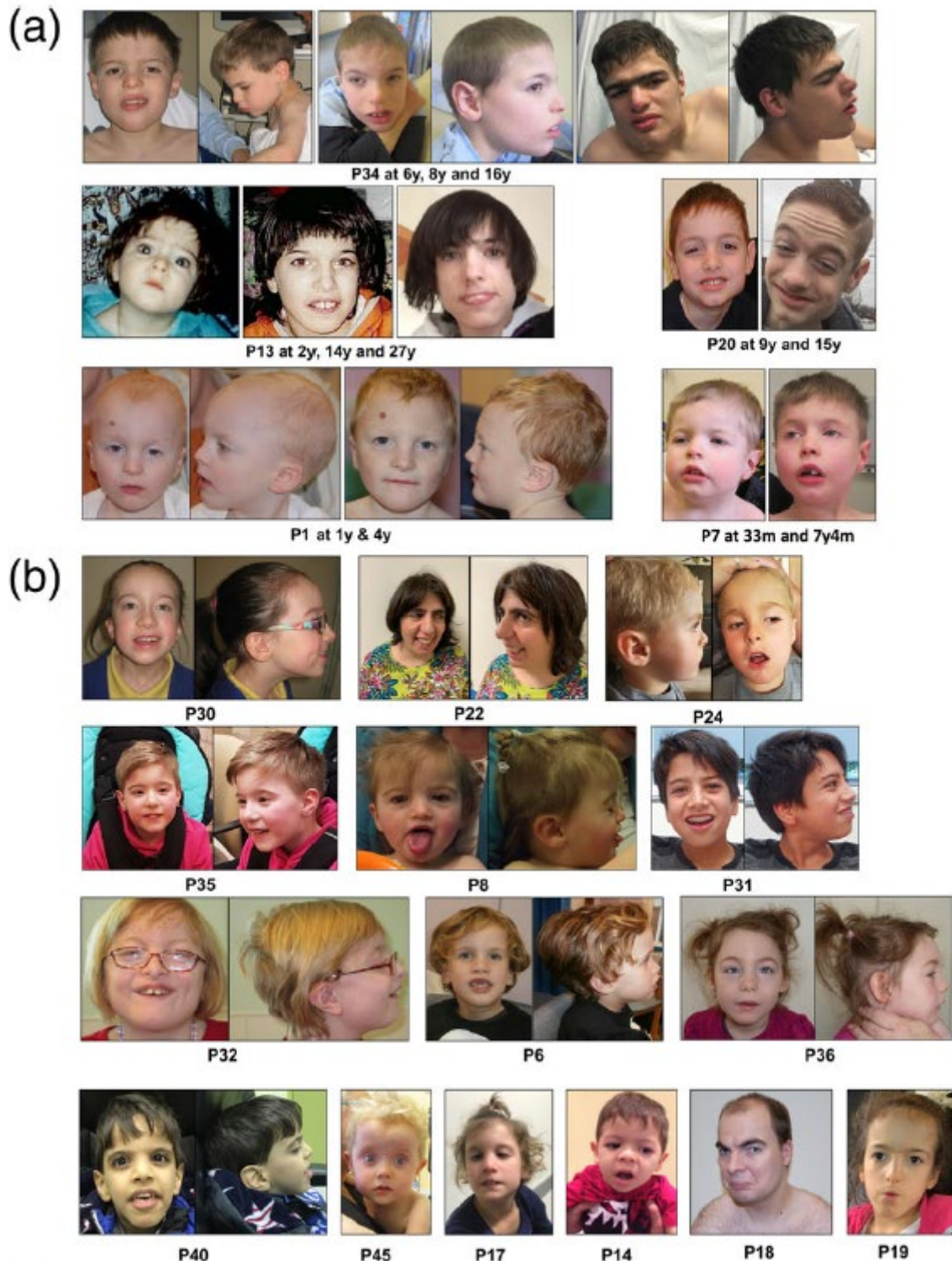
Figure 2 A. The evolving facial appearance in six individuals with *ASXL3*-related syndrome; B. The facial appearance of adults and children with *ASXL3*-related syndrome; C. Evolving facial appearance and unusual body positioning posture with bent elbow, wrists and fingers with slight radial deviation of the hand; D. Composite facial features as generated by facial recognition technology as provided by Face2Gene in control group and individuals with *ASXL3*-related syndrome; E. Dental phenotype characterized by overcrowding, mal-alignment and severe hypodontia; F. Shows tapering finger, clinodactyly and arachnodactyly.

Figure 3 A. Distribution of *ASXL3* variants in our cohort; B. Variant types in our cohort and previously published literature. Protein domains and corresponding codons are as follows, ASXN (1-84), ASXH (250-363), ASXM1 (964-1058), ASXM2 (1741-1765).



Figure 1. Overview of clinical features in individuals with ASXL3-related syndrome.

Figure 2. A. The evolving facial appearance in six individuals with *ASXL3*-related syndrome; B. The facial appearance of adults and children with *ASXL3*-related syndrome; C. Evolving facial appearance and unusual body positioning posture with bent elbow, wrists and fingers with slight radial deviation of the hand; D. Composite facial features as generated by facial recognition technology as provided by Face2Gene in control group and individuals with *ASXL3*-related syndrome; E. Dental phenotype characterized by overcrowding, mal-alignment and severe hypodontia; F. Shows tapering finger, clinodactyly and arachnodactyly.



(c)



(d)



Unaffected controls

ASXL3-related disorder

(e)



(f)



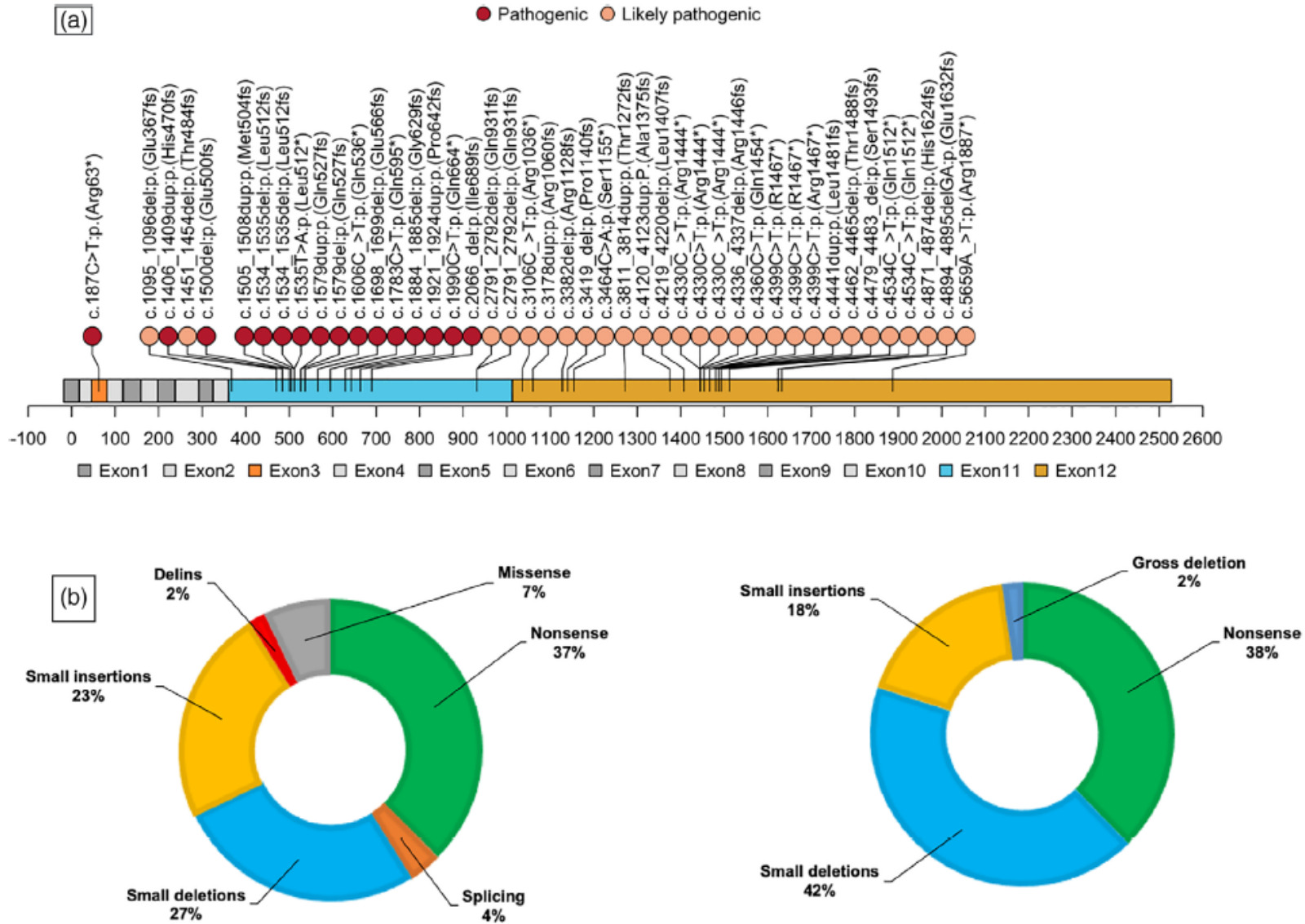


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Table 1.

An overview summary of the common phenotypic features of patients in this study and previously reported patients with ASXL3-related syndrome

Clinical features	Current study (n = 45)	Previous studies (total n = 45)	Total (n = 90)
Feeding difficulties	28/40	35/41	63/81
Hypotonia	33/40	38/42	71/82
Characteristic craniofacial features	31/31	41/42	72/73
Downslanting palpebral fissures	21/33	27/40	48/73
Palatal abnormality	21/30	23/36	44/66
Speech delay/absent	43/43	39/39	82/82
Intellectual disability	29/29	39/40	68/69
Behavioral issues	29/36	28/32	57/68
Seizure	11/39	17/38	28/77
Skeletal features	27/35	29/36	56/71

Table 2.

Variants in this study with ACMG classification

ID	Pt no	Nucleotide variant/CNV	Exon	Predicted protein change	Source	ACMG/ACGS	Classification	Comments
258284	P1	c.4479_4483del	12	p.(Ser1493fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
286691	P2	c.4534C>T	12	p.(Gln1512*)	Mat*	PVS1_str, PM2	Likely-path	>10% of protein lost
300784	P3	c.2066del	11	p.(Ile689fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
362009	P4	c.1534_1535del	11	p.(Leu512fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
307301	P5	c.5659A>T	12	p.(Arg1887*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
305747	P6	c.3811_3814dup	12	p.(Thr1272fs)	De novo	PVS1_str, PM2	Likely-path	>10% of protein lost
275275	P7	c.1606C>T	11	p.(Gln536*)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
274520	P8	c.1921_1924dup	11	p.(Pro642fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
286921	P9	c.4330C>T	12	p.(Arg1444*)	De novo	PVS1_str, PM2	Likely-path	>10% of protein lost

P10	c.2791_2792del	11	p.(Gln931fs)	Pat*	PVS1, PM2	Likely-path	NMD likely
P11	c.2791_2792del	11	p.(Gln931fs)	N/A	PVS1, PM2	Likely-path	NMD likely
P12	c.3382del	12	p.(Arg1128fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
P13	c.1095_1096del	11	p.(Glu367fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
P14	c.1884_1885del	11	p.(Gly629fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
P15	c.55_879del	2-8	p.(Ala19_ Gln293del)	De novo	PM4, PM2, PS2_mod	Likely-path	Arr[hg19] 18q12.1 (31172484_31311379)x1 dn. Size of intragenic deletion is 139 kb.
P16	c.1534_1535del	11	p.(Leu512fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
P17	c.1579dup	11	p.(Gln527fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
P18	c.4894_4895del	12	p.(Glu1632fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
p19	c.1451_1454del	11	p.(Thr484fs)	De novo	PVS1, PM2	Likely-path	NMD likely
p20	c.1990C>T	11	p.(Gln664*)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely

p21	c.1698_1699del	11	p.(Glu566fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
p22	c.4441dup	12	p.(Leu1481fs)	Mat	PVS1_str, PM2	Likely-path	>10% of protein lost
p23	c.4462_4465del	12	p.(Thr1488fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
p24	c.1579del	11	p.(Gln527fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
p25	c.1535T>A	11	p.(Leu512*)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
p26	c.4399C>T	12	p.(R1467*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
p27	c.4399C>T	12	p.(R1467*)	Unknown	PVS1_str, PM2	Likely-path	>10% of protein lost
p28	c.1783C>T	11	p.(Gln595*)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
p29	c.4871_4874del	12	p.(His1624fs)	De novo	PVS1_str, PM2	Likely-path	>10% of protein lost
p30	c.3178dup	12	p.(Arg1060fs)	De novo	PVS1_str, PM2	Likely-path	>10% of protein lost
p31	c.4330C>T	12	p.(Arg1444*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost

	p32	c.1500delA	11	p.(Glu500fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
	p33	c.3464C>A	12	p.(Ser1155*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
262694	p34	c.3106C>T	12	p.(Arg1036*)	Unknown	PVS1_str, PM2	Likely-path	>10% of protein lost
	p35	c.4360C>T	12	p.(Gln1454*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
	p36	c.4399C>T	12	p.(Arg1467*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
305643	p37	c.4534C>T	12	p.(Gln1512*)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
	p38	c.1505_1508dup	11	p.(Met504fs)	De novo	PVS1, PM2, PS2_mod	Path	NMD likely
	p39	c.4219_4220del	12	p.(Leu1407fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
	p40	c.4120_4123dup	12	P.(Ala1375fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
	p41	c.4336_4337del	12	p.(Arg1446fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost
274637	p42	c.3419del	12	p.(Pro1140fs)	De novo	PVS1_str, PM2, PS2_mod	Likely-path	>10% of protein lost

264183	p43	c.1406_1409dup	11	p.(His470fs)	De novo	PVS1, PM2	Path	NMD likely
263416	p44	c.4330C>T	12	p.(Arg1444*)	De novo	PVS1_str, PM2	Likely-path	>10% of protein lost
	p45	Nc.187C>T	3	p.(Arg63*)	De novo	PVS1, PM2, PS2_mod	Path	

Notes: ID refers to DECIPHER ID; Path, pathogenic; Pat, paternal; Mat, maternal; NMD, nonsense mediated decay, N/A, not available. *Parent is affected with a phenotype consistent with the ASXL3-related syndrome. 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 to strong for truncating variants within the last exon but predicted to result in >10% loss of protein size (PVS1_str). 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.