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An International *ASXL3* Natural History Study: Deep Phenotypic Analyses Including Detailed Reports of a Milder Phenotype, Novel Associations, and Clinical Recommendations

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ABSTRACT

Natural History Studies can help inform clinician and caregiver expectations, form the basis of management guidelines, and provide a comparator for therapeutic intervention. In rare conditions, where collection of prospective longitudinal data is untimely and impractical, quasi-natural history data—from multiple individuals of different ages—provides an alternative approach. A detailed genotype–phenotype analysis of 64 individuals with pathogenic or likely pathogenic *ASXL3* variants was carried out, comprising qualitative and quantitative data. The majority of data was collected through direct clinic consultation with the individual and/—or caregiver(s). We report significant phenotypic variability, but improvement trends in feeding, hypotonia, verbalisation, and motor skills over time. Findings include: an increased prevalence of antenatal and neonatal structural anomalies, an emerging renal phenotype, a tendency for poor post-natal growth (with novel reports of obesity later in childhood), and a lower-than-expected prevalence of seizures (compared to the existing literature). We also provide the first qualitative descriptions of several mildly affected probands, at different ages. Our recommendations include: baseline renal imaging after diagnosis, and Dental and Ophthalmological follow-up for all. We describe the largest-to-date cohort of individuals with *ASXL3*-related disorder, including 24 novel variants, novel clinical findings, quasi-natural history trends, management insights, and recommendations.

1 | Introduction

ASXL3-related disorder is a neurodevelopmental syndrome, often comprising delayed development (or intellectual disability), behavioral problems, feeding and growth problems, and musculoskeletal abnormalities (Schirwani et al. 2021). It refers to the rare genetic disorder caused by loss-of-function variants in the *ASXL3* gene, located at 18q12.1, which was first described by (Bainbridge et al. 2013). Since then, an increasingly variable phenotype has emerged, as more cases have been documented in the medical literature (Woods et al. 2024).

ASXL3 is a chromatin modifying gene; the ASXL3 protein plays a role in epigenetic mechanisms and transcriptional processes (Katoh 2015; Katoh and Katoh 2004). However, specific molecular pathways are not yet fully understood. Due to the phenotypic variability seen in some families with inherited *ASXL3* (Schirwani et al. 2023), there are likely additional genetic or non-genetic factors at play that influence gene expressivity.

As a rare genetic syndrome, global efforts are required to improve our understanding. Natural history data is crucial for providing a basis and comparator for possible future therapeutic intervention. We aim to expand the genotypic and phenotypic spectrum of the condition and elucidate the natural history of *ASXL3*-related disorder by conducting clinic appointments with individuals and their families on an international scale, with subsequent plans for follow-up over time.

Here we describe the findings from 1 year of an international natural history study, with a focus on the deep quasi-natural history data of 64 individuals of different ages with loss-of-function *ASXL3* variants. We describe the vast clinical spectrum, including some novel associations.

2 | Methods

Individuals were identified through patient self-enrolment, direct clinician contact, or the local NHS Genetics Laboratory with invites to participate directly (Data S1). Natural History Study details were disseminated by the ASXL Rare Research Endowment (ARRE) Foundation, at UK Clinical Genetics meetings, Society groups, and at a UK-based Conference. Meetings were carried out and data were collected between 1 August 2023 and 1 August 2024.

2.1 | Natural History Study

This analysis comprises 57 individuals enrolled in the Natural History Study. Individuals of any age, from any country, were eligible for recruitment. Relevant study consent was obtained from individuals or their families (IRAS: 316055). All individuals had a confirmed pathogenic, or likely pathogenic, *ASXL3* variant and absence of a dual genetic diagnosis. Variants were classified as per the relevant international ACMG standards (Richards et al. 2015; Riggs et al. 2020) and the UK ACGS best practice guidelines (Durkie et al. 2023).

A detailed clinical assessment was carried out by face-to-face or virtual consultation by one member of the research team (EW), obtaining deep qualitative and quantitative phenotypic data for 55/57 individuals. Reports were occasionally sought from Genomic Medicine Services, Laboratories or local clinicians, as necessary. Data for two individuals were provided by their clinicians only, by means of the standardized study proforma.

2.2 | Additional Individuals

Clinician-reported data from an additional seven individuals, with a pathogenic or likely pathogenic *ASXL3* variant, not enrolled in the Natural History Study, are also included in this analysis. Six of these individuals were excluded from the Natural History Study due to a significant language barrier, and one individual was excluded from the Natural History Study due to a dual diagnosis of recurrent de novo 17q23.1q23.2 interstitial deletion (MIM #613355). Though these clinician reports may introduce an element of data variability, data were provided by means of the standardized study proforma, for uniformity. Any missing or unknown data for any individual was accounted for in the analysis. Consent for publication was sought outside of the Natural History Study. These individuals will not receive subsequent longitudinal follow-up.

3 | Discussion

3.1 | Summary of Individuals

This analysis comprised 64 individuals from 13 countries internationally spanning Europe, Australia, Canada, United States of America, and Brazil (57 enrolled in the Natural History Study and seven additional individuals). Of all individuals, 61% were male, and 75% (48) were not involved in any previous publication. There were six novel unrelated families with inherited *ASXL3*. In addition, there were two pairs of siblings (one newly described) with the same apparently de novo variant, suggestive of gonadal mosaicism.

The mean age of individuals at the time of assessment was 10 years. The mean age of diagnosis was 7.7 years, in keeping with *ASXL3*-related disorder as a neurodevelopmental disorder (Bainbridge et al. 2013). The average age of diagnosis will likely decrease over time, with the increasing availability of new genomic technologies (Whole Genome Sequencing). Only three of the individuals over 18 years were probands; the remaining adults were part of the inherited *ASXL3* families. Two of the three adult probands were diagnosed in adulthood (after initial assessment in childhood prior to gene-disease association).

3.2 | Summary of Variants

Table 1 and Figure 1 summarize the location of *ASXL3* variants in this cohort. Of these, 24 variants were novel. Four comprised copy number changes; the remainder were single nucleotide variants or small insertions/deletions. The majority were de novo variants in exons 11 or 12. Recurrent variants were

TABLE 1 | Summary of variants in this cohort.

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Germline variant also seen
New (unpublished) individuals									
UK	U1	/	c.1643C>A p.(Ser548*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely	/
UK	U2	/	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod PS4_mod	Pathogenic	> 10% protein lost	Schirwani et al. (2021) x 3 (PP10 + two others), Balasubramanian et al. (2017), Srivastava et al. (2016); (Fu et al. 2021), N1
USA	U3	/	c.4744C>T p.(Gln1582*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	> 10% protein lost	/
Canada	U4	/	c.3596_3612del p.(Ser1199*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	> 10% protein lost	/
UK	U5	/	c.1082dup p.(Leu362fs)	10	De novo	PVS1, PM2, PS2_ mod, PS4_sup	Pathogenic	NMD likely	Balasubramanian et al. (2017)
USA	U6	/	c.1378dup p.(Thr460fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely	/
USA	U7	/	c.670A>T p.(Lys224*)	7	Unknown	PVS1, PM2	Pathogenic	NMD likely	/
UK	U8	/	c.1568C>G p.(Ser523*)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely	/
Luxembourg	U9	/	18q12 .1q12.3(25897038_37400276)×1	1–12	De novo	CNV guidelines – 1A, 2A (1), 3C (0.9), 5F—total 1.9	Pathogenic	Multi-gene deletion	/
UK	U10	/	c.3349C>T p.(Arg1117*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	> 10% protein lost	Cuddapah et al. (2021), Hegde et al. (2017), Zhang et al. (2018)
UK	U11	357,881	c.3332_3333del p.(Phe1111fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	> 10% protein lost	/
UK	U12	/	c.3039 + 2T>C	Inton 11	De novo	PVS1_str, PM2, PS2_mod, PS1_mod	Pathogenic	Predicted skipping of exon 11, frameshift, loss of > 10% protein	/

(Continues)

TABLE 1 | (Continued)

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Germline variant also seen
USA	U13	/	c.4899T>A p.(Tyr1633*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	> 10% protein lost	/
UK	U14	/	c.6110dup p.(Pro2038fs)	12	De novo	PVS1_mod, PS2_mod, PP4	Likely pathogenic	< 10% protein lost	/
UK	U15	/	c.4399C>T p.(Arg1467*)	12	De novo	PVS1_str, PS2_mod, PS4_mod	Likely pathogenic	> 10% protein lost	Schirwani et al. (2021) x 3, Yu et al. (2021)
UK	U16	/	c.3364C>T p.(Gln1122*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	> 10% protein lost	Srivastava et al. (2016), Hyder et al. (2021)
Australia	U17	/	c.4156del p.(Ser1386fs)	12	Unknown	PVS1_str, PM2	Likely pathogenic	> 10% protein lost	/
France	U18	/	c.4219_4220del p.(Leu1407fs)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	> 10% protein lost	Schirwani et al. (2021), U23
Brazil	U19	/	c.4322C>G p.(Ser1441*)	12	De novo	PVS1_str, PS4_sup, PM2, PM6_sup	Likely pathogenic	> 10% protein lost	Cuddapah et al. (2021)
USA	U20	/	c.1897_1898del p.(Gln633fs)	11	Unknown	PVS1, PM2, PS2_mod, PS4_mod	Pathogenic	NMD likely	Dinwiddie et al. (2013), Awamleh et al. (2022)
Lithuania	U21	/	c.2041_2042insA p.(Ser681fs)	11	De novo	PVS1. PM2, PM6_sup	Pathogenic	NMD likely	/
UK	U22	/	18q12.1(33687447_33846149)×1	9–12	Unknown	CNV guidelines – 1A, 2D-4 (1), 3A, 5F – total 1	Pathogenic	Multi-exon deletion	/
UK	U23	/	c.4219_4220del p.(Leu1407fs)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	> 10% protein lost	Schirwani et al. (2021), U18

(Continues)

TABLE 1 | (Continued)

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Germline variant also seen
USA	U24	/	c.3106C>T p.(Arg1036*)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_mod	Pathogenic	> 10% of protein lost	Schirwani et al. (2021), Koboldt et al. (2018), Kuechler et al. (2017), Myers et al. (2018), Heide et al. (2020), Duan et al. (2021)
USA	U25	/	c.2237dup p.(Leu746fs)	11	De novo	PVS1, PM2, PM6_sup	Pathogenic	NMD likely	/
Spain	U26	/	c.1612G>T p.(Glu538*)	11	Unknown	PVS1, PM2, PM6_sup, PS4_sup	Pathogenic	NMD likely	Khan et al. (2022)
USA	U27	/	c.1801_1802del p.(Gln601fs)	11	De novo	PVS1, PM2, PM6_sup	Pathogenic	NMD likely	/
Canada	U28	/	c.1550_1554del p.(Val517fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely	/
Russia	N1	/	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod PS4_mod	Pathogenic	> 10% protein lost	Schirwani et al. (2021) x 2 (PP10 + two others), Balasubramanian et al. (2017), Srivastava et al. (2016), U2
USA	M1	/	c.1990C>T p.(Gln664*)	11	Maternal mosaicism	PVS1, PM2, PS2_mod, PS4_mod	Pathogenic	NMD likely	Schirwani et al. (2021), Cuddapah et al. (2021)
USA	M2	/	c.2070del p.(Glu691fs)	11	Paternal mosaicism	PVS1, PM2	Pathogenic	NMD likely	/
Sweden	S2a	/	c.1210C>T p.(Gln404*)	11	Apparent De novo (likely gonadal mosaicism)	PVS1, PM2, PS2_mod, PS4_mod	Pathogenic	NMD likely	In relative, Švantnerová et al. (2022), Bainbridge et al. (2013)
Sweden	S2b	/	c.1210C>T p.(Gln404*)	11	Apparent De novo (likely gonadal mosaicism)	PVS1, PM2, PS2_mod, PS4_mod	Pathogenic	NMD likely	In relative, Švantnerová et al. (2022), Bainbridge et al. (2013)

(Continues)

TABLE 1 | (Continued)

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Germline variant also seen
USA	F1a	/	c.138-1G>A	Intron 2	Maternal	PVS1, PM2	Pathogenic	Predicted skipping of exon 3, NMD likely	In relative
USA	F1b	/	c.138-1G>A	Intron 2	Unknown	PVS1, PM2	Pathogenic	Predicted skipping of exon 3, NMD likely	In relative
USA	F1c	/	c.138-1G>A	Intron 2	Maternal	PVS1, PM2	Pathogenic	Predicted skipping of exon 3, NMD likely	In relative
UK	F2a	/	18q12.1(32218967_33612863)×1	1–2	Paternal	CNV guidelines—1A, 2C-1 (0.9), 3A – total 0.9	Likely pathogenic	Start loss	In relative
UK	F2b	/	18q12.1(32218967_33612863)×1	1–2	Unknown	CNV guidelines—1A, 2C-1 (0.9), 3A – total 0.9	Likely pathogenic	Start loss	In relative
France	F3a/N2	/	c.2902G>T p.(Glu968*)	11	Maternal	PVS1, PM2	Pathogenic	NMD likely	In relative
France	F3b/N3	/	c.2902G>T p.(Glu968*)	11	Unknown	PVS1, PM2	Pathogenic	NMD likely	In relative
Greece	F4a/N4	/	c.4611del p.(Thr1538fs)	12	Paternal	PVS1_str, PM2, PP1	Likely pathogenic	>10% protein lost	In relative
Greece	F4b/N5	/	c.4611del p.(Thr1538fs)	12	Paternal	PVS1_str, PM2, PP1	Likely pathogenic	>10% protein lost	In relative
Greece	F4c/N6	/	c.4611del p.(Thr1538fs)	12	Unknown	PVS1_str, PM2, PP1	Likely pathogenic	>10% protein lost	In relative
Greece	F4d/N7	/	c.4611del p.(Thr1538fs)	12	Paternal	PVS1_str, PM2, PP1	Likely pathogenic	>10% protein lost	In relative
Netherlands	F7a	/	c.6199_6202del p.(Leu2067fs)	12	Maternal	PVS1_mod, PM2, PS2_mod	Likely pathogenic	<10% protein lost	In relative
Netherlands	F7b	/	c.6199_6202del p.(Leu2067fs)	12	De novo	PVS1_mod, PM2, PS2_mod	Likely pathogenic	<10% protein lost	In relative

(Continues)

TABLE 1 | (Continued)

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Germline variant also seen
UK	F8a	481,364	18q12.1(33697293_33839862)×1	9–12	Maternal	CNV guidelines – 1A, 2D-4 (1), 3A, 5D (0) – total q	Pathogenic	Multi-exon deletion	In relative
UK	F8b	/	18q12.1(33697293_33839862)×1	9–12	Unknown	CNV guidelines – 1A, 2D-4 (1), 3A, 5D (0)—total 1	Pathogenic	Multi-exon deletion	In relative

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Paper previously published
Previously published individuals									
UK	PP1	307,301	c.5659A>T p.(Arg1887*)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Schirwani et al. (2021)
UK	PP2	/	c.1505_1508dup p.(Met504fs)	11	De novo	PVS1, PM2, PS2_mod	Pathogenic	NMD likely	Schirwani et al. (2021)
UK	PP3	/	c.4871_4874del p.(His1624fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Schirwani et al. (2021)
UK	PP4	271,709	c.3039+1G>A	Intron 11	De novo	PVS1_str, PM2, PS2_mod, PS4_mod, PS1	Pathogenic	Predicted skipping of exon 11 > 10% protein lost	Myers et al. (2018)
UK	PP5	258,284	c.4479_4483del p.(Ser1493fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Schirwani et al. (2021)
USA	PP6	/	c.4060_4061del p.(Ser1354fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Ayoub et al. (2023)
USA	PP7	/	c.4788_4816delinsT p.(Cys1597fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Ayoub et al. (2023)
USA	PP8	/	c.4034_4035dup p.(Ile1346fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Ayoub et al. (2023)
USA	PP9	/	c.4462_4465del p.(Thr1488fs)	12	De novo	PVS1_str, PM2, PS2_mod, PS4_sup	Likely pathogenic	>10% protein lost	Schirwani et al. (2021)

(Continues)

TABLE 1 | (Continued)

Country	Patient	Decipher	Variant	Exon	Inheritance	ACMG criteria	Classification	Effect	Paper previously published
UK	PP10	286,921	c.4330C>T p.(Arg1444*)	12	De novo	PVS1_str, PM2, PS2_mod PS4_mod	Pathogenic	>10% protein lost	Schirwani et al. (2021)
UK	PP11	265,854	c.3355dup p.(His1119fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Balasubramanian et al. (2017)
UK	S1a	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. (2021)
UK	S1b	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. (2021)
Netherlands	F9a	/	c.2791_2792del p.(Gln931fs)	11	Paternal	PVS1, PM2	Pathogenic	NMD likely	Schirwani et al. (2021) and Schirwani et al. (2023)
USA	PP12	/	c.1978_1981del p.(Asp660fs)	11	De novo	PVS1, PM2, PS2_mod, PS4_sup	Pathogenic	NMD likely	Bainbridge et al. (2013)
UK	PP13	265,908	c.3127_3128dup p.(Gly1045fs)	12	De novo	PVS1_str, PM2, PS2_mod	Likely pathogenic	>10% protein lost	Balasubramanian et al. (2017)

Note: De novo—confirmed with parental genetic testing. SNV 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. PM6_sup: Assumed De novo, but without confirmation of paternity and maternity, used at supporting due to non-specificity of the phenotype. 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) or ≤10% loss of protein size (PVS1_mod). PM2: Absent from controls in gnomAD database, 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 and not seen in gnomAD. PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease, applied in line with used in line with Jarvik and Browning (2016). PS1_mod: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change, applied in line with Walker et al. (2023). CNV ACMG Criterion applied in line with UK ACGS guidelines (Durkie et al. 2023).

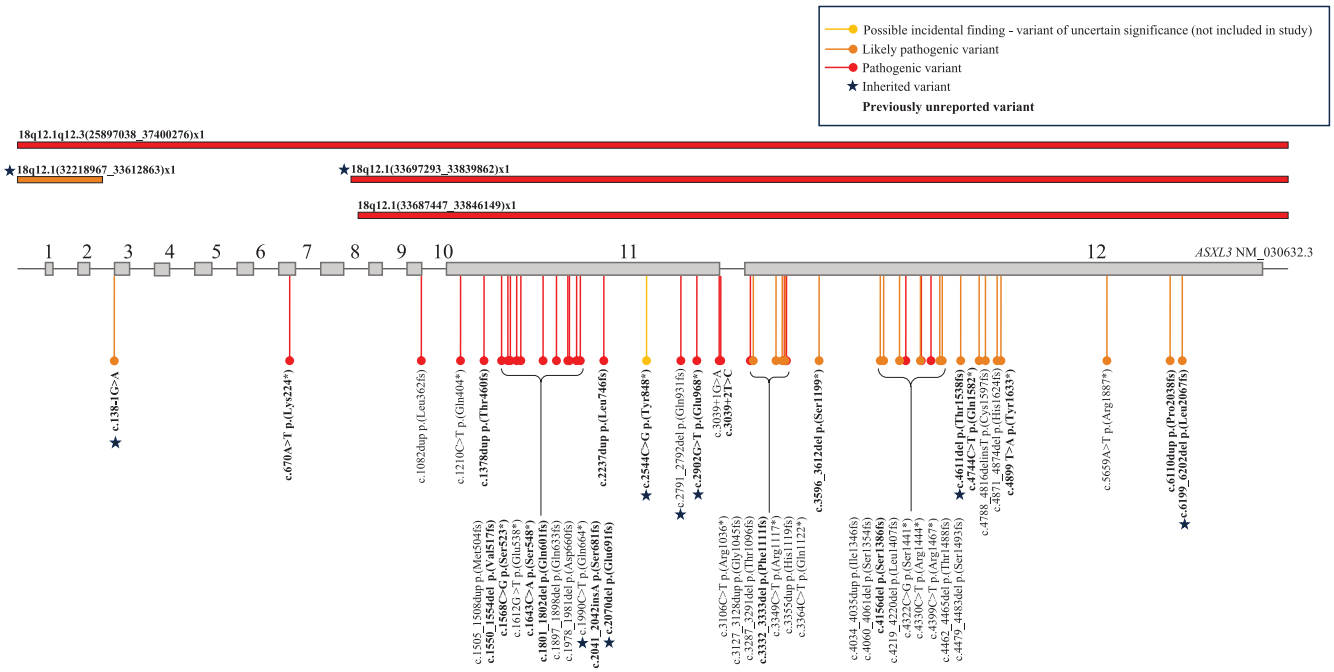

FIGURE 1 | Illustrates the location of *ASXL3* variants in this study. All variants are pathogenic or likely pathogenic as per ACMG criterion, with the exception of one VUS as described/highlighted. This VUS was not included in the study. Inheritance status was confirmed for all variants marked as 'inherited'. Respective parents of inherited variants c.1990C>T p.(Gln664*) and c.2070del p.(691 fs) were not included in the study due to parental mosaicism.

identified through cross-referencing of the existing medical literature. Novel variants—outside the typical exon 11 and exon 12 clusters—included an exon seven nonsense variant, an intron two splice site variant, and an intron 11 splice site variant.

3.3 | Pregnancy

In total, 59% of pregnancies experienced complications (9% maternal spotting or bleeding; 9% reduced foetal movements; 3% gestational cholestasis; 3% gestational diabetes; 2% gestational hypothyroidism; 5% hyperemesis; 3% gestational hypertension; 3% eclampsia or pre-eclampsia; 3% deemed high risk for trisomy with normal karyotype; 9% thromboembolism risk on treatment or prophylaxis; 7% breech position; 15% congenital abnormality of anatomy antenatal scan; 20% growth abnormality on antenatal scan).

Compared to the general population, there was an increased frequency of congenital abnormalities identified antenatally. Four individuals had renal abnormalities (one with mild-to-moderate bilateral hydronephrosis, one with 'enlarged kidney,' and two with unspecified pyelectasis), three had short femur length, one had asymmetric ventricular horns, and one had a cleft lip and palate.

3.4 | Gestation and Birth

The mean gestation was 39 weeks, to the nearest whole week. There was no difference in the prematurity rate, with 10% of babies born prematurely; the earliest at 33 weeks gestation. The majority of births were vaginal (55%), while 31% were elective caesarean-sections, and 14% were emergency caesarean-sections, reflective of the general population when considering births in developed countries.

3.5 | Birth Measurements

There was no significant abnormality in mean birth growth parameters. Mean birth weight was 3.1 kg (95% CI [4.2, 2.0]), but there was negative skew on Z scores (when accounting for gender and prematurity), with a median value of −0.75 (Figure 2). Mean birth head circumference was 34.4 cm (95% CI [38.2, 30.6], median Z score 0.42), although fewer data points were available for head circumference (Figure 2).

3.6 | Neonatal Complications

There were more complications in the neonatal period than expected, with 64% experiencing a neonatal complication. A quarter of babies were admitted to the Neonatal Unit (NICU). Reasons for admission were: hypotonia or lethargy requiring feeding support, respiratory distress syndrome requiring either invasive or non-invasive ventilation, choking episodes, suspected sepsis, prematurity, and hypoglycaemia.

Almost half of all babies (47%) had difficulty with initial feeding comprising poor suck, poor latch, or inability to breastfeed. There was significant weight loss, or static weight, in 20%. Any element of respiratory distress—regardless of NICU admission—was reported in 17%.

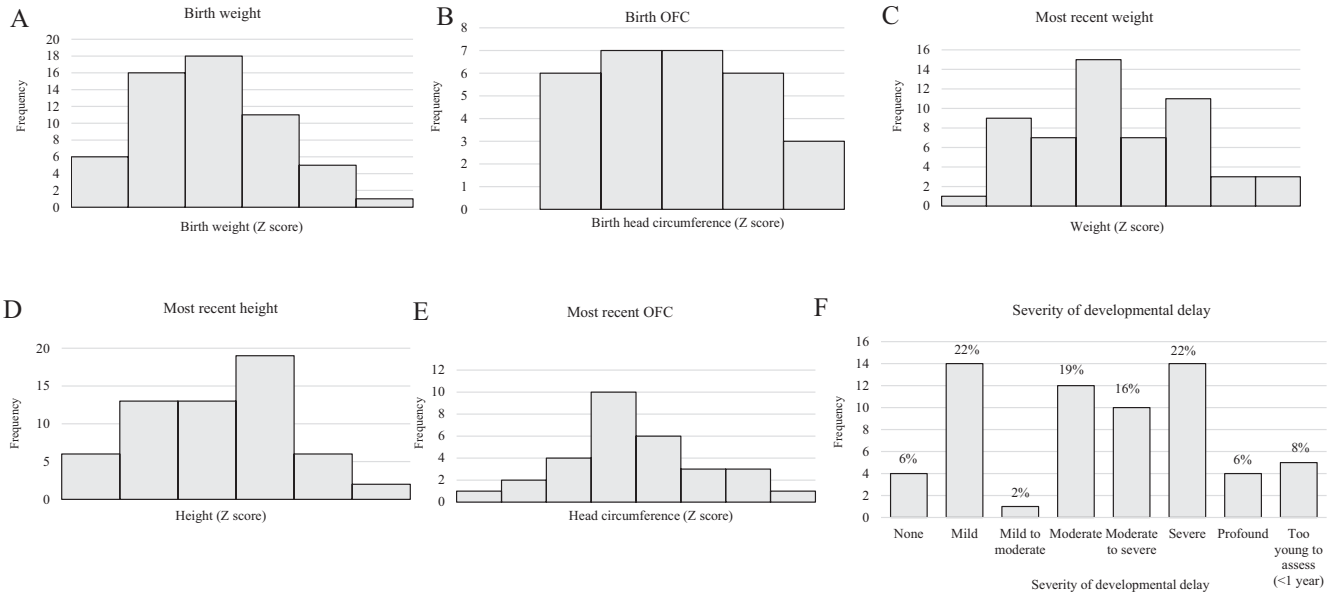

FIGURE 2 | Histograms of general characteristics of the cohort. Z scores indicate how much a given value deviates from the standard deviation norm, and accounts for gender and prematurity. (A) Birth weight Z score, $n = 57$, reflecting a normal distribution with negative skew, with mean of 3.1 kg (95% CI [4.2,2.0]), median Z score of −0.75. (B) Birth OFC Z score, $n = 29$, reflecting normal distribution, with mean OFC of 34.4 cm (95% CI [38.2, 30.6]), medial Z score of 0.42. (C) Most recent weight by Z score, $n = 56$, with median Z score of −1.2. (D) Most recent height by Z score, $n = 59$, with median Z score of −1.2. (E) Most recent head circumference by Z score, $n = 30$, with median Z score of −1.3. (F) Clinical impression of severity of developmental delay.

Structural or physical abnormalities were noticed in 17% of individuals after birth. These included dysmorphic craniofacial features (abnormal head shape, prominent forehead, downslanting palpebral fissures, synophyrs, high arched palate, ear lobe pit, folded ears), pedal edema, and joint contractures. A greater occurrence of congenital abnormalities was seen in this cohort than would be expected in the general population (Zile-Velika et al. 2023).

3.7 | Growth

The trajectory of the quasi-natural history data showed that individuals continued to grow in height and weight throughout childhood. There was poor post-natal growth in many, with half of all individuals measuring < 10th centile in two or more parameters. In total, 38% of individuals were diagnosed with failure-to-thrive at some point in childhood; the majority in infancy, but occasionally later-on. The majority of individuals remained relatively small for age, but Body Mass Index (BMI) improved with feeding intervention and time. Of note, three probands had significantly increased BMI (> 99th centile) later in childhood likely due to a combination of dysregulated eating behaviors, side effects of medication, and reduced mobility.

Across all ages, median Z score for weight was −1.2, median Z score for height was −1.2, and median Z score for head circumference (OFC) was −1.3 (Figure 2). OFC measurements were generally in keeping with proportionate post-natal microcephaly, although 16% had relative microcephaly (greater than 2 centile difference in OFC compared to weight/height). Z scores were calculated on UK-WHO reference data in pediatrics (Cole et al. 1998; Freeman et al. 1995; WHO Multicentre Growth Reference Study Group 2006; WHO 2007), and CDC reference data for adults (Kuczmarski et al. 2000).

3.8 | Social Development

Of all individuals, 35% had a delayed social smile after 8 weeks of age. The mean age at first smile was 9.7 weeks (95% CI [7.7, 11.7]), and median age was 6 weeks (95% CI [5.4, 6.5]) (Figure 3, Data S2).

In total, 73% exhibited some sort of abnormality of social development at their current age. 74% demonstrated autistic traits, whilst 39% demonstrated traits of attention deficit or hyperactivity disorder (ADD/ADHD). Only 30% had a formal diagnosis of autism spectrum disorder (with mean age at diagnosis of 8.3 years, and range of 1.5 to 41 years), whilst 14% had a formal diagnosis of ADD/ADHD (with a mean age at diagnosis of 6.8 years, and range of four to 9 years).

Features of neurodiversity are thought to be a symptom of the underlying genetic condition. Several factors were reported by families when considering whether to pursue a formal autism assessment. These included the age at which symptoms first appeared relative to the age of genetic diagnosis, concerns about receiving a diagnostic 'label' without adequate consideration of a potential underlying cause, perceived benefits related to access to educational and financial support, the desire for a deeper understanding of behaviors, and the overall perceived value of a formal diagnosis for the individual, their caregivers, and others involved in their care.

A

Age	Individuals	Percentage	Cumulative fraction
<1y	-	-	-
1y to 2y	4	0	0.00
2y to 3y	4	25	0.13
3y to 4y	4	75	0.33
4y to 5y	5	100	0.53
5y to 6y	3	100	0.60
6y to 7y	5	100	0.68
7y to 8y	3	67	0.68
8y to 9y	3	100	0.71
9y to 10y	3	67	0.71
10y to 11y	3	100	0.73
11y to 12y	2	100	0.74
12y to 13y	2	100	0.76
13y to 14y	1	100	0.76
14y to 15y	1	100	0.77
15y to 16y	3	100	0.78
16y to 17y	1	100	0.79
17y to 18y	2	100	0.80
18y to 19y	1	0.00	0.78
>19y	4	75.00	0.78

B

Age	Individuals	Percentage	Cumulative fraction
<1y	-	-	-
1y to 2y	4	100	1.00
2y to 3y	4	50	0.75
3y to 4y	4	0	0.50
4y to 5y	5	60	0.53
5y to 6y	3	33	0.50
6y to 7y	5	60	0.52
7y to 8y	3	66	0.54
8y to 9y	3	0	0.48
9y to 10y	3	33	0.47
10y to 11y	3	0	0.43
11y to 12y	2	50	0.44
12y to 13y	2	0	0.41
13y to 14y	1	100	0.43
14y to 15y	1	0	0.42
15y to 16y	3	33	0.41
16y to 17y	1	100	0.43
17y to 18y	2	50	0.43
18y to 19y	1	100	0.44
>19y	9	11	0.39

C

Age	Individuals	Percentage	Cumulative fraction
<1y	5	100	1.00
1y to 2y	4	100	1.00
2y to 3y	4	100	1.00
3y to 4y	4	75	0.94
4y to 5y	5	60	0.86
5y to 6y	3	67	0.76
6y to 7y	5	60	0.80
7y to 8y	3	100	0.82
8y to 9y	3	100	0.83
9y to 10y	3	33	0.79
10y to 11y	3	100	0.81
11y to 12y	2	100	0.77
12y to 13y	2	50	0.80
13y to 14y	1	100	0.79
14y to 15y	1	0	0.79
15y to 16y	2	67	0.76
16y to 17y	1	100	0.80
17y to 18y	2	100	0.79
18y to 19y	1	100	0.83
>19y	6	17	0.75

D

Age	Individuals	Percentage	Cumulative fraction
<1y	4	75	0.75
1y to 2y	4	50	0.63
2y to 3y	4	100	0.75
3y to 4y	4	25	0.63
4y to 5y	5	0	0.48
5y to 6y	3	0	0.42
6y to 7y	5	20	0.38
7y to 8y	3	33	0.38
8y to 9y	3	33	0.37
9y to 10y	3	0	0.34
10y to 11y	3	33	0.34
11y to 12y	2	50	0.35
12y to 13y	2	0	0.33
13y to 14y	1	0	0.33
14y to 15y	1	0	0.32
15y to 16y	3	33	0.32
16y to 17y	1	0	0.31
17y to 18y	2	50	0.32
>18y	7	29	0.32

E

Age	Individuals	Total score	Average score
<1y	-	-	-
1y-2y	4	12	3
2y-3y	4	16	4
3y-4y	4	15	3.75
4y-5y	5	13	2.6
5y-6y	3	7	2.33
6y-7y	5	13	2.6
7y-8y	3	16	5.33
8y-9y	3	9	3
9y-10y	3	7	2.33
10y-11y	3	5	1.67
11y-12y	2	10	5
12y-13y	2	4	2
13y-14y	1	5	5
14y-15y	1	0	0
15y-16y	3	12	4
16y-17y	1	5	5
17y-18y	2	10	5
18y-19y	1	6	6
19y+	-	-	-

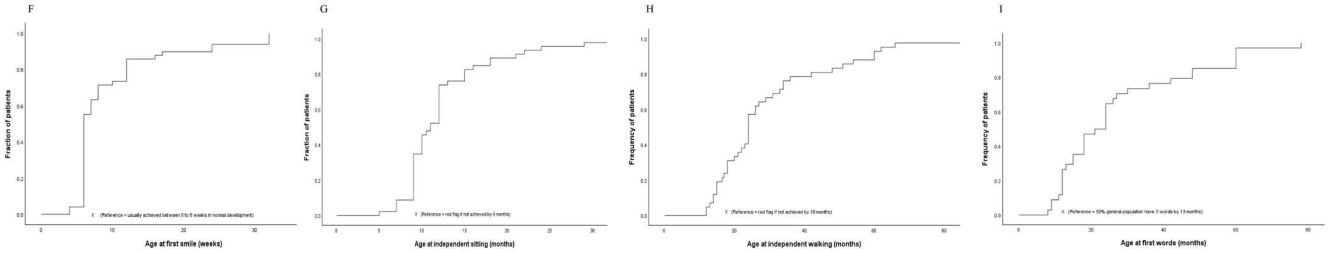

FIGURE 3 | Quasi-natural history data. (A) Data reflecting independent walkers. (B) Data reflecting non-verbal individuals. (C) Data reflecting presence of hypotonia. (D) Data reflecting presence of feeding issues. (E) Data reflecting clinical assessment of severity of developmental delay, scoring criteria comprised 0 = no delay, 1 = mild delay, 2 = mild-to-moderate, 3 = moderate, 4 = moderate-to-severe, 5 = severe, 6 = profound. (–) denotes where values could not be assessed due to age. (F) Kaplan–Meier curve demonstrating age at first smile, *n* = *49*. (G) Kaplan–Meier curve demonstrating age when first independently sitting, *n* = *46*. (H) Kaplan–Meier curve demonstrating age when first independently walking, *n* = *42*. (I) Kaplan–Meier curve demonstrating age at first words, *n* = *34*.

3.9 | Motor Development

In total, 69% had delayed independent sitting after 9 months of age, whilst 71% had delayed independent walking after 18 months age. Of all individuals old enough to determine (and excluding those who were still unable to achieve this milestone), mean age of sitting unsupported was 13 months (95% CI [10.7,15.2]), with a median age of 11 months (95% CI [9.9, 12.1]) (Figure 3, Data S2). For independent walking, mean age was 30.4 months (95% CI [24.6, 36.3]), with median age 24 months (95% CI [23.1, 24.9]). The oldest age at first independent steps was 5.5 years (Data S2).

Figure 3 provides a visual representation of quasi-natural history data, illustrating the proportion of independently mobile individuals at different ages. Of those individuals, 41% had significant ongoing mobility issues, which included: frequent falls, broad-based gait, reduced exercise tolerance, significant instability, or reliance/preference for walking aid/wheelchair for distance.

Of those individuals not limited by specific mobility issues, some were described to have poor coordination with specific motor tasks (such as catching and throwing), an element of clumsiness or instability, weaker core strength, lower confidence with motor tasks, or lack of motor skills progression (such as not being able to run or ride a bicycle). Some children had expected motor skills for age but had been slower to achieve these skills.

Although more difficult to quantify without detailed practical assessment, 76% were also reported to have a delay in fine motor skills.

3.10 | Speech and Language Development

In verbal individuals, the mean age of first word was 27.3 months (95% CI [21, 36.6]), with a median age of 21 months (95% CI [15.9, 26.1]) (Figure 3; Data S2). For individuals older than 15 months, 38% were non-verbal at their current age. Figure 3 provides a visual representation of the quasi-natural history data of non-verbal individuals by age. In individuals old enough to assess, 85% had ongoing speech and language difficulties. However, the majority of the unaffected individuals were parents identified through family testing.

For verbal individuals who had an element of speech delay, this ranged from mild to severe. On the severe end of the spectrum, some individuals had limited words, with a preference for alternate methods of communication. On the milder end of the spectrum, individuals could speak in full sentences but had difficulty with articulation and clarity of speech. A high arched palate and overcrowding of teeth affected articulation in some individuals.

The majority of individuals continued to make progress with their speech and language development, except for in 8% of cases where parents described a developmental language arrest (although the long-term implications cannot be foreseen) and loss of words in 7% (with acute illness or concurrent development of additional features of autism). Regression in other developmental domains was not described.

Various methods of alternate communication methods were described in both verbal and non-verbal individuals: 25% of

individuals used a device (such as an Application on a handheld tablet or communication cards), 31% of individuals used signs (such as Makaton), 28% of individuals regularly used gesturing or pointing, and 28% of individuals communicated with regular use of noises. Receptive language skills exceed expressive language skills, which led to frustration with communication.

3.11 | Developmental Delay Spectrum

In this cohort, there was evidence of a wider clinical spectrum ranging from mild to profound developmental delay (Figure 2). Although the majority of individuals had varying degrees of global delay, some individuals only had delay in one developmental domain, while others had none. A clinical scoring system was employed to assess potential longitudinal trends in the severity of developmental delay; however, no consistent correlation was observed over time (Figure 3).

3.12 | Neurology

In total, 77% of all individuals had been diagnosed with hypotonia at some point (comprising 16% with a diagnosis of global hypotonia, 16% with a diagnosis of central hypotonia, and 67% unspecified hypotonia). In some, hypotonia was reported to improve over time. This is also reflected in the quasi-natural history data (Figure 3).

In total, 13% of individuals had an additional neurological diagnosis. Diagnoses included dystonia, cerebral palsy (hemiplegic), neuropathic pain, migraine, and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS).

Current long-term medication included painkillers (Gabapentin, Pregabalin), anti-spasmodics (Baclofen), and anti-epileptics (Lamotrigine, Topiramate, Levetiracetam, Oxcarbazepine, Clonazepam).

3.13 | Seizures and EEG

Seizures were diagnosed in 16% of individuals, whilst epilepsy was diagnosed in 13%, which is significantly less than previously reported in the existing literature (Woods et al. 2024). The majority of epilepsy diagnoses comprised generalized idiopathic epilepsy, with one diagnosis of cryptogenic partial epilepsy. Seizure types included generalized tonic–clonic, absence, partial, and mixed. The majority of first seizures occurred in childhood (range of 4 years to 15 years), whilst one individual experienced a seizure for the first time in adulthood, but was not diagnosed with epilepsy. Seizure frequency varied, but in most cases, was well controlled by medication.

In addition, 20% of children had absence episodes that were not classified as seizures. Absence episodes were described as 'staring spells,' 'daydreaming,' or 'zoning-out,' and is a known phenomenon in children with autism spectrum disorder (Goenka et al. 2023). In some, such episodes warranted further investigation into possible absence seizures, especially when seen alongside repetitive movements or stimming (self-stimulatory) behaviors. Some children had been investigated with an EEG, whereas some children were not investigated due to other reassuring signs, such as being easily distractable during such episodes.

In this cohort, 44% had an electroencephalogram (EEG) for any reason, and 29% of these had an abnormal finding (Data S3).

3.14 | Structural Brain Abnormalities

The majority of individuals (71%) had some form of brain imaging. Of these, 38% had a reported abnormal finding. These were non-specific and did not require any particular treatment. Structural brain abnormalities can sometimes be associated with epilepsy, but there was no clear correlation in this cohort (Data S3).

3.15 | Musculoskeletal

Hypermobility often goes alongside hypotonia, as increased flexibility is often a result of low muscle tone. In total, 56% of individuals had an element of hypermobility. Of these, 88% had generalized hypermobility, and the remaining 12% had hypermobility of limited joints. As a result, some individuals had pes planus and positional ankle weakness or deformity that required ankle supports or orthotics. No one in this cohort had an alternate or contributing cause of hypermobility.

10% of individuals had contractures, the majority of which were recognized and diagnosed at birth, including: finger contractures, wrist contractures, and torticollis (contracture of sternocleidomastoid). Contractures of the hips and knees were diagnosed in one older child. Some individuals (8%) had tightness of muscles. Treatments included physiotherapy, Botox, splinting, casting, or multiple tendon releases.

Pectus excavatum was present in 11% of individuals; all were congenital or diagnosed before 2 years of age. Scoliosis was diagnosed in 8%; some were congenital, and some were diagnosed later in childhood and thought to have a postural element (as a result of wheelchair use). All cases of scoliosis involved the thoracic spine and some involved the lumbar spine. One case was treated surgically with spinal rods, and one required a spinal brace. In addition, some individuals had marked lumbar lordosis. Craniosynostosis was diagnosed in 5% of individuals. There were also some less frequently reported fixed deformities including hip dysplasia, hemivertebrae and genu varum (Data S3).

3.16 | Feeding

Feeding problems were common, with 70% of individuals having ever experienced feeding difficulties.

Many neonates (16%) had difficulty establishing feeds, poor latch, and many were unable to breastfeed. This may have been exacerbated by hypotonia or an anatomical problem (such as tongue tie, lip tie, retrognathia, or high arched palate). Some

babies required additional feeding support, conversion to bottle feeding, or a high calorie or alternate formula milk.

Anecdotal and quasi-natural history data illustrated that feeding issues generally improved with time (Figure 3). Overall, 54% of individuals continued to have feeding difficulties into infancy or childhood. Continued feeding difficulties included dysphagia, unsafe swallow, oromotor dyspraxia, frequent regurgitation, problems with weaning, sensory issues with food, food aversion, poor appetite, and insufficient intake.

In addition, there were some older children who had difficulties with dysregulated eating behavior. There were also individuals with sensory eating behaviors only, and children who had isolated uncoordinated mastication (not requiring intervention as not causing concern with regards to intake or weight). These individuals were not considered to have feeding issues for the purpose of analysis.

In terms of feeding adjuncts, a nasogastric tube (NGT) was required in 25% of individuals. These were first inserted at a median age of 3 months (range zero to 24 months), and removed at a median age of 11.5 months (range one to 30 months). Some were removed as oral feeding was established, but some were removed in place of a percutaneous endoscopic gastrostomy (PEG). In total, 22% of children had required PEG feeding. Only one child received a PEG without having a prior NGT. The median age of PEG insertion was 12 months (range one to 125 months).

3.17 | Gastrointestinal

In total, 31% of individuals had ever experienced regular constipation (for which the vast majority took a Macrogol). Gastroesophageal reflux was diagnosed in 48%, which self-resolved in some, but varying medications were trialed in others including; antacids (such as Gaviscon), protein pump inhibitors (such as Omeprazole), histamine H2 antagonists (such as Ranitidine). Others had only been on milk thickeners. Rarely, surgical intervention with a fundoplication was required.

Frequent or cyclical vomiting was diagnosed in 11%. Some had investigations which revealed slow gastric emptying or slow gut motility, whilst the cause in others was thought to be behavioral. Medications trialed for vomiting or nausea included Cyproheptadine, Domperidone, Alimemazine, Erythromycin, Amitriptyline, and Ondansetron.

Additional reported gastrointestinal (GI) findings included enlarged liver and non-alcoholic fatty liver disease (NAFLD) of unknown etiology, medication-induced NAFLD, medication-induced pancreatitis, eosinophilic esophagitis, Crohn's disease, redundant duodenal segment, dolichocolon, and one individual with a low alkaline phosphatase (ALP) level of unknown cause (which may not be GI related).

3.18 | Vision and Hearing

In total, 70% had an ophthalmological finding. Significant eye findings were: divergent or convergent strabismus, reported in 45%, ptosis reported in 7%, and nystagmus in 7%. Some children were prescribed glasses (36%) which many did not tolerate wearing. Some individuals had eye surgery to correct strabismus. Compared to population prevalence, an expected number of children had middle ear congestion, and some required a hearing aid as a result. Regular ophthalmological follow-up will ensure the timely treatment of correctable pathology, and reduce the possibility of visual problems contributing to delayed development.

3.19 | Respiratory

Respiratory problems were commonly seen, as expected in the general pediatric population. Overall, 11% of individuals had respiratory problems at birth, in the form of respiratory distress, laryngomalacia, tracheomalacia, or concern of restricted upper airways (such as stridor). In total, 34% reported at least one current respiratory problem, but symptoms were generally mild. These comprised viral wheeze (2%), asthma (5%), recurrent otitis media with or without effusion (8%), enlarged adenoids/tonsils (23%), and recurrent respiratory tract infections (20%). Some individuals were described as having an increased susceptibility to infections, as reported by the caregiver or clinician, but in the absence of a known underlying immunodeficiency or alternate explanation.

3.20 | Sleep and Sleep Apnoea

In total, 57% of individuals had ever experienced sleep difficulties, with 49% experiencing sleep problems at their current age. Sleep problems were defined as difficulty getting to sleep, difficulty staying asleep, poor-quality sleep, or other non-specific sleep issues. Anecdotally, some individuals' sleep did improve with age; however, quasi-natural history data did not suggest a clear general trend or significant improvement over time.

In total, 23% of individuals had been diagnosed with, or had evidence of, central or obstructive sleep apnoea. Some were diagnosed following polysomnography, while others had a compatible clinical history. Of these individuals, five (36%) required continuous positive airway pressure (CPAP) or bi-level positive airway pressure (BiPAP) at night. Other individuals had curative adeno/tonsillectomies.

Sleep interventions included sleep therapist input, initiation of good sleep hygiene, and medication (Melatonin, Promethazine). Side effects of other medication also proved beneficial in some (Trazadone). One individual had tried Cannabidiol (CBD) but saw no improvement. One individual's sleep improved significantly once their generalized pain was under control.

3.21 | Cardiovascular

No one in this cohort was diagnosed with a structural pathological cardiac abnormality, dysrhythmia, or cardiomyopathy. In total, 44% of individuals had an echocardiogram for any reason. All detected heart murmurs were deemed non-concerning

following investigations or follow-up, and most likely represented physiological changes.

One individual was diagnosed with vascular instability, and one individual with hypotension. One individual experienced adult-onset palpitations of unknown cause. One individual had an incidental subclavian aneurysm, as previously described in Schirwani et al. (2023), which was stable on subsequent imaging.

3.22 | Renal

Structural renal and urological abnormalities were previously thought to be very rare. Bilateral renal dysplasia was the only previously reported renal abnormality, in one individual reported by (Siu Xiao et al. 2022). However, in this cohort antenatal renal findings were detected in 7%, and postnatal renal findings were identified in an additional 15% (Table 2). Pathological postnatal abnormalities included refluxed urethral meatus, duplex kidney, dysplastic kidneys, and hydronephrosis. Some findings may have been non-pathological, and seen in the general population, such as simple kidney cysts.

In total, 15% of all individuals had multiple urinary tract infections. Most—but not all—of these individuals had a renal ultrasound scan to further investigate. The vast majority of asymptomatic individuals had never had a renal ultrasound scan, so prevalence of asymptomatic reno-urological structural abnormalities could be underrepresented.

Based on these findings, we recommend a baseline renal ultrasound scan to look for congenital abnormalities, particularly if there are any concurrent signs or symptoms such as multiple urinary tract infections.

3.23 | Skin and Hair

A variety of birth marks and skin conditions were reported, as seen in the general population, including café-au-lait, haemangioma, naevus simplex, congenital dermal melanosis, seborrheic dermatitis, and eczema. Forehead/glabella/eyelid naevus flammeus was reported in 13% of individuals, which is a common finding in *ASXL1*-related disorder (Bohring-Opitz syndrome) (Russell et al. 2015). Many were reported to significantly fade over time. Hypertrichosis was reported in 27% of individuals.

3.24 | Puberty and Pregnancy

One individual had investigations into precocious puberty at 6 years of age, but these were reassuring. One individual had delayed puberty, with no secondary sexual characteristics at 14 years of age. There were no reported pregnancies in probands.

3.25 | Dysmorphology

Some features were present at greater frequency, but there was no recognizable facial gestalt specific to, or pathognomonic of, *ASXL3*-related disorder. The commonest features were prominent forehead (57%), down-slanting palpebral fissures (44%), wide nasal bridge (43%), and high or narrow palate (62%). A summary of features, seen in more than one individual, and a compilation of facial photographs of probands are shown in Figure 4.

In addition to physical teeth abnormalities (overcrowding, large incisors, overbite), some children had tooth decay. This was thought to be the result of feeding difficulties or sensory difficulties associated with teeth brushing. Some children had staining of the teeth, thought to be due to certain medications or resistance to brushing. Some children had misaligned teeth that required orthodontic treatment such as braces.

3.26 | Behavior and Mental Health

In this cohort, 62% of individuals were reported to have problematic behaviors. Many children were described to have immaturity of behavior, lack of safety awareness, and an element of impulsivity. Often, behaviors were reactive, and they were reportedly worse with communication frustration, tiredness, over-stimulation, or inability to implement a preferred routine. Anecdotally, for many, the severity of behavioral difficulties fluctuated, but generally improved somewhat with age.

- Difficulty with emotional regulation was reported in 46%, manifesting as temper tantrums in younger children or outbursts of swearing or screaming in some older children;
- Stereotypies were seen in 41%, including wringing of hands, hand clapping, arm waving, head banging, spinning, and rocking;
- Aggressive behaviors—directed towards others—were reported in 40%, and were most often directed towards caregivers rather than strangers;
- Self-injurious behaviors were seen in 38% of individuals, including biting, hitting, scratching and pinching;
- Outbursts of unprecipitated or inappropriate uncontrollable laughter were described in 14%.
- Pica was reported in 8%.

Only including formal diagnoses, 11% of individuals had anxiety disorder, 5% had obsessive compulsive disorder (OCD), and 3% had depression. One individual had a diagnosis of disruptive mood dysregulation disorder (DMDD), and one had a diagnosis of post-traumatic stress disorder (PTSD). Regular medications included Fluoxetine, Sertraline, Citalopram, Trazodone, Olanzapine, and Venlafaxine. Medications for behavioral issues included Risperidone, ADHD medication (Guanfacine, Methylphenidate, Viloxazine), and a minority had additional 'as required' medications including Hydroxyzine, Trazadone, and Lorazepam for severe episodes of anxiety or aggression.

Many children were described to be happy, friendly, affectionate, and placid.

TABLE 2 | Antenatal and postnatal structural and functional renal anomalies.

Individual	Antenatal structural anomaly	Postnatal structural or functional anomaly	Symptoms	Management
1	Mild-to-moderate bilateral hydronephrosis	No abnormality detected	None	None
2	Enlarged single kidney	Right refluxed urethral meatus, without signs of posterior valves	None	Prophylactic antibiotics (Cefalexin) Nephrology follow-up
3	Pyelectasis	Left extra renal pelvis	None	None
4	Pyelocaliceal dilatation	Moderate pyelo-calcific dilatation (unilateral) Ectopic testes	None	Follow-up scans Orchidopexy
5	No abnormality detected	Left duplex kidney	None	None
6	No abnormality detected	Left duplex kidney Grade 4 vesicoureteric reflux Moderate left sided hydronephrosis Left 52%; Right 44% functioning	Recurrent urinary tract infections	Follow-up scans Prophylactic antibiotics (now stopped)
7	No abnormality detected	Twists neck of bladder (incidental finding on abdominal ultrasound; normal appearance of kidneys)	None	None
8	No abnormality detected	1 cm kidney cyst	None	Follow-up/monitoring
9	No abnormality detected	Increased kidney volume	None	Not known
10	No abnormality detected	Dysplastic kidneys Renal agenesis (No chronic kidney disease)	None	None
11	No abnormality detected	Grade 1 pyelectasis	One E.Coli UTI	Nephrology follow-up, since discharged
12	No abnormality detected	Mild hydronephrosis	None	Nephrology follow-up, since discharged
13	No abnormality detected	Simple kidney cyst	Multiple urinary tract infections	Nephrology follow-up

Note: Summary of antenatal and postnatal renal findings in this cohort.

A

Feature	Percentage
Forehead	
Prominent forehead	57% (36/63)
Synophyrs	22% (13/63)
Metopic ridge	17% (11/64)
Prominent eyebrows (not synophyrs)	8% (5/63)
Eyes	
Down-slanting palpebral fissures	44% (28/63)
Epicanthic folds	22% (14/63)
Hypertelorism or telecanthus	17% (11/63)
Deep-set or sunken eyes	6% (4/63)
Long eyelashes	5% (3/63)
Narrow palpebral fissures	5% (3/63)
Up-slanting palpebral fissures	3% (2/63)
Nose	
Wide nasal bridge	43% (27/63)
Broad nasal tip	22% (14/63)
Upturned nose	8% (5/63)
Smooth philtrum	3% (2/63)
Short nose	3% (2/63)
Mouth	
Palate abnormality (high and/or narrow palate)	62% (39/63)
Micrognathia or retrognathia	35% (22/63)
Overcrowding or predicted overcrowding of teeth	33% (21/63)
Tongue tie	22% (14/63)
Large central incisors	16% (10/63)
Thin lip(s)	13% (8/63)
Lip tie	10% (6/63)
Overbite or prominent maxilla	10% (6/63)
Wide mouth	5% (3/63)
Tented upper lip	3% (2/63)
Small teeth	3% (2/63)
Ears	
Low set ears	24% (15/63)
Pointed ears	8% (5/63)
Rotated ears	3% (2/63)
Head	
Dolichocephaly	5% (3/63)
Trigonocephaly	5% (3/63)
Long face	3% (2/63)
Hand and feet	
Long slender hands or feet	17% (11/63)
Flat feet	14% (9/63)
Overlapping toes	8% (5/63)
High arched feet	5% (3/63)
5th finger clinodactyly	5% (3/63)
Curled toes	3% (2/63)
Single palmar crease	3% (2/63)
Sandal gap	3% (2/63)
Small hands and feet	3% (2/63)
Other	
Inverted nipples	3% (2/63)

B

FIGURE 4 | (A) Summary of dysmorphology in this cohort. (B) Facial photographs of probands in this study who consented to publication of images, in ascending order of age (left-to-right), illustrating the range of dysmorphology.

3.27 | Education

The greatest proportion of individuals (of school age or older) attended Special Education (42%), whilst 25% attended mainstream school with specialist education support, 15% attended mainstream school without support, and 8% received home schooling only.

Many children in the UK had an Educational Health and Care Plan (EHCP) or received Early Years Support. For those in Special Education, depending on learning level, some individuals did basic Maths and English lessons, others did Life Skills, and others did sensory classes.

Many children also received therapies in school (Physiotherapy, Occupational Therapy, Speech and Language Therapy). In some instances, children initially went to a mainstream school, but were then switched to either home-schooling or a Special School as their individual needs and learning needs became apparent. Some children attended a mix of Special Education and mainstream education, as integration and interaction with other children was deemed important by caregivers.

3.28 | Healthcare Specialty Involvement

Figure 5 shows a heatmap of current Specialty input, by age of the individual, as well as Healthcare Specialty recommendations. Not inclusive of Primary Care (as presumably all individuals had access), the median number of Specialties involved in an individual's care in this cohort was six, with a range of zero to 15. The more commonly involved professionals in younger children include Pediatrics, Neurology, and Therapies (Physiotherapy, Occupational Therapy, Speech and Language Therapy). With increasing age, the number of Specialties decreased.

3.29 | Clinical Genetics Input

Probands were universally seen in Clinical Genetics. In 50% of cases, individuals (or parents) had discussions regarding Prenatal Diagnosis (PND), and in 36% there were discussions regarding Pre-implantation Genetic Diagnosis (PGD). In most cases, discussions were not had if there were no plans for further children. Only 5% of parents had testing in a subsequent pregnancy. In both cases, the proband had an apparent de novo variant.

A

Age	Frequency	Paeds	SLT	PT	OT	Neuro	Gastro	Dietic	Ophth	ENT	Ortho	Orthot	Dent	Sleep	Cardio	Resp	Renal	Urol	Audiol	N.surg	Psych	Psychol	Endo	Pain	Al/Im	OBG	Cleft	Plast	Derm	Metab
<1y	5.00	1.00	0.60	0.80	0.60	1.00	0.60	0.80	0.40	0.60	0.20	0.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
1-2 years	4.00	1.00	0.75	0.75	0.75	0.75	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-3 years	4.00	0.75	1.00	1.00	1.00	0.75	0.75	0.50	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
3-4 years	4.00	1.00	0.75	0.75	0.50	0.50	0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.25	0.25	0.25	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00
4-5 years	4.00	1.00	0.75	0.75	1.00	0.50	0.25	0.00	0.50	0.25	0.00	0.25	0.25	0.25	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00
5-6 years	3.00	1.00	1.00	0.67	0.67	0.33	0.00	0.00	0.67	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
6-7 years	5.00	1.00	0.60	0.80	0.60	0.40	0.20	0.00	0.20	0.20	0.40	0.00	0.00	0.00	0.20	0.00	0.20	0.00	0.00	0.20	0.00	0.00	0.40	0.00	0.20	0.00	0.00	0.00	0.20	0.00
7-8 years	3.00	1.00	1.00	1.00	0.67	1.00	0.33	0.33	0.67	0.33	0.67	0.67	0.00	0.00	0.33	0.33	0.00	0.00	0.33	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
8-9 years	3.00	1.00	0.33	0.67	0.67	0.67	0.00	0.33	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9-10 years	3.00	1.00	0.33	0.00	0.33	0.00	0.00	0.00	0.67	0.33	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
10-11 years	3.00	1.00	1.00	0.67	0.33	0.00	0.33	0.33	0.33	0.00	0.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11-12 years	2.00	1.00	0.50	1.00	1.00	0.50	0.50	0.50	0.50	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12-13 years	2.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13-14 years	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14-15 years	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15-16 years	2.00	1.00	0.00	0.50	0.00	0.50	0.50	0.50	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00
16-17 years	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17-18 years	2.00	1.00	1.00	0.50	1.00	0.00	0.50	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
>18 years	7.00	0.00	0.14	0.14	0.14	0.29	0.29	0.14	0.14	0.14	0.14	0.00	0.00	0.00	0.00	0.14	0.00	0.14	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00

B

Medical Specialty	Timing of assessment	Evaluation	Ongoing assessment in childhood
Paediatrician	After diagnosis	Feeding	Yes
		Growth	Yes
		Development	Yes
		Behaviour and mental health	Yes
		Sleep	Yes
		Tone (hypotonia)	Yes
		Neurological assessment (seizures/ abnormal neurology)	Yes
		Musculoskeletal	Yes
		Renal (CAKUT)	As required
Optician	After diagnosis	Vision	Yes
Ophthalmology	As required if abnormality	Vision/ eye assessment	As required
Dentist	After diagnosis	Dental health[a]	Yes
Orthodontist	As required		As required
Gastroenterologist	As indicated	GI symptoms	As required
Dietician/ feeding support	As required if abnormality	Feeding and growth	As required
Therapies (PT/OT/SLT)	As indicated	Development	As required
Psychology/ Psychiatry	As indicated	Mental health	As required
Sleep team/ ENT	As indicated	Sleep evaluation including sleep apnoea	As required
Neurology	As indicated	Seizures/ abnormal neurology	As required
Orthopaedics/Spinal	As indicated	Musculoskeletal	As required
Orthotics	As required		
Nephrology	As indicated	CAKUT	As required
Urology	As indicated		

FIGURE 5 | (A) Current Specialty involvement included in analysis only. Past (discharged) or planned (future) Specialty involvement not included. Specialty by name: Pediatrics (Paeds), Speech and Language Therapy (SLT), Physiotherapy (PT), Occupational Therapy (OT), Neurology (Neuro), Gastroenterology (Gastro), Dietician (Dietic), Ophthalmology (Ophth), Ears Nose and Throat (ENT), Orthopedics (Ortho), Orthotics (Orthot), Specialist Dentist (Dent), Sleep Team (Sleep), Cardiology (Cardio), Respiratory (Resp), Renal (Renal), Urology (Urol), Audiology (Audiol), Neurosurgery (N.Surg), Psychiatry (Psych), Psychology (Psychol), Endocrinology (Endo), Pain Team (Pain), Allergy/Immunology (Al/Im), Obstetrics and Gynecology (OBG), Cleft Team (Cleft), Plastic surgery (Plast), Dermatology (Derm), Metabolic medicine (Metab). (B) Recommendations for Specialty involvement based on findings in this cohort.

3.30 | The Increasingly Emerging Milder Spectrum of ASXL3-Related Disorder

ASXL3-related disorder is a spectrum disorder. As with many neurodevelopmental conditions, gene-disease association is initially recognized in relatively severely affected individuals. With time, the extent of the wider clinical spectrum becomes apparent. When compared to previous reports, many individuals in this cohort were reported to have a less significant developmental delay, with fewer associated medical issues, comprising an overall milder phenotype. This is particularly important for caregivers who, perhaps due to literature bias, have difficulty recognizing the genetic diagnosis in a child with relatively few difficulties. Intrafamilial variability has been previously reported with inherited *ASXL3* (Schirwani et al. 2023). Novel clinical descriptions of probands in this study, with a relatively milder phenotype, are summarized in Data S3.

3.31 | A Novel Incidental Finding

We are aware of a 5-year-old female child with an incidentally discovered maternally inherited c.2544C>A p.(Tyr848*) *ASXL3* variant. This was discovered on rapid Whole Genome Sequencing to investigate an acute (now resolved) otherwise unexplained epileptic encephalopathy. The child and mother were fit and well, with normal development, and no clinical features of *ASXL3*-related disorder. This variant was classified by the local reporting laboratory as a Variant of Uncertain Significance due to lack of phenotypic fit, and was therefore not included in this study. However, the variant is not dissimilar to the other null variants in exon 11 that would otherwise be classified as pathogenic, as per ACMG criteria and ACGS guidelines, based on this being a null variant with loss of function proven mechanism, and absence in population databases. To our knowledge, this is the first incidentally discovered high-candidate *ASXL3* variant. It may further reflect the emerging asymptomatic phenotype or may have contributed to the unexplained encephalopathic presentation. This further indicates that there are additional genetic or non-genetic mechanisms influencing gene expressivity.

4 | Study Limitations

As this cohort involved individuals from different countries and healthcare systems, it was not possible to compare the numbers or type of specialists involved or draw conclusions about relevance to phenotypic severity. Accessibility to healthcare and threshold for investigation and intervention may have varied. Similarly, all data in this cohort (e.g., growth parameters) were compared by the same standards.

Data collection was primarily sought from parents, which resulted in a wealth of qualitative and quantitative insights and allowed a retrospective natural history of the individual. In most cases, the description of clinical dysmorphology was based on photographs provided by the families. Where available, any additional dysmorphology descriptions from the clinician were also included.

Prevalence data was a snapshot of the entire cohort and did not take into consideration the age of the individual. When considering seizures, for example, the most likely age of first seizure was later in childhood. Therefore, reported prevalence rates do not necessarily reflect lifetime prevalence.

The quasi-natural history data comprised multiple individuals of different chronological ages with the same genetic diagnosis. Although it may reflect general trends, the nature of the cross-sectional study design means that it does not reflect the

specific progress of the individual. In age groups represented by fewer individuals, findings may have been somewhat skewed.

Despite general trends to undertake more genetic testing, there is currently a lack of adult probands; instead, the majority of adults in this cohort represent parents of an affected child. Therefore, data in the adult cohort may not be truly representative.

5 | Conclusion

Here we summarize the detailed clinical findings of the largest-to-date *ASXL3* cohort, with a specific focus on quasi-natural history data. In relatively newly discovered rare conditions, this methodology allows the study of trends over time by analyzing the data of individuals at different ages. True longitudinal data would take many years to collate, with a significant risk of research-fatigue for individuals and their families.

We describe 24 novel variants, including six inherited *ASXL3* families, and the first likely incidental finding in an individual without a clinical phenotype. The relatively high proportion of inherited *ASXL3* and significant variability between individuals highlight the emerging probability of reduced penetrance. This cohort illustrates the vast clinical spectrum of the condition and, for the first time, describes the clinical details of several individuals with a milder phenotype.

Novel findings include a higher than anticipated prevalence of renal findings and a lower than anticipated prevalence of epilepsy. Furthermore, *ASXL3*-related disorder was previously associated with poor postnatal growth; however, we now recognize that there may be a risk, to some, of obesity later in childhood, likely associated with dysregulated eating. We provide the first detailed insight into aspects of developmental progression, and for the first time, systematically summarize the pathology and any interventions in a review of all body systems. Recommendations include early involvement of therapies, ophthalmological and dental follow-up, and a baseline renal scan.

Although non-specific to the individual, these findings will help form the basis of general management expectations, future management guidelines, and provide a natural history comparator for possible therapeutic intervention.

Author Contributions

E.W. wrote the manuscript, carried out data analysis, conducted research interviews, and arranged patient recruitment to the study. N.H. provided variant interpretation and made the variant location diagram. M.B. conceptualized the research study and provided guidance and support as Principal Investigator. A.D., N.B., C.G., B.I., C.W.O., E.P., H.S., A.V., V.J.M.V., M.V., R.L., E.R., A.D., A.S. provided clinical details of their patients. All authors contributed to review and editing.

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GeneMatcher helped match one family to this research (Sobreira et al. 2015). This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust. Firth et al. (2009) (dx.doi.org/10/1016/j.ajhg.2009.03.101 https://www.deciphergenomics.org/). Although no collaborations were made via GeneDx, we thank their continued support in sharing study details alongside reports.

Ethics Statement

Ethical approval for the Natural History Study was sought through the online Integrated Research Application System (IRAS no. 316055). Health Research Authority (HRA) approval was given on 02.06.2023. The Research and Innovation Care Group (REC) at the sponsor site, Sheffield Children's NHS Foundation Trust, completed their Capacity and Capability review for the study and confirmed authorization for the study to be undertaken within the Trust on 08.06.2023 (REC no. 23/SC/0151). All participants of the Natural History Study gave informed consent. Consent for publication was sought by the individual's named clinician(s) for the seven individuals whose data was included for analysis, but where language barriers limited direct participation in the Natural History Study.

Consent

Informed consent was obtained from all individual participants, or their parents, included in the study. The authors affirm that all human research participants in Figure 4b provided informed consent for publication of their images.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.