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ARTICLE

Malan syndrome: Sotos-like overgrowth with *de novo* *NFIX* sequence variants and deletions in six new patients and a review of the literature

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De novo monoallelic variants in *NFIX* cause two distinct syndromes. Whole gene deletions, nonsense variants and missense variants affecting the DNA-binding domain have been seen in association with a Sotos-like phenotype that we propose is referred to as Malan syndrome. Frameshift and splice-site variants thought to avoid nonsense-mediated RNA decay have been seen in Marshall–Smith syndrome. We report six additional patients with Malan syndrome and *de novo NFIX* deletions or sequence variants and review the 20 patients now reported. The phenotype is characterised by moderate postnatal overgrowth and macrocephaly. Median height and head circumference in childhood are 2.0 and 2.3 standard deviations (SD) above the mean, respectively. There is overlap of the facial phenotype with *NSD1*-positive Sotos syndrome in some cases including a prominent forehead, high anterior hairline, downslanting palpebral fissures and prominent chin. Neonatal feeding difficulties and/or hypotonia have been reported in 30% of patients. Developmental delay/learning disability have been reported in all cases and are typically moderate. Ocular phenotypes are common, including strabismus (65%), nystagmus (25%) and optic disc pallor/hypoplasia (25%). Other recurrent features include pectus excavatum (40%) and scoliosis (25%). Eight reported patients have a deletion also encompassing *CACNA1A*, haploinsufficiency of which causes episodic ataxia type 2 or familial hemiplegic migraine. One previous case had episodic ataxia and one case we report has had cyclical vomiting responsive to pizotifen. In individuals with this contiguous gene deletion syndrome, awareness of possible later neurological manifestations is important, although their penetrance is not yet clear.
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INTRODUCTION

Overgrowth syndromes comprise a group of genetic disorders that combine height greater than or equal to two SDs above the mean with variable other features. Amongst the best recognised are Sotos syndrome (MIM 117550), Weaver syndrome (MIM 277590), Beckwith–Wiedemann syndrome (MIM 130650) and Simpson–Golabi–Behmel syndrome (MIM 312870).[1] Other characteristics often present include macrocephaly, developmental delay, advanced bone age, facial dysmorphic features, hemihypertrophy, vascular malformations and, in some disorders, an increased risk of neoplasia.

Sotos syndrome is caused by haploinsufficiency of the *NSD1* (Nuclear receptor Set Domain containing protein 1; MIM 606681) gene and is characterised by learning difficulties, variable medical problems including cardiac anomalies, renal anomalies and seizures and a recognisable facial gestalt, which includes dolichocephaly with a broad forehead, sparse frontotemporal hair, a prominent chin and downslanting palpebral fissures. Up to 95% of individuals with classical features of Sotos syndrome have an identifiable pathogenic

NSD1 variant, but there are a substantial number of *NSD1*-negative individuals with overgrowth and some Sotos-like features but who do not have the classical facial gestalt of Sotos syndrome.[2,3]

Recently, pathogenic *de novo* monoallelic variants in the *NFIX* (Nuclear Factor I/X; MIM 164005) gene have been identified in individuals with a Sotos-like phenotype as well as in individuals with Marshall–Smith syndrome, a disorder associated with advanced skeletal maturation, life-threatening upper airway compromise and failure to thrive. These two distinct phenotypes seem to result from two distinct classes of causative variant. Marshall–Smith syndrome has only been seen in those with frameshift and splice site variants in exons 6–8, which seem to evade nonsense-mediated RNA decay. A variety of variant types have been seen in association with the Sotos-like phenotype, including whole gene deletions (10 patients), one nonsense variant and missense variants and in-frame deletions affecting the DNA-binding domain (3 patients).[4–10] Five of the reported *NFIX* deletions also encompass the nearby *CACNA1A* gene, haploinsufficiency of which causes episodic ataxia type 2 or familial hemiplegic migraine.

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In this paper, we describe six additional patients with a Sotos-like phenotype and *de novo NFIX* deletions or sequence variants and review the literature.

MATERIALS AND METHODS

High-resolution aCGH was performed on genomic DNA and analysed versus reference DNA (Kreatech, Amsterdam, The Netherlands). NimbleGen 135 k CGH microarray was used with a calculated functional resolution of 0.2 Mb (95% confidence limits). The DNA samples were labelled (test with Cy3 and reference with Cy5) and co-hybridised to the microarray in accordance with the manufacturer's instructions (NimbleGen Arrays User's Guide: CGH and CGH/LOH Arrays v9.1, Roche NimbleGen, Madison, WI, USA). The microarray was washed and then scanned on an Axon GenePix 4400A Scanner using GenePix Pro 7 software (Molecular Devices, Sunnyvale, CA, USA). Raw data were normalised, LOESS correction applied and the data ratios calculated using DEVA v1.01 Software (Roche NimbleGen). The normalised data were processed using Infoquant Fusion v6.0 software (Infoquant, London, UK) with analysis call settings of three consecutive probes $+/-0.4$ Cy3/Cy5 ratio.

One patient (Patient 4) initially underwent aCGH as part of the DDD study using a custom Agilent 2×1 M array-CGH (Agilent UK, Wokingham, Berkshire, UK), with five probes per exon and a mean backbone spacing of ~4 kb. Patient samples were processed in batches of 95, labelled in Cy5 using Agilent labelling reagents (Agilent UK), and hybridised against a pool of 500 developmentally normal males labelled in Cy3. All laboratory procedures were supported by a bespoke LIMS, which interacted with DECIPHER to facilitate sample tracking. Following feature extraction, CNVs were called using a novel in-house analysis (CNsolidate) that combines 12 weighted change point detection algorithms. Patient data were annotated with allele frequencies based on overlap with a set of controls analysed on the same platform (570 population controls from the UK Blood Service and 455 developmentally normal individuals from the Scottish Family Health Study).

In all cases, for confirmation of the deletion and for parental testing, real-time quantitative PCR was carried out using primers (Sigma-Aldrich, St Louis, MO, USA) from within intron 2 of the *NFIX* gene. Primers were checked for specificity by melt curve analysis and run on a StepOne Plus Real Time PCR system (Life Technologies Applied Biosystems, Paisley, UK) using the SYBR Green comparative $\Delta\Delta$CT method. Primers from within genes *ACTBL2* and *MANEA* were used as endogenous control sequence targets. Results were processed using StepOne Plus Software v2.2 with copy number loss or gain indicated by relative quantitation (RQ) values.

Conventional Sanger sequencing of the *NFIX* gene was performed on genomic DNA extracted from peripheral blood cells. All coding exons and intron/exon boundaries were included, based on transcript NM_002501.2. Exon numbering is as used by Malan *et al.*[7] also based on this transcript. Primer sequences and PCR conditions are available on request. PCR products were amplified using standard protocols. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Cheshire, UK) and separated on the ABI 3730 (Applied Biosystems). Results were analyzed using SeqScape v2.5 software (Applied Biosystems).

Details of the deletions identified have been submitted to the Decipher database. Details of the sequence variants have been submitted to the HGMD database.

RESULTS
Case descriptions
Patient 1. This 3-year-old boy was referred to the Clinical Genetics clinic with developmental delay and dysmorphic features. He is the first child of healthy unrelated British parents. Vaginal bleeding at 9 weeks and premature contractions at 28 weeks gestation complicated the pregnancy. His birth weight was 2490 g at 37 weeks gestation (-1.3 SD below the mean; UK90 growth charts). Motor milestones were delayed. He sat unsupported at 1 year, crawled at 18 months and walked at around 2.5–3 years. At the age of 3, he spoke several words, no sentences. He had a prominent forehead and chin and slightly downslanting palpebral fissures (Figure 1). He had plagiocephaly and

mild facial asymmetry. Head circumference was 52 cm ($+0.3$ SD), height 103 cm ($+1.9$ SD) and weight 19.3 kg ($+2.6$ SD). There was mild scoliosis of the thoracic spine with 1 cm leg asymmetry and a mild pectus excavatum. Bone age was 3.5 years at a chronological age of 3 years (1 SD above mean). On the basis of these clinical features, he was given a working diagnosis of Sotos syndrome. *NSD1* sequencing and karyotype were normal. At last follow-up, aged 10 years, his height was 152.0 cm ($+2.2$ SD), his weight 45.5 kg ($+1.7$ SD) and his head circumference 55.5 cm ($+0.7$ SD). He had behavioural difficulties and a diagnosis of autistic spectrum disorder.

Patient 2. This 3.5-year-old girl was referred to the Clinical Genetics clinic with global developmental delay, hypotonia and overgrowth. She is the only child of healthy unrelated Chinese parents and was born by forceps delivery at 42 weeks gestation. She first sat unsupported at the age of 12 months and made her first independent steps by the age of 2.5 years. Aged 3.5 years, she said 'dada', but no other clear words. On physical examination, at age 2.5 years, her height was 94.5 cm ($+1.0$ SD using British charts), head circumference 51.1 cm ($+1.0$ SD) and weight 14.1 kg ($+0.7$ SD). She had scaphocephaly, a prominent forehead, depressed nasal bridge and deep-set eyes. Her palate was high arched and there was significant drooling. There was no scoliosis.

Patient 3. This 2-years and 8-month-old girl was referred to the Clinical Genetics clinic because of abnormal array CGH results. Array was performed because of global developmental delay and oculomotor dyspraxia. She had had one febrile seizure. She was born at term after an uneventful pregnancy to healthy unrelated Italian parents weighing 3.6 kg ($+0.5$ SD). She first walked at 2.5 years. She had been found to have virtually undetectable biotinidase levels for which biotin was given for a brief period. A repeat measurement of biotinidase levels – several months after biotin treatment was stopped – was normal. Her height was 93.4 cm ($+0.5$ SD), head circumference 52 cm ($+1.6$ SD) and weight 15.4 kg ($+1.3$ SD). She has intermittent end-gaze horizontal nystagmus and an intermittent convergent squint. She had a prominent forehead and slightly downslanted palpebral fissures. A brain MRI showed no abnormalities.

Patient 4. This 13-month-old boy was referred to the Clinical Genetics clinic because of global developmental delay and overgrowth. He is the only child of healthy parents of British and Afro-Caribbean ancestry. He was born at 39 weeks gestation weighing 4.1 kg ($+1.1$ SD). At birth, he was hypertonic with flexed posture and adducted thumbs. There were mild contractures of small joints, which resolved spontaneously in the first year of life. He had global developmental delay, first standing up at 2.5 years, walking at 4.5 years. At the age of 5.5 years, he could babble but had no words. There was a left divergent squint and a visual evoked potential examination showed post-retinal dysfunction bilaterally. At the age of 4 years, he presented with cyclical vomiting. This resolved following commencement of pizotifen. He had scaphocephaly and a prominent forehead. Aged 5.5 years, his height was 128 cm ($+2.8$ SD), his head circumference 59 cm ($+2.9$ SD) and his weight 30.5 kg ($+3.9$ SD). Several investigations had been performed, including plasma creatinine kinase, very long chain fatty acids, karyotype, 1 Mb resolution array CGH, and *PTEN* and *NSD1* sequencing. All results were normal. A brain MRI performed at the age of 8 weeks showed delayed myelination.

Figure 1 Facial features of patients. (**a**) Patient 1 at age 3 and 10; (**b**) Patient 2 at age 3.5; (**c**): Patient 5 at age 2.5 and 7. Note the pectus excavatum at 7 years; (**d**) Patient 6 at age 3.

Patient 5. This 2-years and 5-month-old girl was referred to the Clinical Genetics clinic because of global developmental delay and hypotonia. She was born at term to healthy unrelated Dutch parents. Her birth weight was reported as 'average'. She had an uneventful neonatal period, except for muscular hypotonia. There was a global developmental delay with walking and several single words at 2 years. There were recurrent upper airway infections for which grommets were inserted. Physical examination showed macrocephaly with a head circumference of 52 cm (+3.3 SD) and height and weight were both 2–3 SD above the mean. There was frontal bossing, mild downslanting palpebral fissures, a small mouth, pointed chin and a pectus excavatum. Several investigations had been performed, including karyotype, MLPA of subtelomeric regions and microdeletions, high-resolution array and *NSD1* sequencing, all of which gave normal results. Bone age was advanced. A brain MRI performed at the age of 3 years showed periventricular white matter hyperintensity, slightly enlarged ventricles, possible heterotopias and a persistent cavum septum pellucidum. Sanger sequencing of *NFIX* was requested based on this clinical presentation.

Patient 6. This 3-year-old boy was referred to the Clinical Genetics clinic because of macrocephaly and developmental delay. He was born at term to healthy unrelated Dutch parents after an uneventful pregnancy. His birth weight was 3.25 kg at term (−0.3 SD). During the first few months of his life, he suffered from gastro-oesophageal reflux disease, which resolved with medication. His development was delayed with walking at 2.5–3 years and first single words at the age of 2.5. On physical examination, he had macrocephaly with a mild facial asymmetry and downslanting palpebral fissures. Head circumference

was +1.0 SD, height was +0.7 SD and weight was on the 50th percentile. Previously, several investigations were performed, including a high-density array and *FMR1* expansion testing and *NSD1* sequencing. A brain MRI performed at the age of 2 showed no abnormalities. The bone age was 3.5–4 years at the age of 3. Sanger sequencing of *NFIX* was requested based on this clinical presentation.

Molecular results

Array CGH detected deletions in Patients 1–4 (Table 1). In all patients, the deletion had arisen *de novo*, as confirmed by parental array CGH, FISH and/or quantitative PCR. Array CGH in Patient 1 revealed a 110–170-kb deletion on chromosome 19: chr19.hg19:g.(13,060,729_13,097,782)_(13,209,504_13,232,425). The deletion minimally encompasses the whole *NFIX* gene. Maximally, it also deletes or partially deletes six other RefSeq genes with no known haploinsufficiency phenotype. Array CGH on Patient 2 showed a larger deletion of approximately 740-810 kb chr19.hg19:g.(12,724,476 _12,774,135)_(13,511,059_13,534,643). This deletion encompasses the whole *NFIX* gene and all but the final three exons of *CACNA1A* as well as between 32 and 34 other RefSeq genes with no known haploinsufficiency phenotype. FISH analysis using probe RP11-35L15 confirmed the presence of this deletion. Array CGH on Patient 3 showed a 410–470-kb deletion chr19.hg19:g.(13,060,729_13,097,782)_(13,511,059_13,534,643). This deletion encompasses the whole *NFIX* gene and all but the final three exons of *CACNA1A* as well as between five and seven other RefSeq genes with no known haploinsufficient phenotype. Array CGH on Patient 4 identified a 560–640-kb deletion chr19.hg19:g.(12,745,959_12,812,594)_(13,376,997_13,383,138). This deletion encompasses *NFIX* and the first 26 exons of *CACNA1A*

Table 1 Clinical features and details of pathogenic *NFIX* deletions and sequence variants in Patients 1–6

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Mechanism	Deletion	Deletion	Deletion	Deletion	Frameshift variant	Nonsense variant
Genes deleted/affected	*NFIX*	*NFIX + CACNA1A*[a]	*NFIX + CACNA1A*[a]	*NFIX + CACNA1A*[a]	*NFIX*	*NFIX*
Minimal breakpoints/variant	13 097 782_13 209 504	12 724 476_13 511 059	13 097 782_13 511 059	12 812 594_13 376 997	c.90_99del, p.(Trp30fs)	c.1012C>T, p.(Gln338[a])
Size	110–170 kb	740–810 kb	410–470 kb	560–640 kb	—	—
Age at last assessment	10.0 years	3.5 years	2.7 years	5.5 years	5 years	3 years
Birthweight (SD)	−1.3	N/A	+0.5	+1.1	N/A	−0.25
Length/height (SD)[b]	+2.2	+1.0	+0.5	+2.8	+2-3	+0.7
Weight (SD)[b]	+1.7	+1.0	+1.3	+2.9	+2.3	0
OFC (SD)[b]	+0.7	+0.7	+1.6	+3.9	+3.3	+1.0
Dysmorphic features	Plagiocephaly, prominent forehead, facial asymmetry, low posterior hairline	Scaphocephaly, prominent forehead, depressed nasal bridge, deep-set eyes, high arched palate	Prominent forehead, slightly downslanting palpebral fissures	Scaphocephaly, prominent forehead	Prominent forehead, slightly downslanting palpebral fissures, elongated face, small mouth, pointed chin	Frontal bossing, mild facial asymmetry, slightly downslanting palpebral fissures
Developmental delay	Moderate	Moderate	Moderate	Severe	Severe	Severe
Neonatal feeding difficulties/hypotonia	−	−	−	−	−	−
Seizures	+	−	−	−	−	−
Episodic ataxia	−	−	−	−	−	−
Migraine	−	−	−	+	−	−
Nystagmus	−	+	−	−	−	−
Strabismus	−	+	+	+	+	+
Optic disc pallor/hypoplasia	−	−	−	−	−	−
Advanced bone age	−	N/A	N/A	N/A	+	N/A
Scoliosis	+	−	−	−	−	−
Pectus excavatum	+	+	+	−	+	−

Abbreviations: OFC, occipital-frontal circumference; N/A, not available; SD, standard deviations above (+) or below (−) mean.
[a]Also deleted in all cases are five annotated genes with no known human phenotype located between *NFIX* and *CANCA1A* (*LYL1, TRMT1, NACC1, STX10* and *IER2*). Genomic positions are given according to build HG19.
[b]Measurement at last assessment in clinic.

and between 26 and 31 other Refseq genes with no known haploinsufficiency phenotype.

In Patient 5, a *de novo* heterozygous frameshift variant was identified in the *NFIX* gene: c.90_99del, p.(Trp30fs). In Patient 6, a *de novo* heterozygous nonsense variant was identified in exon 7 of the *NFIX* gene: c.1012C>T, p.(Gln338*).

DISCUSSION
Recently, there have been several reports of patients with Sotos-like features who carry a *de novo* sequence variant or deletion of *NFIX* at 19p13.2.[5,7–11] Here, we report six additional patients with this disorder who have *de novo* disruption of the *NFIX* gene by either deletions (Patients 1–4; Table 1) or sequence variants (Patients 5 and 6). In three of the four deletion patients, there is also deletion of *CACNA1A*. This brings the total number of patients reported with the Sotos-like overgrowth disorder caused by *NFIX* to 20, including 14 with deletions (8 encompassing *CACNA1A*), 3 with truncating variants and 3 with missense substitutions or in-frame deletions (Table 2 and Supplementary Table 1). With 20 patients now reported and a distinctive phenotype emerging, we believe the condition is best referred to as Malan syndrome.[7]

The phenotype is characterised by mild-to-moderate overgrowth, macrocephaly with a prominent forehead and moderate developmental delay, all features of *NSD1*-positive Sotos syndrome (Figure 1, Tables 1 and 2 and Supplementary Table 1). Birth weight is often within normal limits among Malan syndrome patients with the median birth weight 0.3 SD above the mean (range −2.3 to +2.1 SD). Overgrowth is therefore often postnatal in onset and is typically less marked than in Sotos syndrome with a median height in Malan syndrome of 2.0 SD above the mean (range −0.5 to +3.8 SD) compared to a median of > +2.5 SD in Sotos syndrome (Table 3).[12–14] Median head circumference is 2.3 SD above the mean (range −0.7 to 3.9 SD) and may also be more mildly increased than in Sotos syndrome where it is > +2.5 SD. Median weight is 0.6 SD above the mean (range −0.9 to +2.9 SD). Developmental delay has been reported in all cases to date and was reported as moderate in 15 (75%), severe in 4 (20%) and mild in 1 (5%). Autistic traits have been reported in five cases (25%). A common early presentation is with neonatal hypotonia and/or feeding difficulties, which have been specifically commented upon in five patients (25%). Seizures have been reported in five patients (25%).

A number of the reported cases have facial features overlapping those seen in Sotos syndrome including prominent chin and down-slanting palpebral fissures and prominent forehead with a high anterior hairline (Figure 1). However, of our cases, only Patient 1 had sufficient features to have led to a working diagnosis of Sotos syndrome prior to molecular testing. As in Sotos syndrome, advanced bone age is common, present in six of seven from whom data are available.[13] Scoliosis is also a feature of both conditions, reported in five Sotos-like *NFIX* patients (25%).[12,14]

There are often additional findings not commonly seen in classical Sotos syndrome including strabismus (13 patients; 65%; Table 3),

Table 2 Clinical features of Malan syndrome patients with pathogenic *NFIX* variants and deletions reported here and in the literature

	All	*Deletion of NFIX and other genes including CACNA1A*	*Deletion of NFIX excluding CACNA1A*	*Nonsense or frameshift NFIX variant*	*Missense or in-frame NFIX variant*
Number of patients	20	8	6	3	3
Median birthweight (SD)	+0.3	0	+0.9	+0.2	−0.4
Median length/height (SD)[a]	+2.0	+1.4	+1.7	+2.5	+3.8
Median weight (SD)[a]	+0.6	+1.2	+1.2	+1.3	+0.6
Median OFC (SD)[a]	+2.3	+2.1	+2.3	+2.5	+2.8
Developmental delay					
Mild	1 (5%)	0	0	0	1
Moderate	15 (75%)	7	5	2	1
Severe	4 (20%)	1	1	1	1
Neonatal feeding difficulties/hypotonia	6/20 (30%)	2/8	3/6	0/3	1/3
Seizures	5/20 (25%)	1/8	3/6	0/3	1/3
Episodic ataxia	1/20 (5%)	1/8	0/6	0/3	0/3
Migraine	1/20 (5%)	1/8	0/6	0/3	0/3
Nystagmus	5/20 (25%)	2/8	1/6	1/3	1/3
Strabismus	13/20 (65%)	5/8	4/6	2/3	2/3
Optic disc pallor/hypoplasia	5/20 (25%)	1/8	3/6	0/3	1/3
Advanced bone age	6/7 (86%)	1/1	2/3	2/2	2/2
Scoliosis	5/20 (25%)	0/8	2/6	1/3	2/3
Pectus excavatum	8/20 (40%)	2/8	3/6	1/3	2/3

Abbreviations: OFC, occipital-frontal circumference; N/A, not available; SD, standard deviations above (+) or below (−) mean.
Genomic positions are given according to the HG19 build.
[a]Measurement at last assessment in clinic.

nystagmus (5 patients; 25%) and optic disc pallor/nerve hypoplasia (5 patients; 25%).[12,13] Pectus excavatum, uncommon in Sotos syndrome, was present in eight patients (40%) and may be a particularly useful discriminating feature (Table 3). We note that mice deficient in *Nfix* function show, in addition to brain abnormalities, a kyphotic deformation of the spine and impairment of endochondral ossification in the vertebrae and femur.[15,16]

There have been no reports of neoplasia in the condition to date. The small number of cases reported to date hinders identification of genotype–phenotype correlations for *NFIX*. However, a strong correlation has been observed between the classes of variant that cause Malan syndrome and Marshall–Smith syndrome, with Marshall–Smith syndrome seen only with frameshift or splice site variants in exons 6–8. It has been speculated that these evade nonsense-mediated RNA decay and it is this that results in their distinct phenotypic effect. It should also be noted that there are several alternative transcripts of the *NFIX* gene, which may impact on the differential phenotypic effects of variants. Amongst our patients is one child with a Sotos-like phenotype (Patient 6, Figure 1) and a nonsense variant in exon 7 (c.1012C>T, p.(Gln338*)). The same nucleotide is involved in frameshift variants identified in Marshall–Smith syndrome patients (c.1011_1012delTC and c.1008_1012delCTCTC).[7] Suitable samples were not available from our patient to determine if the mutated transcript evades nonsense-mediated RNA decay. Nevertheless, our finding urges caution regarding division of phenotypic effect based on variant position alone.

In the large majority of cases, there appears to be clear separation of the phenotype of patients, with patients either manifesting with the Malan syndrome or Marshall–Smith syndrome. However, we note that the patient with a 21-nucleotide deletion in the DNA-binding domain reported by Priolo *et al*[8] had a combination of a Sotos-like presentation and mild features of Marshall–Smith syndrome

Table 3 Features that assist in distinguishing Malan syndrome patients from those with Sotos syndrome.[12,13]

	Malan syndrome	*Sotos syndrome*
Median height (SD)	+2.0	>+2.5
Median head circumference (SD)	+2.3	>+2.5
Pectus excavatum	40%	<15%
Strabismus	65%	<15%
Ocular nerve hypoplasia/pallor	25%	<15%
Cerebellar ataxia, hemiplegic migraine	At risk if *CACNA1A* deleted	−

Abbreviation: SD, standard deviations above the mean.

including sleep apnoea and bullet-shaped phalanges, suggesting that this separation may not be absolute.

In all of the cases we report, the *NFIX* variants occurred *de novo*. This is the case in the large majority of cases tested and it is clear that the penetrance of the disorder is high. In one family, there was presumed gonadal mosaicism.[9] Yoneda *et al*[6] reported one child with a missense substitution in the DNA-binding domain which had been inherited from a possibly affected mother (c.362G>C, p.(Arg121Pro)); Supplementary Table 2). Although the location and physicochemical properties of this variant and the description of the facial and other phenotypic features of this child are consistent with its pathogenicity, caution is required in the interpretation of missense variants in *NFIX*, particularly when they are inherited.

The literature describes deletions or truncating variants of *CACNA1A* as typically causing episodic ataxia type 2 (EA2, MIM108500).[17] Pathogenic missense variants typically cause familial hemiplegic migraine type 1 (FHM1, MIM141500), in which cerebellar signs and symptoms varying from isolated nystagmus to progressive

ataxia may also be found.[18] Overlap exists between the two phenotypes and hemiplegic migraine has been reported in individuals with truncating variants or deletions.[19,20] CAG-expansion in exon 47 of *CACNA1A* causes the distinct disorder spinocerebellar ataxia 6 (SCA6, MIM 183086), characterised by adult-onset, slowly progressive cerebellar ataxia, dysarthria and nystagmus.

Eight patients have now been reported with contiguous deletion of *NFIX* and *CACNA1A*. The patients have mainly presented with features attributable to *NFIX* rather than *CACNA1A*. One of our patients (Patient 4) aged 4 years presented with cyclical vomiting responsive to pizotifen and one of the patients reported in the literature aged 9 years had episodic ataxia. Nystagmus has been seen in a similar number of *NFIX* patients with and without deletions, and it is likely that in most, it is not a feature of EA2/FHM1 but a result of *NFIX* haploinsufficiency.

Lifetime penetrance for EA2 is thought to be 80–90% but there is a wide range of age of onset – between 2 and 32 years – and it has been suggested that deletions may have an older age of onset.[21] The absence of features of EA2/FHM1 in the majority of *NFIX-CACNA1A* deletion cases may simply reflect the young age of those reported to date (the median age at last assessment is 9 years). Longer-term follow-up of patients with *NFIX-CACNA1A* will provide data on the (age-related) penetrance of *CACNA1A* deletion ascertained independent of family history and may differ from estimates available from familial EA2 and FHM1 cohorts. Until these data are available, caution is advisable when estimating the penetrance of deletion of *CACNA1A* in *NFIX-CACNA1A* deletion patients.[19,21,22]

A number of other genes are deleted in addition to *NFIX* and *CACNA1A* in the patients others and we have reported. To date no other obvious genotype–phenotype correlations have emerged. However, we do note that the child with the largest reported deletion, measuring ~3Mb, had a more complicated phenotype including craniosynostosis and was growth retarded.[10]

There is little overlap in breakpoints between the reported deletions and there is no clustering of breakpoints around low-copy repeats (LCRs) as in a number of common microdeletion/duplication disorders. It is likely that other mechanisms are responsible, for example, non-allelic homologous recombination between Alu elements. Alu elements comprise an estimated 10–15% of the human genome but are overrepresented on chromosome 19, comprising 25% of its sequence.[22,23] They mediate a number of reported copy number aberrations on chromosome 19 and occupy 30% of the genomic footprint of *CACNA1A*, supporting a possible role in *NFIX-CACNA1A* deletions.

In conclusion, *NFIX* analysis should be considered in patients presenting with overgrowth, macrocephaly and developmental delay including those in whom Sotos syndrome has been considered clinically but are negative for pathogenic *NSD1* variants. Medical follow-up should include developmental support, ophthalmological assessment, surveillance for scoliosis and a low threshold for investigation of possible seizures. In cases where *CACNA1A* is deleted together with *NFIX*, awareness of possible later neurological manifestations is important.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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