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ORIGINAL ARTICLE

A novel 11 base pair deletion in *KMT2C* resulting in Kleefstra syndrome 2

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Abstract

Background: Haploinsufficiency of the Lysine Methyltransferase 2C (KMT2C) gene results in the autosomal dominant disorder, Kleefstra syndrome 2. It is an extremely rare neurodevelopmental condition, with 14 previous reports describing varied clinical manifestations including dysmorphic features, delayed psychomotor development and delayed growth.

Methods: Here, we describe a female with global developmental delay, attention deficit disorder, dyspraxia, short stature and subtle non-specific dysmorphic features. To identify causative mutations, whole exome sequencing was performed on the proband and her younger brother with discrete clinical presentation.

Results: Whole exome sequencing identified a novel de novo heterozygous 11 bp deletion in *KMT2C* (c.1759_1769del), resulting in a frameshift mutation and early termination of the protein (p.Gln587SerfsTer7). This variant is the second-most N-terminal reported mutation, located 4171 amino acids upstream of the critical enzymatically active SET domain (required for chromatin modification and histone methylation).

Conclusion: The majority of the other reported mutations are frameshift mutations upstream of the SET domain and are predicted to result in protein truncation. It is thought that truncation of the SET domain, results functionally in an inability to modify chromatin through histone methylation. This report expands the clinical and genetic characterisation of Kleefstra syndrome 2.

KEYWORDS
COMPASS complex disorder, Kleefstra syndrome 2, KMT2C

1 | INTRODUCTION

Lysine Methyltransferase 2C (KMT2C, MIM: 606833) is a methyltransferase that modifies chromatin and regulates gene expression by methylating histones. Specifically, KMT2C methylates the lysine-4 position of histone H3 (H3K4) at enhancers and promoters, leading to transcriptional activation of downstream genes (Hu et al., 2013; Sedkov et al., 2003). Correct KMT2C function is, therefore, critical for the appropriate spatial and

temporal expression of genes from early development through to adulthood. Kleefstra et al. (2012) identified an autosomal dominant nonsense mutation (p.Arg1481*) in *KMT2C* resulting in a neurodevelopmental disorder presenting with intellectual disability, delayed growth, mild dysmorphic features (including prominent eyebrows, thick ear helices and teeth misalignment) and neuropsychiatric traits including hyperactivity and aggressiveness (Kleefstra et al., 2012). They subsequently coined the condition, Kleefstra syndrome 2 (MIM: 617768). Fourteen other cases have subsequently been described, resulting from haploinsufficiency of KMT2C (Brunet et al., 2021; Cheema et al., 2022; Faundes et al., 2018; Koemans et al., 2017; Schoch et al., 2020; Siano et al., 2022; Wu & Li, 2022).

Kleefstra syndrome 2 is member of the Kleefstra syndrome phenotypic spectrum (KSS), a collection of disorders with the core symptoms of Kleefstra syndrome 1 (MIM: 610253). Kleefstra syndrome 1 is caused by mutations in the gene *EHMT1* (MIM: 607001). Specifically, the KSS is phenotypically characterised by intellectual disability (ID), childhood hypotonia and distinctive facial features including microcephaly, brachycephaly, hypertelorism, synophrys, mid-face hypoplasia, protruding tongue, eversion of the lower lip and prognathism (Kleefstra et al., 2006, 2009; Stewart et al., 2004). Taken as a whole Kleefstra syndrome phenotypic spectrum result from loss of function mutations in genes encoding chromatin modifiers: *EHMT1* (Kleefstra syndrome 1), *MBD5*, *SMARCB1*, *NR1I3* and *KMT2C* (Kleefstra et al., 2012; Koemans et al., 2017) (Kleefstra syndrome 2). Interestingly, these genes include both transcriptional repressors (e.g. *EHMT1*) and activators (e.g. *KMT2C*) which have antagonistic functions.

Frega et al. (2020) shed light on the biology underlying KSS by demonstrating that knockout of KSS genes in cellular models results in hyperactive neuronal networks (Frega et al., 2020). The hyperactive networks result in increased neuronal excitability and differentially expressed genes, mainly associated with cellular components of the axon, dendrite, synapse and postsynaptic density. Therefore, the precise control of epigenetic modifications is critical for neurological development and supporting neural function.

Methylation of H3K4 by KMT2C in eukaryotes is largely catalysed by a protein complex termed Complex Proteins Associated with Set1 (COMPASS) (Hu et al., 2013; Lavery et al., 2020). The COMPASS complexes are built around a protein containing a Set1 domain, such as KMT2C. Mutations in COMPASS complex genes result in aberrant chromatin modification and subsequent reduced transcriptional activation, collectively called COMPASS complex disorders. Thus, Kleefstra syndrome 2 falls under the umbrella of both a KSS due to the clinical presentation and as a COMPASS complex disorder due to the underlying biology.

A number of *KMT2C* knockout animal models have been generated to investigate the varied function of the protein. For example, murine knockout models were generated to investigate the non-neuronal roles of KMT2C in adipogenesis and as a tumour suppressor. The models display phenotypes that reflect the clinical presentation of Kleefstra syndrome 2, including decreased body weight, increased locomotor activity and stunted growth (Lee et al., 2006; Lee et al., 2008). Additional murine and cell models have revealed KMT2C to be a ubiquitously expressed histone methyltransferase, with a role in a wide variety of biological processes, including adipogenesis (Lee et al., 2008) and bile acid homeostasis (Kim et al., 2011), as well as acting as a tumour suppressor implicated in multiple cancer types, including acute myeloid leukaemia (Chen et al., 2014), ureter carcinoma (Lee et al., 2009) and pancreatic ductal adenocarcinoma (Dawkins et al., 2016). To explicitly model the neurobiology of Kleefstra syndrome 2, a brain-specific knockout of *KMT2C* was generated using CRISPR-Cas9 with adeno-associated viruses as delivery vectors (Brauer et al., 2023). The knockout mice demonstrated repetitive behaviours and severe deficits in memory formation, consistent with phenotypes previously reported in individuals diagnosed with Kleefstra syndrome 2.

To date, there have been 14 published descriptions of Kleefstra syndrome 2 spanning exons 12-57 of the 59 exons comprising the canonical transcript (NM_170606.2), all resulting from unique mutations in *KMT2C* (Table 1). All reported causative variants are heterozygous, primarily resulting in early termination of the KMT2C protein. Reported variants span the breadth of the genetic mutational spectrum, including five single nucleotide variants, seven indels and two structural variants (deletions) as shown in Table 1.

Here we report a New Zealand European female with a history of global developmental delay, global learning difficulties, attention deficit hyperactivity disorder, short stature and subtle non-specific dysmorphic facial features. Following whole exome sequencing, a de novo 11 base pair deletion (NM_170606.2:p.Gln587SerfsTer7) in exon 13 was identified in the *KMT2C* gene, not previously reported in the literature. The affected proband has two older male siblings. The younger of the two brothers displayed a discrete clinical presentation of attention deficit hyperactivity disorder (ADHD), autism and intellectual disability and does not have the *KMT2C* frameshift variant. The eldest sibling is healthy with no history of developmental delay or learning difficulties but was not available for genetic analysis.

2 | RESULTS

2.1 | Ethical compliance

Ethical approval was obtained by the New Zealand Northern B Health and Disability Ethics Committee (12/NTB/59) prior to acquiring, sequencing and analysing all human genetic information. All procedures were performed in accordance with the ethical standards of the institutional and national responsible committees on human experimentation and with the 1975 Helsinki Declaration (as revised in 2000).

Informed consent for the study and publication was obtained for all participants in this study.

2.2 | Clinical presentation and family history

The proband (II.3) was the third child to non-consanguineous New Zealand European parents. One older brother (II.2) was diagnosed with autism and intellectual disability. A maternal uncle had Trisomy 21. The proband was born at 36 weeks after emergency lower segment caesarean section for intrauterine growth restriction. Her birth weight was 1950 g (2-9th centile). She was in good condition at birth with Apgar scores of 8 and 9. She was slow to establish feeding and required a short period of nasogastric feeding. She has developmental dysplasia of the hips treated with Pavlik Harness. She had a congenital torticollis and underwent open release of the right sternocleidomastoid muscle at 5 months of age. She was first assessed by a paediatrician and neurodevelopmental therapist at 23 months who noted mild developmental delay and hypotonia on physical examination. Subsequently, she was noted to have global learning difficulties and was diagnosed with dyspraxia and attention deficit hyperactivity disorder in childhood. She was noted to have hypernasal speech and palatal function was investigated with videofluroscopy at 7 years of age and diagnosed as normal. Her speech improved with speech language therapy although clinically she was noted to have a degree of velopharyngeal insufficiency when fatigued. She had a Class I, division one dental malocclusion and underwent extraction of multiple teeth and orthodontics at 14 years. She had short stature with delayed bone age, which was thought to represent constitutional delay. In her teenage years, she was diagnosed with an anxiety disorder, which was treated with a selective serotonin reuptake inhibitor. Height and weight was measured at 15 year 2 months as 146 cm (−2.1 SD) and 36.6 kg (≤2.7 SD), respectively. Her pubertal development was delayed with menarche at 17 years. She has hyperopia corrected with glasses since age 4 years. Her audiometry assessments in childhood were normal.

She was assessed by a clinical geneticist at the age of 14 years. There were no specific non-familial facial dysmorphic features. She had short nailbeds. Investigations for structural variants and copy number variants completed prior to whole exome sequencing included a G-band karyotype and single nucleotide polymorphism microarray (Affymetrix CytoScan 750 k Cytogenetics array), which were both normal.

2.3 | Whole exome sequencing analysis

The DNA of II.2 and II.3 was extracted from saliva using the Oragene prepIT L2P saliva extraction kit (DNA Genotek Inc, Ottawa, Canada) according to the manufacturer's protocols. The DNA of II.1 was not collected or investigated. Whole exome sequencing was performed by Macrogen using the SureSelect XT Human All Exon v6 (Agilent, Santa Clara, CA, USA) and Illumina Novaseq 6000, generating 100 bp paired-end reads. Following variant analysis (Data S1), a heterozygous 11 bp deletion within the gene *KMT2C* (NM_170606.2: c.1759_1769del, p.Gln587SerfsTer7, ClinVar accession SCV002601604) was identified in II.3, confirmed as de novo by PCR and Sanger sequencing (Figure 1). The deletion was not present in II.2.

The deletion occurs within exon 13 of *KMT2C*, substituting a glycine codon for serine codon, followed by a frameshift resulting in the truncation of the protein encoded by the canonical transcript (NM_170606.3) at amino acid 549/4911. The identification of this variant subsequently led to a diagnosis of Kleefstra syndrome 2. The variant is absent from the gnomAD, HapMap, 1000 Genomes Project databases and our in-house dataset of 46 additional individuals diagnosed with neurodevelopmental disorders for which whole exome sequencing and read alignment had been performed at the same time using the same method as II.3. The breakpoints for the deletion occur within two identical hexamer motifs separated by 5 bp of sequence (5′ GAAGAGCAACAGAAGAG 3′). The deletion results in a single copy of the hexamer repeat and loss of the intervening sequence (5′ GAAGAG 3′).

There have been 14 disease-causing variants in *KMT2C* previously reported, resulting in the diagnosis of Kleefstra syndrome 2 (Table 1). Mutations are found within all domains of the protein, with no apparent mutational hotspots (Figure 2). The clinical presentation of developmental delay, reduced height and weight and facial dysmorphisms in II.3 was consistent with the common characteristics of previously reported cases of Kleefstra

TABLE 1 Summary of reported mutations in *KMT2C* resulting in loss of protein function or Kleefstra syndrome 2 diagnosis, ordered by location in the gene (5′-3′).

Sex	Age	Coordinates (GRCh37p13)	(NM_170606.2)	Protein	DD or ID	Neuropsychiatric disorders and CNS anomalies
M	NR	7:151962134	c.1173C>A	p.Cys391*		Autism
NR	NR	7:151949721	c.1378delT	p. Tyr460Thrfs*35		Autism
M	15 years	7:151947983	c.1690A>T	p.Lys564*	Moderate DD (IQ = 50)	PDD-NOS, ADHD
F	NR	7:151947040	c.1736-2A>G		ASD	
F	**17**	**7:151947005-151947015**	**c.1759_1769del**	**p.Gln587Serfs*7**	**Mild**	**Global learning difficulties, dyspraxia, ADHD, anxiety**
M	4.3	7:151945567-151945568	c.1951_1952del	p.Glu651Lysfs*3	Global DD and ID	Cerebral atrophy, widened subarachnoid space, hypotonia
M	NR	7:151945256	c.2263C>T	p.Gln755*	Moderate ID (verbal IQ = 43, non-verbal IQ = 36)	Autism
NR	NR	7:151932982	c.2689C>T	p.Arg897*		Autism
NR	NR	7:151932977	c.2693del	p.Gly898Valfs*15		Autism
F	NR	7:151921614-151921630	c.3048-3064del17	p.Thr1017Profs*4	No ID (verbal IQ = 81, non-verbal IQ = 93)	Autism
F	11.14	7: 151904384	c.3841+1G>A	p.Gly1281Aspfs*15		
F	15 years	7:151891591	c.4441C>T	p.Arg1481*	Moderate DD (IQ = 35)	Hyperactivity, aggressiveness
F	17 years	7:151884849	c.4744G>T	p.Gly1582*	Severe	Elective mutism
M	NR	7:151880219	c.5103_5104del	p.R1701Sfs*6		ASD
M	29 years	7:151880108	c.5216del	p.Pro1739Leufs*2	Moderate	Autistic traits
F	NR	7:151879601	c.5344C>T	p.Q1782*		ASD
M	NR	7:151879459	c.5481_5485del	p.K1827Nfs*17		ASD

Malformations and anomalies	Height (SD)/weight (SD)/ OFC (SD)	Craniofacial dysmorphisms	Other medical issues	Reported clinical diagnosis of Kleefstra syndrome 2	Ref
				No	Iossifov et al., 2015
				No	Yuen et al., 2017
-	160 cm (−2 SD), 55 kg (+1.7 SD), −0.5 SD	Prominent eyebrows, thick ear helices	Bifid uvula, hypospadias, bilateral inguinal hernia	Yes	Koemans et al., 2017
				No	Zhou et al., 2022
Developmental dysplasia of the hips	**At 15 years 2 months 146 cm (−2.1 SD) Weight 36.6 kg (≤2.7 SD)**		**Congenital torticollis, delayed puberty, delayed bone age, hyperopia, Class I, division one dental malocclusion**	**Yes**	**This report**
Macrocephaly				Yes	Brunet et al., 2021
				No	Iossifov et al., 2015
				No	Alonso-Gonzalez et al., 2021
				No	De Rubeis et al., 2014
				No	Iossifov et al., 2015
	−2.05 SD, NR, NR		Primordial uterus, congenital spina bifida	No	Li et al., 2022
-	148 cm (−2.5 SD), 41 kg (0 SD), −2 SD		-	Yes	Kleefstra et al., 2012
Duplicated right thumb and left preauricular tag	Short stature (−2.1), low weight (−2.74), microcephaly (−2.42)	Yes	Hearing loss and delayed puberty	Yes	Faundes et al., 2018
				No	Zhou et al., 2022
Thoracal kyphosis	171.5 cm (−1.7 SD), 63.5 kg (+0.6 SD), −0.5 SD	Flattened mid-face, prominent eyebrows, thick ear helices	Phenylketonuria (PKU), recurrent respiratory infections	Yes	Koemans et al., 2017
				No	Zhou et al., 2022
				No	Zhou et al., 2022

(Continues)

TABLE 1 (Continued)

Sex	Age	Coordinates (GRCh37p13)	(NM_170606.2)	Protein	DD or ID	Neuropsychiatric disorders and CNS anomalies
F	NR	7:151879278	c.5667dup	p.Arg1890Thrfs*24	ID	ASD
NR		7:151879229	c.5716C>T	p.Arg1906*		Tourette syndrome
F	NR	7:151878327	c.6617del	p.P2206Lfs*33		ASD
M[a]	8 months	7:151877103_151877114del	c.7247_7258del	p.His2416_Pro2419del	Global DD and ID	Motor delayed, delayed social skills
M	31 years	7:151874988	c.7550C>G	p.Ser2517*	Mild	Autism
M	NR	7:151874822	c.7715_7716insC	p.Pro2573Alafs*15	Moderate ID (verbal IQ = 36, non-verbal IQ = 51)	Autism
M	NR	7:151874312	c.8224_8225dup	p.D2742Efs*9		ASD
M	NR	7:151874286	c.8252C>G	p.S2751*		ASD
F	4 years	7:151873688-151873689	c.8849_8850delAT	p.His2950Argfs*17	Severe	Hydrocephalus and hypoplasia of cerebellar vermis
F[a]	6 years	7:151873294	c.9294C>T	p.Pro3082Ser	Severe	Autism, hyperactivity, aggressiveness, language delay, motor delay
M	10		c.9284delC	p.P3095Lfs*2	Comprehensive developmental delay	ADHD
M	NR	7:151868428	c.9375-1G>A			ASD
F	10 months	7:151868411	c.9391C>T	p.Gln3131*	DD	
M	NR	7:151864359	c.9621dup	p.S3208Ifs*2		ASD
F	7 years	7:151859847_151859850	c.10812_10815del	p.Lys3605Glufs*24	Mild DD (IQ = 63)	Autism, sleeping disorder
M	NR	7:151859501	c.11161G>T	p.E3721*		ASD
M	NR	7:151853340	c.11760_11762delinsGG	p.F3920Lfs*14		ASD
F	NR	7:151851508	c.11983C>T	p.R3995*		
M	NR	7:151851508	c.11983C>T	p.R3995*		
M	NR	7:151845580	c.13432C>T	p.Arg4478*	Mild ID (verbal IQ = 62, non-verbal IQ = 59)	Autism

Malformations and anomalies	Height (SD)/weight (SD)/ OFC (SD)	Craniofacial dysmorphisms	Other medical issues	Reported clinical diagnosis of Kleefstra syndrome 2	Ref
				No	Brea-Fernández et al., 2022
				No	Liu et al., 2019
				No	Zhou et al., 2022
	66 cm (−3 SD), 6 kgs (−3 SD), 36.5 cm (≤2 SD)	Hypertelorism, brachycephaly, mid-face hypoplasia, everted lower lip and head-tilt	Seizures, bilateral sensory neural deafness	Yes	Cheema et al., 2022
Scoliosis	179 cm (−0.5 SD), 53.8 kg (−1.5 SD), 57 cm (−0.5 SD)		Strabismus, cryptorchidism	Yes	Koemans et al., 2017
				No	Iossifov et al., 2015
				No	Zhou et al., 2022
				No	Zhou et al., 2022
No	Short stature (−2), low weight (−2 SD), microcephaly (−1.97 SD)	Yes	no	Yes	Faundes et al., 2018
Ligamentous hyperlaxity	+0.67 SD, +0.67 SD, +0.67 SD	Broad and rounded forehead, hypertelorism, nose with saddle bridge and bulbous tip		Yes	Siano et al., 2022
	−0.77 SD at 4 years 6 months, −2.14 SD at 4 years 6 months, −1 SD at 3 years 1 months	Feeding difficulties, anaemia and eczema	Bushy brows, mandibular retrusion	Yes	Wu & Li, 2022
				No	Zhou et al., 2022
		Dysmorphic features	Upper respiratory tract infection	No	Mahfouz et al., 2020
				No	Zhou et al., 2022
-	109 cm (−3 SD), 16 kg (−1.5 SD), −2.25 SD	Flattened mid-face, everted lower lip	Recurrent respiratory infections, dry skin, hoarse voice	Yes	Koemans et al., 2017
				No	Zhou et al., 2022
				No	Zhou et al., 2022
				No	Zhou et al., 2022
				No	Zhou et al., 2022
				No	Iossifov et al., 2015

(Continues)

TABLE 1 (Continued)

Sex	Age	Coordinates (GRCh37p13)	(NM_170606.2)	Protein	DD or ID	Neuropsychiatric disorders and CNS anomalies
NR	NR	7:151842339	c.14073C>A	p.Tyr4691*		Autism
M	NR	7:151836877	c.14344-1G>A			ASD
F	5 years	7:151836279	c.14526dupG	p.Pro4843Alafs*12	Severe (motor delay was mild)	Autistic traits, developmental regression, insensitivity to pain, abnormal gait
F	10 years	7q36.1 (151858920-152062163)×1			Severe	Auto-mutilation
F	6 years, 2 months	7q36.1 (151839151-151965981)×1			Mild ID (IQ = 81)	Severe impairment in verbal comprehension

Note: Cases ordered by coding transcript location (5′ to 3′). The case described in this report is bolded.

Abbreviations: ADD, attention deficit disorder; ADHD, attention deficit hyperactivity disorder; CNS, central nervous system; DD, developmental delay; ID intellectual disability; NR, not reported; OFC, occipital frontal circumference; PDD-NOS, pervasive developmental disorder not otherwise specified; SD, standard deviation.

[a]Indicates cases possessing non-truncating mutations and diagnosed with Kleefstra syndrome 2.

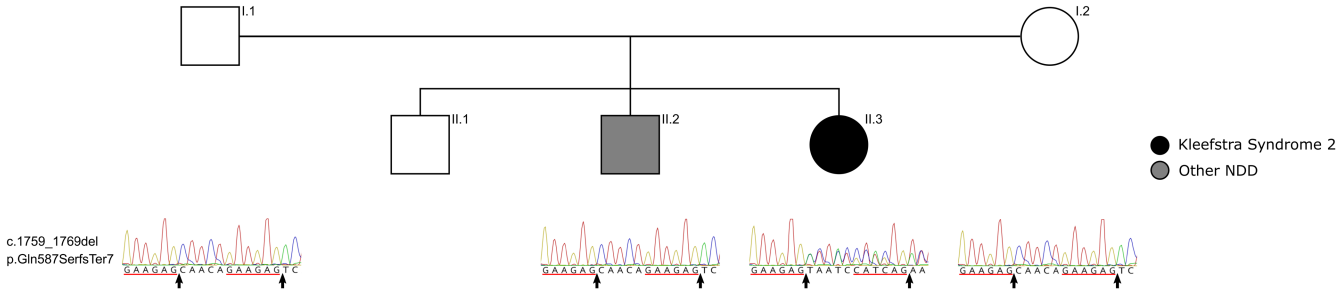

FIGURE 1 Family pedigree and corresponding Sanger sequencing electropherograms showing the c.1759_1769del in II.3. The breakpoints for the deletion are indicated on the electropherograms by the black arrows. The 6 nucleotide repeat that flanks the deletion is indicated by the red line below each electropherogram. II.1 was not sequenced.

syndrome 2, and only one previously published pathogenic variant (NM_170606.2:c.1690A>T, p.Lys564*) is located more 5′ of the p.Gln587Serfs*7 variant identified in the case described here.

3 | DISCUSSION

We describe a girl with a history of global developmental delay, global learning difficulties, attention

Malformations and anomalies	Height (SD)/weight (SD)/ OFC (SD)	Craniofacial dysmorphisms	Other medical issues	Reported clinical diagnosis of Kleefstra syndrome 2	Ref
				No	Iossifov et al., 2014; Sanders et al., 2012; O'Roak et al., 2012, Satterstrom et al., 2020
				No	Zhou et al., 2022
No	Normal (+0.4 SD), normal (+0.18 SD), normal (−1 SD)	Yes	Constipation	Yes	Faundes et al., 2018
Kyphosis	NR, 20 kg (−2.5 SD), −2 SD		Plagiocephaly	Yes	Koemans et al., 2017
NR	NR, NR, +2 SD	Macrocephaly, prominent forehead, deep set eyes, short nose with pinched nasal tip, narrow philtrum, thin upper lip, down-turned mouth and misaligned teeth	Torticollis, plagiocephaly	Yes	Schoch et al., 2020

deficit hyperactivity disorder, short stature and subtle non-specific dysmorphic facial features. Whole exome sequencing and genetic variant analysis identified an 11 bp deletion in II.3 located within exon 13 of the gene *KMT2C*, resulting in a frameshift mutation and predicted truncation of the protein (NM_170606.2:c.1759_1769del, p.Gln587Serfs*7). The clinical features of II.3 and identification of the mutation led to a diagnosis of Kleefstra syndrome 2. This is the fifteenth case of Kleefstra syndrome 2 reported, with only one other mutation (NM_170606.2: c.1690A>T, p.Lys564*) located more 5′ in the transcript. An older brother with a discrete neurodevelopmental disorder does not harbour this mutation.

Review of the clinical (Table 1) and genetic (Figure 2) characteristics of the previous 14 reported Kleefstra syndrome 2 cases revealed clinical phenotypes that can be divided into four main categories: (1) developmental or intellectual delay (13 cases), (2) neuropsychiatric disorders such as autism and ADHD (14 cases), (3) reduced height (9 cases), weight (7 cases) and head circumference (11 cases) and (4) craniofacial dysmorphisms (10 cases). Interestingly, there does not appear to be an obvious concordance between phenotype and mutation type or location across the gene. Other skeletal malformations and health issues are also frequently observed, including kyphosis, torticollis, delayed puberty, strabismus and plagiocephaly.

Notably, the majority (13/15, including the case described here) of reported mutations for Kleefstra syndrome 2 are heterozygous and result in premature translation termination (PTC) codons via nonsense mutations, indels or deletions with breakpoints within the gene. These PTC could prevent the synthesis of the KMT2C protein via nonsense mediated decay (NMD) or create a truncated protein. Nonsense mediated decay is a mechanism under which mRNAs containing PTC

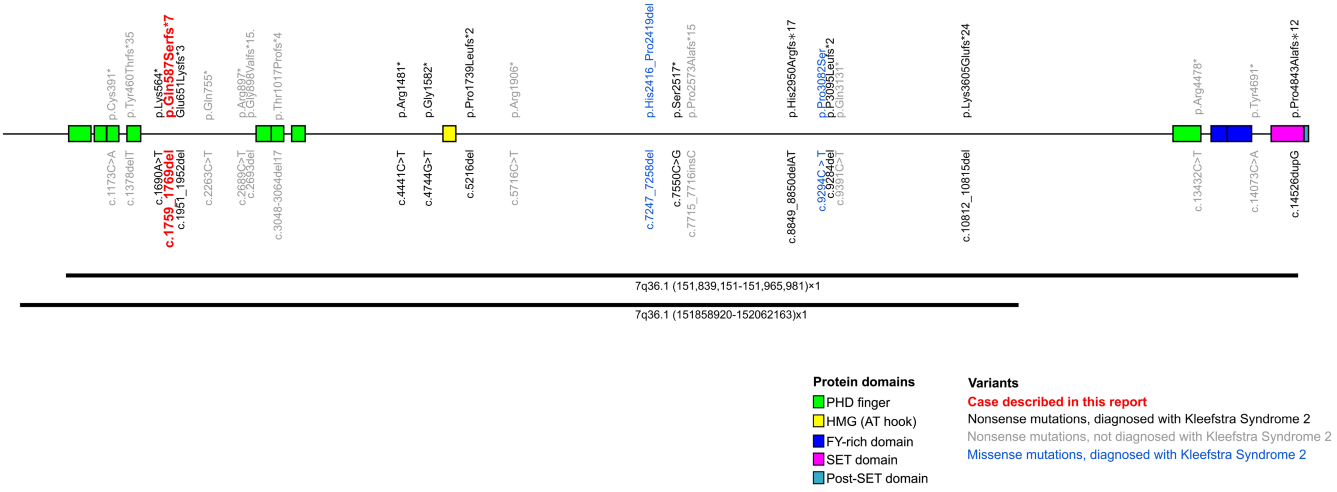

FIGURE 2 Reported loss of function mutations in *KMT2C*. Individuals diagnosed with Kleefstra syndrome 2 resulting from nonsense mutations are indicated in black text, with individuals who have not received a formal diagnosis in grey. The extent of genomic deletions of the two causative copy number variants are indicated below the protein, with a black line. The two cases diagnosed with Kleefstra syndrome 2 which harbour missense mutations are indicated in blue text. The mutation documented in this report appears in red.

codons are degraded, preventing the synthesis of truncated and potentially harmful proteins with mRNA from the wild type allele remaining intact. Typically, NMD occurs if the PTC is greater than 50–55 nucleotides upstream of the subsequent exon-exon boundary in the mRNA (Nagy & Maquat, 1998). RNA sequencing or mRNA amplification has not been performed for any of the previous case reports of Kleefstra syndrome 2. Therefore, it is possible that the causative nonsense could result in NMD. However, for 8/13 reported causative nonsense mutations (including the case described herewith), the PTC is within 50 nucleotides of the downstream exon-exon boundary. Given there is no clearly discernible phenotypic distinctions between cases where the PTC is greater or less than 50 nucleotides from the exon-exon boundary, here doesn't appear to be a functional distinction between the mechanisms by which early termination and NMD result in haploinsufficiency of the KMT2C protein.

The enzymatically active domain of all KMT2 proteins is the highly conserved SET domain (Figure 2) (Schubert et al., 2003) located at the 3′ end of the gene (amino acids 4771-4893 of 4911 encoded by NM_170606.2). All reported PTC-inducing mutations that result in Kleefstra syndrome 2, including the case detailed in this report, (if translated) would truncate the SET domain, eradicating the methyltransferase activity of the truncated protein. Furthermore, the two most 5′ mutations (the variant reported here in exon 13/59 and that mutation in exon 12/59 described by Koemans et al., 2017), result in the absence of 4 of the 8 metal binding plant homeodomain (PHD) finger domains, the DNA-binding high-mobility group (HMG) AT-hook domain, the protein–protein interaction FY-rich domain and the critical SET and post-SET domains (Koemans et al., 2017). These domains are highly conserved across species (Eissenberg & Shilatifard, 2010). Thus, for the case presented here, it is not only the catalytic capability of the resultant protein that is affected, but likely also the metal binding, DNA-binding and protein–protein interaction of the mutant KMT2C protein. However, there does not appear to be an association between phenotypic severity and length of protein truncation. For example, the case with the most 3′ truncating mutation, located within the SET domain itself (NM_170606.2:p.Pro4843Alafs*12), was reported to exhibit severe developmental delay, microcephaly and other dysmorphic features (Faundes et al., 2018). Thus, abolishing the enzymatic action of KMT2C alone appears to be sufficient for the development of Kleefstra syndrome 2.

Only two non-truncating mutations in KMT2C resulting in Kleefstra syndrome 2 have been described (Cheema et al., 2022; Siano et al., 2022). A missense variant (NM_170606.2: c.9294C>T, p.Pro3082Ser) and a three amino acid in-frame deletion (NM_170606.2: c.7247_7258del, p.His2416_Pro2419del), both impact protein regions outside of any known functional domains (Figure 2) and are both N-terminal of the SET domain. The in-frame deletion was reported in a child born to consanguineous parents, who presented with seizures, developmental delay, hypertelorism, brachycephaly, mid-face hypoplasia and reduced head circumference, weight and body length. The child had a brother with developmental delay and a second-degree cousin with a history of psychomotor retardation. Unfortunately, the report does not clarify whether the brother and cousin also carry the three

amino acid in-frame *KMT2C* deletion. The deletion is located 2352 amino acids upstream from the SET domain and the deleted amino acids are not evolutionarily conserved (Pollard et al., 2010). The case harbouring the missense variant presented with childhood autism and developmental delay, a history of hypotonia and minor face anomalies. Unlike the majority of Kleefstra syndrome 2 cases, this individual was reported to have slightly elevated head circumference, weight and height. The proposed causative variant (NM_170606.2: c.9294C>T, p.Pro3082Ser) results in the substitution of proline 3082 for serine, 1686 amino acids N-terminal of the SET domain. Although, this particular variant is not present in population databases (such as gnomAD), another variant that results in a substitution of proline 3082 for leucine (p.Pro3082Leu) is relatively common in the gnomAD database, with a minor allele frequency 3.43e-3 (950 alleles, including 25 homozygotes) (Karczewski et al., 2020). The p.Pro3082Leu substitution is listed as benign in ClinVar (Landrum et al., 2014) and is predicted to be more deleterious than the p.Pro3082Ser substitution by both SIFT (0 vs. 0.47) (Ng & Henikoff, 2003) and Polyphen (0.994 vs. 0.991) (Adzhubei et al., 2013). Additionally, the p.Pro3082Leu substitution represents a greater difference in side chain atomic composition than the p.Pro3082Ser substitution (Grantham scores 98 and 74, respectively; Grantham, 1974). As the majority of reported cases of Kleefstra syndrome 2 harbour mutations which truncate the SET domain of KMT2C, it will be important to functionally characterise these two missense mutations to determine their impact on KMT2C and their role in the condition.

Missense mutations in *KMT2C* have been reported as pathogenic for related neurodevelopmental disorders (but not specifically Kleefstra syndrome 2 based on the provided clinical details), including participants in large cohort studies (Alonso-Gonzalez et al., 2021; Brea-Fernández et al., 2022; De Rubeis et al., 2014; Guo et al., 2018; Iossifov et al., 2014; Krumm et al., 2015; Krupp et al., 2017; Satterstrom et al., 2020; Yuen et al., 2017; Zhou et al., 2022). Of these missense mutations, only two of 16 occur within the critical SET domain. Target specificity of H3K4 methyltransferases, such as KMT2C, are dependent on the interaction of the KMT2 COMPASS complex proteins with multiple protein cofactors (Lavery et al., 2020). Therefore, missense mutations in *KMT2C* that occur outside of the SET domain may affect the methyltransferase activity of the KMT2C protein if they alter binding to the chromatin substrate or affect recruitment of members of the KMT2 COMPASS complex, particularly if they occur within the protein–protein interaction FY-rich domains or DNA-binding AT-hook domain. It is, therefore, feasible that missense mutations that do not abolish the enzymatic action of the SET domain result in a less severe phenotype than the typically described Kleefstra syndrome 2, with only a subset of the core traits presenting in the affected individual as indicated by the generalised description of phenotype of individuals within the large cohort studies.

Truncating mutations that encompass the SET domain of KMT2C have been reported in 27 additional individuals not explicitly described as having Kleefstra syndrome 2 (Table 1; Figure 2). One of these cases, included as part of a study investigating the utility of clinical exome sequencing in a complex Emirati paediatric cohort (Mahfouz et al., 2020), presented with altered neurodevelopment and dysmorphic features reminiscent of the Kleefstra syndrome 2 phenotype. The 26 other cases were described in large cohort studies focusing on single disorders including Tourette's syndrome (Liu et al., 2019), autism (Alonso-Gonzalez et al., 2021; De Rubeis et al., 2014; Iossifov et al., 2014, 2015; O'Roak et al., 2012; Satterstrom et al., 2020; Yuen et al., 2017) and short stature (Li et al., 2022). However, the specific clinical features for each of these cases were not provided. From the previously described cases of Kleefstra syndrome 2, heterozygous loss of the SET domain of KMT2C is sufficient to cause the condition. Therefore, it would be interesting to know the full clinical descriptions of each of these individuals to determine the extent of phenotypic overlap between these individuals with truncating mutations (without a known Kleefstra syndrome 2 diagnosis) and those previously diagnosed with Kleefstra syndrome 2. The absence of a diagnosis in these individuals may reflect the difficulties of diagnosing Kleefstra syndrome 2 based on phenotype alone, given the significant phenotypic overlap with other neurodevelopmental disorders.

Despite extensive analysis of genetic variants called from whole exome sequence reads, we were unable to identify a causative variant that could explain the phenotype of the proband's brother, II.2.

It is important to understand the clinical and genetic spectrum of rare diseases such as Kleefstra syndrome 2, to enable appropriate clinical management and evaluate efficacious treatment options. This report describes the fifteenth case of Kleefstra syndrome 2, contributing to a growing knowledge of the core traits of the disorder and the growing genetic spectrum of variants that underlie the condition, both of which will help shed light on possible therapeutic targets.

AUTHOR CONTRIBUTIONS

Jessie C. Jacobsen, Klaus Lehnert and Russell G. Snell conceived the experiments. Whitney Whitford performed DNA-based laboratory experiments. Whitney Whitford,

Klaus Lehnert and Jessie C. Jacobsen performed data and bioinformatics analysis. Juliet Taylor, Ian Hayes and Warwick Smith conducted the clinical evaluation. Whitney Whitford wrote the initial manuscript, and Jessie C. Jacobsen critically reviewed the manuscript. All authors edited and reviewed the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Consent was not obtained to deposit full variant data publicly; however, the variant has been deposited to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) under the accession number SCV002601604.

ETHICS STATEMENT

Ethical approval was obtained by the New Zealand Northern B Health and Disability Ethics Committee (12/NTB/59) prior to acquiring, sequencing and analysing all human genetic information. All procedures were performed in accordance with the ethical standards of the institutional and national responsible committees on human experimentation and with the 1975 Helsinki Declaration (as revised in 2000).

CONSENT

Informed consent for the study and publication was obtained for all participants in this study.

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SUPPORTING INFORMATION

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

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