

KAT6A Syndrome: genotype–phenotype correlation in 76 patients with pathogenic KAT6A variants

Joanna Kennedy, BSc MBBS MRCPCH, Ruth Newbury-Ecob, MB ChB MD et al.
A full list of authors and their affiliations appears at the end of the paper.

Purpose: Pathogenic variants in *KAT6A* have recently been identified as a cause of syndromic developmental delay. Within 2 years, the number of patients identified with pathogenic *KAT6A* variants has rapidly expanded and the full extent and variability of the clinical phenotype has not been reported.

Methods: We obtained data for patients with *KAT6A* pathogenic variants through three sources: treating clinicians, an online family survey distributed through social media, and a literature review.

Results: We identified 52 unreported cases, bringing the total number of published cases to 76. Our results expand the genotypic spectrum of pathogenic variants to include missense and splicing mutations. We functionally validated a pathogenic splice-site variant and identified a likely hotspot location for *de novo* missense variants. The majority of clinical features in *KAT6A* syndrome have highly variable penetrance. For core

features such as intellectual disability, speech delay, microcephaly, cardiac anomalies, and gastrointestinal complications, genotype–phenotype correlations show that late-truncating pathogenic variants (exons 16–17) are significantly more prevalent. We highlight novel associations, including an increased risk of gastrointestinal obstruction.

Conclusion: Our data expand the genotypic and phenotypic spectrum for individuals with genetic pathogenic variants in *KAT6A* and we outline appropriate clinical management.

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INTRODUCTION

Lysine (K) acetyltransferase 6 A (*KAT6A*, a.k.a. *MOZ*, *MYST3*) belongs to the MYST family of histone acetyltransferases that are defined by the presence of a highly conserved MYST domain consisting of acetyl-CoA binding motif and a zinc finger.¹ The MYST family of proteins (*KAT6A*, *KAT6B*, *KAT5*, and *KAT7*) take part in a wide range of core cellular functions, such as chromatin remodeling, gene regulation, protein translation, metabolism, and cellular replication.² Use of exome sequencing in patients with syndromic intellectual disability has revealed causative pathogenic variants in several genes that function as parts of chromatin remodeling complexes.^{3–6}

De novo, rare, and protein-truncating genomic variants in a number of genes have been associated with cases of intellectual disability with speech delay.^{7,8} Genes involved in highly penetrant and syndromic developmental delay have high probability of loss-of-function intolerance (pLI) scores,⁹ a metric indicating that these same genes are not found to have predicted protein-truncating variants in a control population. Protein-truncating variants in two of the four

MYST family genes, paralogs *KAT6A* (OMIM 6162680) and *KAT6B* (OMIM 606170 and 603736) have been associated with syndromic developmental delay. The phenotypic heterogeneity of pathogenic variants in both genes is striking. *De novo* truncating variants in *KAT6B* cause a spectrum of disorders, including genitopatellar syndrome (MIM 606170), Ohdo syndrome (Say–Barber–Biesecker–Young–Simpson SBBYS variant MIM 603736) and a Noonan syndrome–like disorder.^{10–12} Reported cases of *KAT6A* syndrome have been identified primarily through clinical or research exome sequencing in a gene-centric approach. A few cases have been identified through consortia exploring broad clinical phenotypes such as neutropenia.¹³

KAT6A and *KAT6B* each function in a multisubunit complex with three other proteins: BRPF1/2/3, ING5, and hEAF6¹⁴. These proteins form a complex to acetylate lysine residues on histone H3 tails, thereby promoting a wide range of developmental programs. The importance of the *KAT6A/B* complex has been further highlighted by recently identified pathogenic variants in the binding partner, *BRPF1*^{15,16} resulting in clinical features that overlap

Correspondence: Valerie A. Arboleda (varboleda@mednet.ucla.edu) or Ruth Newbury-Ecob (Ruth.Newbury-Ecob@UHBristol.nhs.uk).
These authors contributed equally: Valerie A. Arboleda and Ruth Newbury-Ecob.

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with syndromes caused by pathogenic variants in *KAT6A* and *KAT6B*.

The use of model organisms to investigate *KAT6A* function has provided insight into the role of *KAT6A* in vivo. Complete knockout mouse models result in embryonic lethality, due to a failure of hematopoiesis. A knock-in pathogenic variant that eliminates the *KAT6A*'s acetyltransferase function results in decreased life span, decreased body weight, and proliferation defects.¹⁷ Tissue- or cell-specific knockout has shown that *KAT6A* regulates transcriptional programs important for skeletogenesis, hematopoiesis, and splenic and thymic function.^{17–19} Further studies demonstrated that *KAT6A*-mediated acetylation promotes memory B-cell formation and the CD8 T-cell response to viral infection.^{20,21} Transcriptomic profiles of human fibroblast cell lines derived from patients harboring heterozygous *KAT6A* truncating pathogenic variants demonstrated altered expression of p53-associated genes.⁴

To date, eight papers^{4–6,13,22–25} describe 24 patients with pathogenic variants in *KAT6A*. In this paper, we add 52 novel and comprehensively phenotyped cases and review all previously published cases.

MATERIALS AND METHODS

Research cohort

Phenotypic information from patients with likely causative *KAT6A* pathogenic variants was obtained by three methods: through clinical geneticists, through an online survey to families, and through literature review. The cohorts were independently identified and therefore some individuals were identified through two methods. In these cases data were combined. All patients/families included in the study provided consent through the treating clinician or through the institutional review board–approved patient/family survey. Written consent for publication of patient photos was also obtained.

In the first cohort ($N=33$), information was obtained from the primary clinical geneticist using a targeted phenotypic questionnaire designed to identify a spectrum of clinical phenotypes in patients with convincing de novo pathogenic variants in *KAT6A*. In this cohort some patients were identified through the Deciphering Developmental Disorders (DDD) Study, and the DDD Complementary Analysis Project allowed us to access initial phenotypic data.⁷ Additional patients in Australia, Holland, Japan, Finland, Norway, and the United States were identified through communication of the lead authors with treating physicians and *KAT6A* variants were identified through clinical or research exome sequencing.

A second cohort ($N=43$) was recruited through social media and patient advocacy groups. This was performed independently. We collected information allowing for us to match cases. A parent or family member was asked to complete an online survey spanning birth history, developmental milestones, current treatments, and associated medical conditions. Detailed information about the clinically identified genetic change in *KAT6A* gene was also collected.

A third cohort ($N=24$) was identified from the published medical literature. Approximately 40% of families where the proband case was previously published provided updated information via online survey.

Splice-site variant analysis

RNA was extracted from peripheral blood samples and underwent reverse transcription using a High Capacity cDNA RT kit (Thermo Fisher Scientific). Polymerase chain reaction (PCR) amplification was performed using custom designed primers (see Supplemental information for additional details) and Sanger sequencing was performed using ABI3730 DNA sequencer (Applied Biosystems).

Statistical analysis

To assess significant differences between our early- and late-truncating variant cohorts, we performed a two-tailed Fisher's exact test²⁶ to determine if the differences within groups were significant.

RESULTS

Our study comprised a total of 75 patients with pathogenic or likely pathogenic variants in the gene *KAT6A* and 1 patient with a variant of unknown significance (VUS). Of these, 70% (52/76) are novel cases that have not been previously reported in the literature. Their ages range from 1 to 32 years of age and the cohort is 49% female and 51% male.

Genotype analysis

Within the 52 novel cases, we identified 44 novel genetic variants, of which 88% (39/44) are predicted to result in a truncating frameshift or nonsense variant. Some locations have recurrent truncating changes, primarily located in the acidic domain, which is rich in arginine residues (amino acid positions 1019, 1024, and 1129).

Five novel pathogenic variants are missense changes at highly conserved residues (Supplemental Fig. 2). Four of the five missense changes have been classified as likely pathogenic because they are de novo, rare,⁹ match the clinical phenotype, and are predicted to be deleterious based on in silico algorithms.^{27–29} The remaining missense change, p.S371Y, is classified as a VUS as the patient fulfilled the criteria above except the variant was ultimately found to be maternally inherited from an unaffected parent. Although the missense Z -score⁹ for *KAT6A* is nonsignificant at $Z=2.14$, this score represents a depletion across the entire gene and does not account for regional variation. In our missense cases, three of the individuals have de novo missense changes within a highly conserved region and are known to bind to *RUNX1/2*, a gene important in transcriptional transactivation (Fig. 1c).

We have identified 4 individuals with predicted splice-site changes due to substitutions in canonical splice site of *KAT6A* exons. For patient 17 with pathogenic variant at c.1364-2 A>T, sequencing of *KAT6A* cDNA from a patient blood sample demonstrated that the splice variant resulted in an 8-

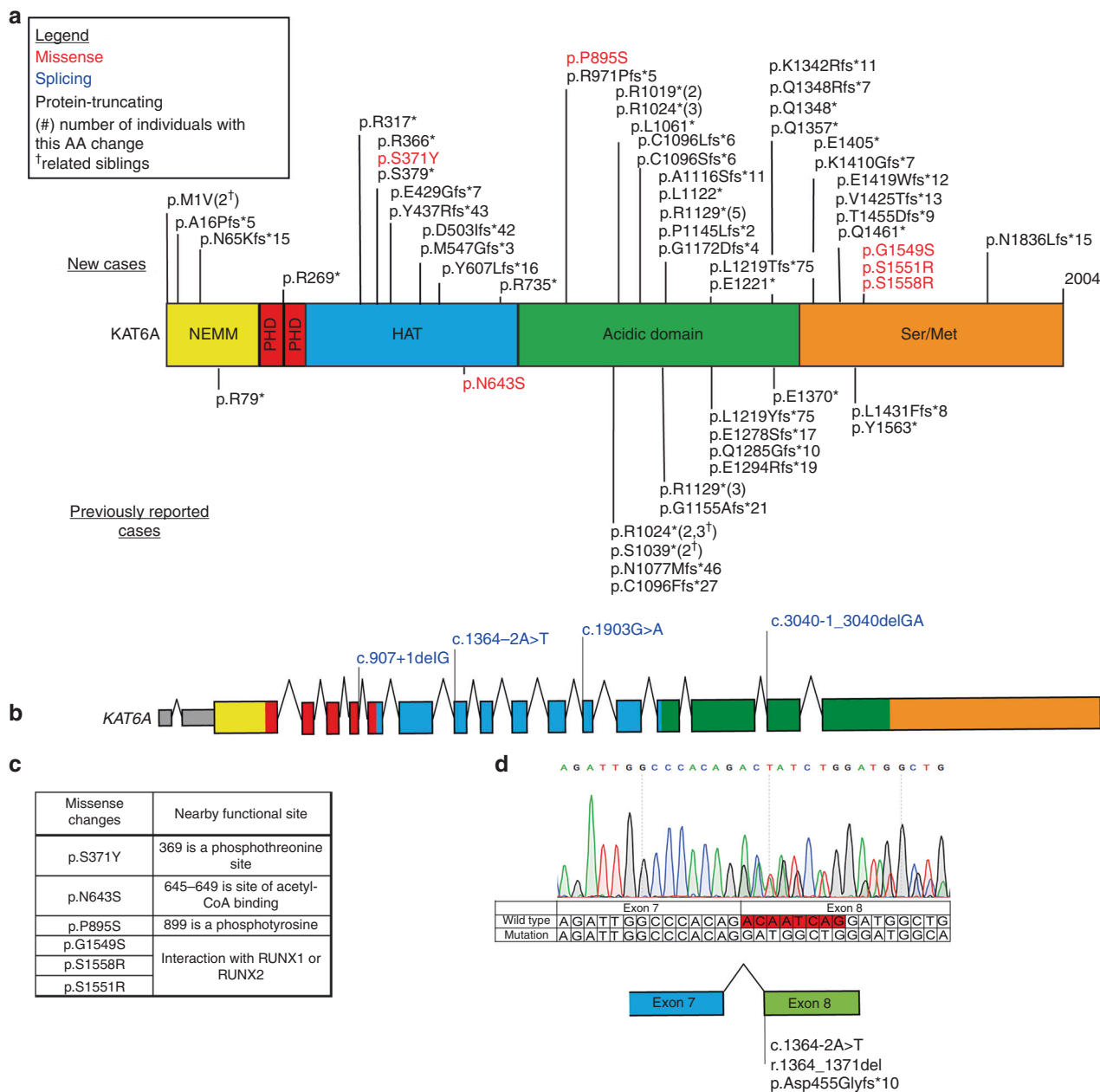


Fig. 1 KAT6A domains and the distribution of pathogenic genetic variants. **a** We observed a total of 76 patients with 61 unique genetic variants across the 2004–amino acid protein. The pathogenic variants in new patients are shown above the gene; previously reported variants are displayed below the gene. Missense pathogenic variants are denoted in red while protein-truncating pathogenic variants are denoted in black font. Protein ID for KAT6A is NP_006757.2 and various protein domains are: NEMM domain (AA 1–206), PHD domains (AA 207–313), HAT domain (AA 314–787), acidic domain (788–1414), and Ser/Met domain (1414–2004). **b** Splice changes are denoted in blue demonstrating their approximate intronic location in the gene model. **c** Missense changes are located near important functional domains of KAT6A described in UniProt.³⁴ **d** The functional effect of the identified splice variant in patient 17 was validated from sequencing of the complementary DNA (cDNA) product of blood RNA. The splicing effect resulted in a deletion of eight base pairs and a frameshift change. *WT* wild type

bp deletion in exon 8, which is predicted to cause protein truncation (Fig. 1d).

Clinical features of newly reported patients

Developmental delay and intellectual disability

Intellectual disability and developmental delay are universal. Intellectual disability (ID) varies from mild to severe. One young adult has a driver's license. Hypotonia is common

and contributes to motor delay. Often, truncal hypotonia was associated with limb hypertonia; this was more notable in the neonatal period. Specific speech and language delay combined with motor delay may lead to an overestimate of ID in the early years. To date no individuals have been reported without ID though this may represent a selection bias because most patients have been identified by sequencing patients with ID.

A correlation is observed between the site of the pathogenic variant and the level of ID. In cases in which the level of ID was reported on, 95% of late-truncating cases (exon 16 and 17) were rated as moderate or severe, while 60% of early-truncating cases (exons 1–15) were rated as mild (Supplementary Fig. 3). This pattern was also observed when we considered the age of achieving developmental milestones for affected patients. A previously reported patient with a full-gene deletion had a mild ID (20).

Oromotor dyspraxia

Marked expressive speech delay is universal. Many struggle with articulation. Speech delay is often described as a form of verbal dyspraxia. Receptive language is consistently more developed than expressive language. One patient with mild ID achieved a score typical for receptive language for his age. Use of sign language and communication aids are helpful. Communication difficulties are a source of frustration. Despite significant delays many children do make progress. For example, a patient who spoke four words at the age of 4 now speaks fluently as an adult. However, there are individuals with KAT6A syndrome who remain nonverbal into adulthood.

Feeding difficulties are commonly associated with verbal dyspraxia due to oromotor dysfunction. Seventy-eight percent of patients experienced feeding difficulties. Many patients had difficulty establishing feeding at birth and nasogastric feeding was often required. In addition, several patients have dysphagia and a few have had recurrent aspirations.

Other gastrointestinal problems

The high prevalence of reflux and constipation is suggestive of dysfunctional intestinal motility. Reflux is a significant issue, and while some outgrow this a few report persistent vomiting and retching well beyond infancy. Many patients have had a gastrostomy tube for feedings and small number underwent a fundoplication. Constipation is a significant issue for over half of our patients and many are on long-term laxatives. In addition, four patients in this study (patients 6, 22, 42, and 46) had bowel obstruction. One required surgery for a duodenal web and malrotation, the second also required surgery for a malrotation, the third had a small bowel obstruction leading to the resection of part of the ileum, and the fourth required three laparotomies for a bowel obstruction, and subsequently experienced gastrointestinal failure and was transferred to hospice care. This patient also had anal stenosis and gastroesophageal nerve impairment. Interestingly malrotation and duodenal rupture has been reported in KAT6B related disease³⁰ suggesting shared underlying mechanisms.

Cardiac malformations

Cardiac malformations are present in half (51%) of our cohort. Most frequent are septal defects including atrial septal defects (34%), ventricular septal defects in (8%), and persistence of the fetal anatomy (19%) (patent foreman ovale, and persistent ductus arteriosus). At least 45% of patients with

cardiac malformations required intervention (open heart surgery or via cardiac catheterization). A minority of our patients are awaiting cardiology review and have not yet had an echocardiogram. The prevalence of cardiac lesions highlights the need for early cardiology assessment.

Hematological and immunological associations

While the majority of patients did not report hematological or immunological deficiencies, three individuals report isolated moderate to severe neutropenia.^{5,13} Many patients' parents reported frequent infections, and some were reported to take longer to recover from infections compared with peers. The majority, however, did not report that they experienced frequent infections and those who did tended to report common childhood illnesses including otitis media and upper and lower respiratory tract infections. Many children experience recurrent infections of these types, so this is not necessarily indicative of immunodysfunction.

Some unusual infections have been noted. Patient 7 has recurrent and extensive herpes simplex infections of the face and eyes and patient 13 has had impetigo and a separate staph infection. Patient 2 also has a B-cell and T-cell immunodeficiency, hypogammaglobulinemia for which she receives regular intravenous immunoglobulins, in addition to episodes of suspected perianal streptococcal dermatitis. Patient 20 has hypogammaglobulinemia. Patient 11 mounted a low immune response to HibB and pneumococcal vaccine requiring booster vaccines. Patient 1 has unexplained persistent thrombocytopenia. One hypothesis is that KAT6A syndrome may result in a variety of abnormalities of blood cell lines. Alternately, there may be other genetic or environmental factors in each individual.³¹ Additional research is required to define this further. Structural abnormalities can also predispose to infection. Patients 3 and 10 have renal abnormalities and recurrent urinary tract infections.

Facial features

A broad nasal tip, which may become more obvious with age, and a thin, tented upper lip, are the most consistent facial features in patients with KAT6A syndrome. Other common facial features include bitemporal narrowing, prominence of the nasal bridge, and a short and flat philtrum. Notable features present in a significant minority are epicanthic folds and low-set and posteriorly rotated ears, which are occasionally folded (Fig. 2).

Within the mouth a high arched narrow palate was noted in a few patients and teeth abnormalities were common. Abnormal peg-shaped teeth have been reported previously^{4,5} and are also seen in a number of our newly reported patients. Other dental abnormalities reported are small tooth size, supernumerary teeth, and dental crowding. Cleft palates are not frequently seen (reported in two patients).

Skull and brain abnormalities

Many patients with KAT6A syndrome have had magnetic resonance image (MRI) scans of the brain and major



Fig. 2 Clinical images of 25 newly reported cases show subtle facial features suggestive of KAT6A syndrome

structural anomalies are rare. One patient has been reported with a pituitary malformation and related hormone deficiencies.²⁵ No other pituitary stalk abnormalities have been reported. Some mild abnormalities have been reported including a thin corpus callosum or delayed myelination that resolves over time. Other structural anomalies observed in our cohort include a large cisterna magna in patient 1, a variant venous anatomy including anomalous venous sinus that traverses the falx cerebri in patient 26, and hydrocephalus and a Chiari malformation in patient 33.

Craniosynostosis is reported in a total of six patients.⁵ Initially, microcephaly was reported in 33% of previously published patients⁴⁻⁶ and in our larger cohort, only 25% individuals had microcephaly, which was not always present at birth. Seizure activity has only been reported in seven patients and there is no consistency in seizure type (see Supplementary Table 2).

Eye features

Strabismus is reported in 54% of patients. This can be intermittent and is often worse when fatigued. Some patients have been treated with patching or surgery. Strabismus can result in amblyopia and this is more likely to occur if it is unrecognized and untreated. Amblyopia is reported in several patients.

Refractory errors are reported in a minority of patients. Myopia is more commonly reported than hypermetropia. Delayed visual maturation, cortical visual impairment, and astigmatism are also occasionally reported as well as isolated cases of photophobia (patient 9), latent horizontal nystagmus (patient 11), and Jeavons epilepsy (patient 47).

Behavioral issues

An increased frequency of behavioral difficulties is noted in our cohort. Autism and autistic features have been reported

Table 1 Summary of clinical features for patients with KAT6A syndrome

Feature	Early-truncating cases: exons 1–15/full-gene del (18)	Late-truncating pathogenic variants: exons 16+17 (48)	Total cases excluding missense (70)	Missense cases (6)	Total cases (76)	Extra information
Sex	F = 8 M = 10	F = 25 M = 23	F = 35 M = 35	F = 2 M = 4	F = 37 M = 39	
Pathogenic variant type	Fs = 10 n = 7 del = 1	Fs = 19 n = 29	Fs = 29 n = 36 s = 4 del = 1	M = 6	Fs = 29 n = 36 m = 6 s = 4 del = 1	
SGA	13% (2/15)	18% (8/44)	16% (10/62)	0% (0/4)	15% (10/66)	
Microcephaly	6% (1/18)	44% (20/45)	32% (21/65)	20% (1/5)	31% (22/70)	
Presence of ID	100% (18/18)	100% (44/44)	100% (65/65)	100% (4/4)	100% (69/69)	
Neonatal hypotonia	44% (8/18)	85% (40/47)	75% (52/69)	83% (5/6)	76% (57/75)	
Seizures	12% (2/17)	4% (2/47)	9% (6/68)	17% (1/6)	9% (7/74)	No common type of seizure activity
Speech delay	100% (18/18)	100% (44/44)	100% (66/66)	100% (5/5)	100% (71/71)	
Strabismus	53% (9/17)	57% (27/47)	56% (37/66)	20% (1/5)	54% (38/71)	
Ptosis	17% (3/18)	16% (7/45)	18% (12/67)	0% (0/6)	16% (12/73)	
Visual defect	53% (9/17)	68% (26/38)	65% (37/57)	33% (1/3)	63% (38/60)	
Broad nasal tip	89% (16/18)	88% (35/40)	87% (54/62)	60% (3/5)	85% (57/67)	Prominence of this feature may increase with age
Thin upper lip	41% (7/17)	74% (28/38)	68% (40/59)	50% (2/4)	67% (42/63)	
Feeding difficulties	56% (10/18)	87% (40/46)	79% (52/66)	67% (4/6)	78% (56/72)	
Reflux	39% (7/18)	71% (27/38)	65% (35/54)	50% (3/6)	60% (38/60)	
Constipation	25% (4/16)	64% (18/28)	51% (24/47)	50% (3/6)	51% (27/53)	
Congenital heart defect	28% (5/18)	70% (32/46)	56% (38/68)	0% (0/6)	51% (38/74)	
Frequent Infection	31% (5/16)	71% (24/34)	50% (22/44)	20% (1/5)	47% (23/49) ^a	
Behavioral problems	27% (4/15)	44% (8/18)	33% (11/33)	100% (3/3)	39% (14/36) ^a	
Sleep disturbance	19% (3/16)	54% (15/28)	36% (15/42)	50% (2/4)	37% (17/46) ^a	

F Female, Fs frameshift, ID intellectual disability, M Male, m missense, n nonsense, s splicing, SGA small for gestational age

This table shows the prevalence of features in patients with KAT6A syndrome. If a feature was unknown or untested, the patient was excluded for that feature. For the purposes of this table, two patients with missense variants of unknown significance were excluded

^aPrevious cases were not in total if feature in the information was not present in the published report or available through clinical survey

Table 2 KAT6A clinical advice and guidelines

Medical care: Children should be under the care of a general or community pediatrician to monitor their health and development.
Speech and language: Children have marked expressive speech delay. Articulation is especially challenging. Many children have significantly benefited from the use of sign language and communication aids. Early speech and language therapy (SALT) is recommended.
Gastrointestinal: Feeding difficulties and reflux in infancy are common. Short-term nasogastric feeding may be required. Early feeding difficulties appear to be secondary to oromotor dysfunction; early support is recommended. Constipation is common and can be severe; long-term medical management with laxatives is often required. There appears to be an increased risk of malrotation and acute and subacute bowel obstruction; this should be considered for children in acute pain, with decreased bowel movements, or with increased vomiting/reflux.
Congenital heart disease (CHD): In our cohort approximately 50% of children had congenital heart disease, commonly septal defects and persistent ductus arteriosus. Of those with CHD approximately 50% required surgical intervention. For this reason we recommend all individuals with pathogenic KAT6A mutations should have an electrocardiogram and an echocardiogram. A specialist cardiology review should be considered.
Vision: Regular visual assessments are recommended. Over half of the individuals in our cohort have experienced strabismus. This can lead to permanent amblyopia if not picked up and treated. Ptosis has been noted in some individuals. Overall, visual defects, including refractive errors and cortical visual impairments, seem to be more common in this patient group.
Immunity/Infection: Further work is needed to determine whether immunodeficiency including neutropenia is a rare feature of this condition. This possibility should be considered in individuals with recurrent severe infections. Respiratory infections, urinary tract infections, and ear infections are relatively common features and should be considered if unwell.
Sleep: Many individuals experience difficult initiating and maintaining sleep. Melatonin has been used successfully in some people. Obstructive sleep apnea is more common in this patient group.
Education: An assessment of special educational needs should be carried out so that extra help can be put in place at school. Some children have behavioral difficulties requiring support.

These guidelines are based on the most commonly identified features across KAT6A patients. There is a wide range in variability of the clinical presentation and individual patients should have a personalized plan to reflect their own clinical features

in approximately 25% of newly reported cases. Temper tantrums, inappropriate laughing, hand flapping, and increased anxiety are also mentioned in multiple patients. The majority of patients in our cohort do not report behavioral difficulties and many are described as being good natured, happy, and sociable.

Sleep

Over 30% of patients in this cohort reported sleep disturbance. This included difficulty initiating and maintaining sleep, and five patients reported central obstructive sleep apnea.

Other clinical features and clinical guidelines

A summary of all clinical features described is located in Table 1. Supplementary Tables 2 and 3 contain further details on unique features seen in a few patients. For example, we found a small number of patients had undescended testes (boys), clinodactyly, and/or brachydactyly.

Based on the features described above, we have put together a set of general guidelines for clinicians to help guide the clinical workup for patients with a pathogenic genetic variant in KAT6A (Table 2).

Phenotypic differences between early and late-onset truncating pathogenic variants

We further subdivided the cases between early-truncating pathogenic variants (exons 1–15) and late-truncating pathogenic variants (exons 16–17) to determine if there was a difference in severity of phenotype or prevalence of specific syndromic features. Across 19 features (Table 1) we

performed Fisher's exact test and identified 8 subphenotypes that were significantly more common in patients with late-truncating pathogenic variant (Fig. 3c). These included microcephaly, neonatal hypotonia, feeding difficulties, reflux, constipation, congenital hearing defects, and frequent infections. Our data on the subphenotypes fit with the trend we observed, in which there is more severe global developmental delay and intellectual disability in patients with late-truncating pathogenic variants.

DISCUSSION

The mutational spectrum and the wide age range of patients allowed us to perform a comprehensive phenotypic assessment to elucidate the clinical phenotypes in childhood, adolescence and adulthood.

The wide phenotypic spectrum in individuals with KAT6A variants highlights the continued importance of exome sequencing to identify the genetic etiology for patients with syndromic ID.^{3,7} Patients with KAT6A pathogenic variants were not phenotypically grouped prior to the advent of clinical exome sequencing as there are no unique and unifying features that allowed for easy recognition by physicians. While patients with KAT6A syndrome do share many phenotypic features, many of these are common to a wide range of developmental syndromes. Accurate and detailed reporting of the phenotype is critical for affected families. KAT6A is one of the more common causes of undiagnosed syndromic intellectual disability⁷ with some reports suggesting a rate as high as 1% of undiagnosed syndromic developmental delay.⁴

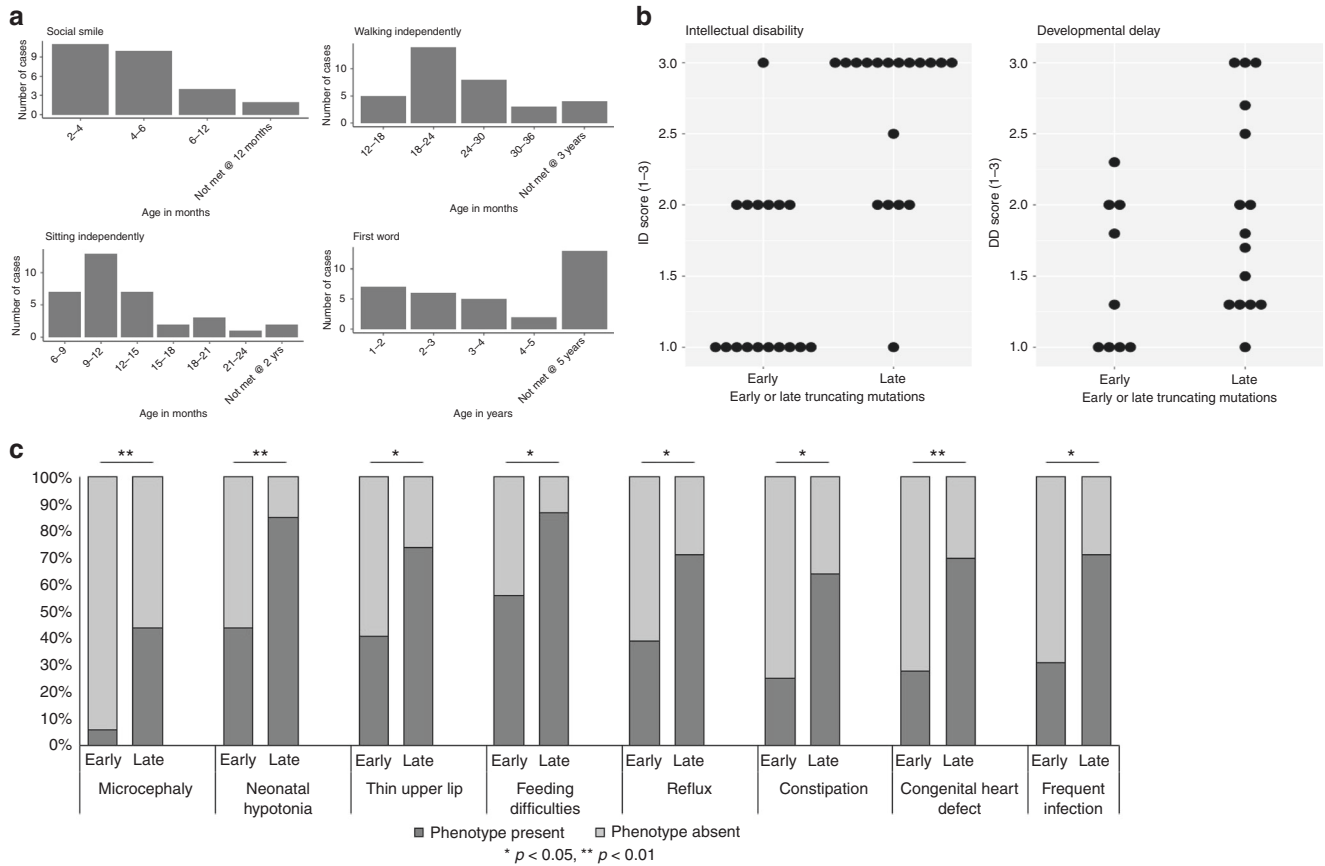


Fig. 3 Developmental delay, intellectual disability, and early and late-truncating pathogenic variant. **a** Developmental milestones in patients with pathogenic variants in *KAT6A*. Delay in childhood milestones are commonly seen, with milder delays in social and motor milestones and more severe delay noted in acquisition of verbal language. **b** Intellectual disability and developmental delay are more severe for truncating pathogenic variant in last two exons. **A**. More severe intellectual disability is more commonly seen in patients with truncating pathogenic variants in the last two exons of *KAT6A* as compared with early-truncating pathogenic variants (in exons 1–15). Contributing clinicians were asked to rate the level of intellectual disability as mild, moderate, or severe on a scale from 1 to 3 (1 = mild to 3 = severe). Patients collected through the survey or through the Deciphering Developmental Disorders (DDD) Study were asked to report the age when key developmental milestones were reached. We rated the severity of delay for four milestones as mild, moderate, or severe: first smile (mild = 2–4 months, mod = 4–6 months, severe = 6 months+); sitting (mild = 6–12 months, mod = 12–18 months, severe = 18 months+); walking (mild 12–24 months, mod = 24–30 months, severe = 30 months+); first word (mild = 1–3 years, mod = 3–5 years, severe = 5 years +). The scores for each individual were then averaged to give a score of 1 to 3. **c** Several syndromic features were seen less commonly in patients with early-truncating pathogenic variants. This includes microcephaly, neonatal hypotonia, gastrointestinal complications, and congenital heart defects.

Prior to this report, the majority of pathogenic variants were found to be *de novo*, truncating, and located in the ultimate and penultimate exons of the gene, which make up over half of the protein. Our analysis identifies hotspot nonsense pathogenic variants within the penultimate exons at amino acid positions 1019, 1024, and 1129 that account for 19.1% (13/68) of pathogenic variants in unrelated individuals. Protein-truncating variants have been identified throughout the length of the gene, and the number of cases we present in this article allows us to consider the genotype–phenotype correlations. We observe a bias of increased severity of developmental delay and an increased frequency of microcephaly, hypotonia, cardiac anomalies, and gastrointestinal complications associated with truncating pathogenic variants in the last two exons. This suggests a potential role for nonsense mediated decay (NMD), where truncating pathogenic variants in the first 15 exons trigger NMD mechanisms and result in haploinsufficiency

while pathogenic variants in exons 16 and 17 would not result in NMD, therefore the messenger RNA (mRNA) would result in a translated but dysfunctional protein that may have gain-of-function or dominant negative effects.

It should be noted that assessment of intellectual disability and developmental delay was based on clinicians rating the level of the patient’s intellectual disability as mild, moderate, or severe rather than from formal IQ testing. However, it is unlikely that this resulted in significant bias because this effect was not predicted before collecting the patient data. A large fraction of patients with *KAT6A* syndrome have had some form of biochemical and metabolic testing as a part of the clinical genetic workup. None of these cases demonstrated a clear and consistent metabolic dysfunction, by standard clinical biochemical genetic testing.

The role of nonsense mediated decay allowing for differing mechanisms of pathogenic variants within the same gene has been observed in pathogenic variants of related gene *KAT6B*,

which results in two distinct syndromes, SBBYS and genitopatellar syndrome.^{10,11} It has been postulated that differential truncating pathogenic variants in exon 18 may have a gain-of-function or even dominant negative effects.¹⁰ The molecular effects of different pathogenic variant localization remain to be validated in careful functional studies.

The lack of distinctive clinical features makes the attribution of KAT6A syndrome to missense variants in *KAT6A* particularly challenging. In our study, we have six patients with missense variants (including one previously reported case), five of which are *de novo* and one maternally inherited. While all of these variants fall into highly conserved regions of the protein (Supplemental Fig. 2) further functional studies are needed to confirm pathogenicity. Although our sample size is small, patients with missense variants have not, to date, shown a cardiac phenotype. We are aware that there are many more individuals with *de novo* missense reported through the DDD study, for which the pathogenicity is unclear. Some of these individuals have multiple potentially pathogenic variants further increasing the difficulty of assigning pathogenicity. Additionally, it is becoming increasingly evident that multiple variants may contribute to the phenotype; however it remains difficult to assess the relative contribution and interaction of multiple variants within an individual.^{31,32}

We note significant clinical variability in our cohort. This is not surprising as *KAT6A* functions as an epigenetic modifier, so its molecular effects are more nuanced and influenced by both background genetic variation and the environment. While we have a fairly large cohort, it is not large enough to confidently identify rare associations. For instance, we cannot yet be certain about the link with *KAT6A* syndrome and immunodeficiency or pituitary anomalies. When individuals with pathogenic *KAT6A* variants are found to have unusual features they should be further assessed for either a second pathogenic variant,³¹ or nongenetic cause, in addition to considering the possibility that the *KAT6A* pathogenic variant is responsible.

An interesting aspect of our study is that we collected data for many of our patients through an online family survey. The family questionnaire was received warmly and many families responded in a short timeframe. It proved to be a useful, relatively low resource, and efficient way to collect data. It allowed us to collect updated information for patients who had been previously reported in the literature. It should be noted however that although many individuals started the survey, only 60% completed the survey and were included in our study. It is not surprising that the patient families are heavily invested in their care. Many maintained a deep working knowledge of their child's medical condition. Collecting information from the family relies on the engagement of the families and a basic understanding of medical terminology, therefore consulting with family members to ensure accessibility of survey questions is critical to designing a successful survey. The family survey and the clinician questionnaire had to be designed with different wording to take into account the different baseline level of

medical knowledge of the two groups. In the family survey, parents were asked to select keywords that the child was noted to have by a physician (e.g., wide-spaced nipples, small jaw, small head, abnormal teeth, etc.). Collecting information from family members about dysmorphic facial features can be offensive and family members are not trained to assess this. We therefore also requested families to share photos so that this could be assessed by trained dysmorphologists. We were able to obtain a photo from 9 of the 15 survey-only individuals. Other studies have found family questionnaires an effective way of investigating disease phenotypes.³³ As the number of genetic syndromes increases, utilization of well-designed clinical surveys can provide invaluable data for clinical definition and for identifying a baseline by which future therapies can be measured.

Our report is the most comprehensive phenotypic evaluation of patients with pathogenic variants in *KAT6A*. We have provided significant insight into the range of phenotypic expressivity observed in patients with *KAT6A* syndrome. Further functional studies assessing the role of genetic pathogenic variants are required to understand the effect of various missense pathogenic variants and how early and late-truncating pathogenic variants ultimately affect downstream molecular processes.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (<https://doi.org/10.1038/s41436-018-0259-2>) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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Joanna Kennedy, BSc MBBS MRCPCH^{1,2}, David Goudie, MBChB³, Edward Blair, BMSc(Hons), MBChB^{4,5}, Kate Chandler, MD MB BChir⁶, Shelagh Joss, MBChB, MEd⁷, Victoria McKay, MBBS⁸, Andrew Green, MB, PhD, FRCP (Edin), FRCPI, FFPATH(RCPI)^{9,10}, Ruth Armstrong, MBChB, MSc, MRCPCH¹¹, Melissa Lees, MBBS, MRCP, MSc, MD, FRACP¹², Benjamin Kamien, MBBS¹³, Bruce Hopper, BSc¹³, Tiong Yang Tan, MBBS, PhD^{14,15}, Patrick Yap, MBChB, MSc^{14,16}, Zornitza Stark, BMBCh DM¹⁴, Nobuhiko Okamoto, MD^{17,18}, Noriko Miyake, MD, PhD¹⁹, Naomichi Matsumoto, MD, PhD¹⁹, Ellen Macnamara, ScM, CGC²⁰, Jennifer L. Murphy, RN²⁰, Elizabeth McCormick, MS, LCGC²¹, Hakon Hakonarson, MD, PhD²², Marni J. Falk, MD²¹, Dong Li, PhD²², Patrick Blackburn, PhD²³, Eric Klee, PhD^{23,24}, Dusica Babovic-Vuksanovic, MD^{23,24}, Susan Schelley, MPH²⁵, Louanne Hudgins, MD²⁵,

Sarina Kant, MD, PhD²⁶, Bertrand Isidor, MD, PhD²⁷, Benjamin Cogne, Pharm.D.²⁷, Kimberley Bradbury, BA MA MBBS²⁸, Mark Williams, RMIT, FHGSA²⁹, Chirag Patel, MBBS, MD³⁰, Helen Heussler, MBBS, DM³¹, Celia Duff-Farrier, PhD³², Phillis Lakeman, MD PhD³³, Ingrid Scurr, MBBS BSc¹, Usha Kini, MD^{4,5}, Mariet Elting, MD PhD³⁴, Margot Reijnders, MD³⁵, Janneke Schuurs-Hoeijmakers, MD, PhD³⁵, Mohamed Wafik, MD^{4,5}, Anne Blomhoff, MD PhD³⁶, Claudia A. L. Ruivenkamp, PhD³⁷, Esther Nibbeling, PhD³⁸, Alexander J. M. Dingemans, MD³⁵, Emilie D. Douine, MS³⁹, Stanley F. Nelson, MD^{39,40}, The DDD Study⁴¹, Valerie A. Arboleda, MD, PhD^{39,40} and Ruth Newbury-Ecob, MB ChB MD^{1,2}

¹Clinical Genetics, University Hospitals Bristol, Southwell St, Bristol, UK. ²University of Bristol, Bristol, UK. ³Clinical Genetics, Ninewells Hospital & Medical School, Dundee, UK. ⁴Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK. ⁵Department of Clinical Genetics, Churchill Hospital, Headington, Oxford, UK. ⁶Manchester Centre for Genomic Medicine, St. Mary's Hospital, Central Manchester Foundation NHS Trust, Manchester Academic Health Science Centre (MAHSC), Manchester, UK. ⁷West of Scotland Genetics Service, Queen Elizabeth University Hospital, Glasgow, UK. ⁸Cheshire & Merseyside Regional Genetics Service, Liverpool Women's NHS Foundation Trust, Crown Street, Liverpool, UK. ⁹Department of Clinical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland. ¹⁰School of Medicine and Medical Science, University College Dublin, Dublin, Ireland. ¹¹East Anglian Medical Genetics Service, Addenbrooke's Hospital, Cambridge, UK. ¹²Clinical Genetics, Great Ormond Street Hospital NHS Trust, London, UK. ¹³Hunter Genetics, Newcastle, Australia. ¹⁴Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Melbourne, Australia. ¹⁵Department of Paediatrics, University of Melbourne, Melbourne, Australia. ¹⁶Genetic Health Service New Zealand, Auckland, New Zealand. ¹⁷Department of Medical Genetics, Osaka Medical Center, Osaka, Japan. ¹⁸Research Institute for Maternal and Child Health, Osaka Medical Center, Osaka, Japan. ¹⁹Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan. ²⁰National Human Genome Research Institute, NIH, Bethesda, MD, USA. ²¹Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA. ²²Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA. ²³Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA. ²⁴Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA. ²⁵Division of Medical Genetics, Department of Pediatrics, Stanford University, Stanford, CA, USA. ²⁶Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands. ²⁷Service de Génétique Médicale, CHU Nantes, Nantes, France. ²⁸Clinical Genetics Guys and St Thomas' NHS Foundation Trust, Guys Hospital, London, UK. ²⁹Molecular Diagnostics, Mater Group, South Brisbane, Queensland, Australia. ³⁰Genetic Health Queensland, Herston, Brisbane, Queensland, Australia. ³¹Child Development Service, Lady Cilento Children's Hospital, Brisbane, Queensland, Australia. ³²Bristol Genetics Laboratory, Southmead Hospital, Bristol, UK. ³³Academic Medical Center, Department of Clinical Genetics, Amsterdam, The Netherlands. ³⁴Klinisch Genetisch, VU Medisch centrum, Amsterdam, The Netherlands. ³⁵Radboud University Medical Center, Department of Human Genetics, Nijmegen, The Netherlands. ³⁶Department of Medical Genetics, Oslo University Hospital, Oslo, Norway. ³⁷Klinische Genetica, Leiden University, Leiden, The Netherlands. ³⁸Department of Genetics, University of Groningen, Groningen, The Netherlands. ³⁹Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. ⁴⁰Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. ⁴¹Wellcome Sanger Institute, Hinxton, Cambridge, UK