De novo variants in KLF7 are a potential novel cause of developmental delay/intellectual disability, neuromuscular and psychiatric symptoms


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Due to small numbers of reported patients with pathogenic variants in single genes, the phenotypic spectrum associated with genes causing neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorder is expanding. Among these genes is KLF7 (Krüppel-like factor 7), which is located at 2q33.3 and has been implicated in several developmental processes. KLF7 has been proposed to be a candidate gene for the phenotype of autism features seen in patients with a 2q33.3q34 deletion. Herein, we report 4 unrelated individuals with de novo KLF7 missense variants who share similar clinical features of developmental delay/ID, hypotonia, feeding/swallowing issues, psychiatric features and neuromuscular symptoms, and add to the knowledge about the phenotypic spectrum associated with KLF7 haploinsufficiency.

KEYWORDS
autism, clinical diagnostics, intellectual disability, KLF7, Krüppel-like transcription factors, whole-exome sequencing, zinc finger DNA-binding protein

1 INTRODUCTION

De novo genetic/genomic variants are increasingly acknowledged as a significant etiology of neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorder (ASD). For genetically heterogeneous disorders such as developmental delay (DD) and ID, diagnostic-exome sequencing (DES) can identify a genetic etiology in up to 30% of cases when traditional genetic testing is inconclusive. During the past decade, substantial advancements have been made in elucidating the genetic causes of neurodevelopmental disorders. However, over half of individuals with neurodevelopmental disorders still do not have an identified genetic etiology. KLF7 (Krüppel-like factor 7, OMIM 604865), encodes a transcription factor belonging to the KLF family characterized by the presence of zinc coordinating (Zn) di-cysteine: di-histidine motif (C2H2) and sequence homology to the Drosophila segmentation gene product Krüppel. KLF7 has been implicated in several developmental processes and may be involved in the regulation of postmitotic differentiation of progenitor cells, neuronal morphogenesis, and/or phenotype maintenance. Emerging evidence suggests that KLF7 haploinsufficiency results in a recognizable neurodevelopmental phenotype. Located at 2q33.3, KLF7 has been proposed to be one of the possible candidate genes for the phenotype associated to the 2q33.3q34 deletion which has been
reported in patients with ASD.\textsuperscript{9-12} Additionally, these patients present with microcephaly, hypotonia, psychomotor retardation and mild dysmorphic features.

Herein, we report 4 unrelated affected individuals with de novo missense variants in KLF7 detected by DES with of DD/ID, neuromuscular and psychiatric complications.

1.1 | Clinical report

Clinical characteristics of the 4 patients are summarized in Table 1.

1.1.1 | Patient 1

Patient 1 was born at 38 weeks, 6 pounds 10 oz by c-section due to breech position to Ashkenazi Jewish, non-consanguineous parents. The pregnancy was uncomplicated with possible decreased fetal movements. He had jaundice requiring phototherapy due to difficulty feeding and also had torticollis. The patient was seen by neurology and began occupational and physical therapy at 6 months of age due to diffuse muscle weakness. He rolled over at 6 months of age, sat unassisted at 11 months and began crawling and pulling to stand at 15 months with the aid of therapies. Vision and hearing evaluations at 8 months was normal. At approximately 1 year, he had mildly elevated creatine kinase levels (353 and 419 units per liter). He was evaluated in genetics clinic at 15 months for possible myopathy due to gross motor delay, proximal muscle weakness and positive Gower's sign. At 21 months, he wore ankle braces for support in ambulation, had mild torticollis, pes planus, positive Gower's sign, and mildly wide-based gait. He did not cooperate with formal strength testing at that time. Language was reported to be mildly delayed and there were reports of some difficulty in swallowing. Muscle weakness later resolved; however, he remained hypotonic with language and cognitive delays. Developmental testing at 3 years 7 months showed a language developmental quotient (DQ) of 71% and cognitive DQ of 73%. He was also diagnosed with ASD and anxiety. The patient was non-dysmorphic with normal growth parameters. Brain MRIs at 20 months and 4 years showed stable mild ventriculomegaly. On follow-up evaluation at age 4 years, his muscle tone continued to be low and his running was slow, but he no longer used a Gower's maneuver and his strength was normal in upper and lower extremities. Family history was non-contributory. Prior normal testing included normal metabolic screening including a skin biopsy for peroxysisomal function, single nucleotide polymorphism (SNP) array, molecular tests for Sotos and Fragile X syndromes, electromyography (EMG) and electroencephalography (EEG).

1.1.3 | Patient 3

Patient 3 had been seen by a pediatric neurologist at regular intervals since age 4.5 years. At 15 years old she is reported to have ID (IQ 64), attention deficit disorder/anxiety, neonatal feeding difficulties, motor dyspraxia, upper extremities hypotonia and lower extremity hypertonia. She was reported as "large for her age." Family history was non-contributory. Prior normal testing included normal metabolic screening including a skin biopsy for peroxysisomal function, single nucleotide polymorphism (SNP) array, molecular tests for Sotos and Fragile X syndromes, electromyography (EMG) and electroencephalography (EEG).

1.1.4 | Patient 4

Patient 4 is a 2 years and 4 months old male with global DD and hypotonia. ID has not been assessed formally, but he spoke his first words at 2 years of age and walked independently at 26 months. He also has hypertelorism. Family history was non-contributory. Prior normal testing included normal metabolic testing and SNP array.

2 | METHODS

2.1 | Exome sequencing

For all patients, trio whole-exome sequencing was performed with proband and both biological parents on genomic DNA isolated from blood. Diagnostically relevant variants were confirmed by Sanger sequencing. For patient 1, DES was performed by Ambry Genetics Laboratory. Samples were prepared using the SeqCapEZ VCR 2.0 (Roche NimbleGen, Madison, Wisconsin) and sequenced on the Illumina HiSeq 2000 Sequencer (Illumina, San Diego, California). Data annotation and interpretation were performed as previously reported.\textsuperscript{4} For patient 2, DES was performed at GeneDx Laboratory with methods as previously reported.\textsuperscript{13}

For patients 3 and 4 exome sequencing and variant calling, a parent-offspring trio approach was used as described previously.\textsuperscript{3} Briefly, the exome was captured using the Agilent SureSelect v4 kit (Agilent, Santa Clara, California). Exome libraries were sequenced on an Illumina HiSeq2000 instrument (Illumina) with 101 bp paired-end reads at a median coverage of \( \times 75 \). Sequence reads were aligned to the hg19 reference genome using BWA version 0.5.9-r16.\textsuperscript{14} Variants were subsequently called by the GATK unified genotyper, version 3.2-2 and annotated using a custom diagnostic annotation pipeline.\textsuperscript{15} All variants were classified utilizing ACMG standards and guidelines.\textsuperscript{16}

Informed consent was obtained from all patients and family members undergoing sequencing. All research described in this case report was conducted in accordance with the World Medical Association Declaration of Helsinki. The clinical information presented herein
<table>
<thead>
<tr>
<th>Alteration details</th>
<th>Genotype</th>
<th>Courtens et al9</th>
<th>Brandau et al10</th>
<th>Rosenfeld et al11</th>
<th>Jang et al12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteration details</td>
<td>Genotype</td>
<td>p.T137M c.410C&gt;T</td>
<td>2q33.3q34 deletion, and paternal supernumerary marker der(15)</td>
<td>2q33.3q34 deletion, and de novo apparently balanced (4;16) translocation</td>
<td>2q33.3q34 deletion</td>
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<tr>
<td>Deletion size</td>
<td>NR</td>
<td>1.4-4.4 Mb</td>
<td>6.3 Mb</td>
<td>5.9 Mb</td>
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<td>Deletion position</td>
<td>NR</td>
<td>207 584 984–213 908 936</td>
<td>206 048 173–211 980 867</td>
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<td>Inheritance</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
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<tr>
<td>Demographics</td>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
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<td>Male</td>
</tr>
<tr>
<td>Age at last exam</td>
<td>4 y</td>
<td>16 y</td>
<td>15 y</td>
<td>2 y</td>
<td>7/12 y</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Birth weight</td>
<td>Normal</td>
<td>Low for 35 wk gestation</td>
<td>NR</td>
<td>Normal</td>
<td>Low with IUGR reported</td>
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<tr>
<td>Development</td>
<td>Motor delay</td>
<td>Yes, although rolled at 6 mo, sat unassisted at 11 mo and began crawling and pulling to stand at 15 mo with therapies</td>
<td>Yes</td>
<td>Yes, walked at 22/12 y</td>
<td>Yes, sat at 1 y, first steps at 18-24 mo</td>
</tr>
<tr>
<td>Speech delay</td>
<td>Mildly delayed (71% at 3 7/12 y)</td>
<td>Yes, non-verbal at 16 y</td>
<td>Yes, first words at 2 y</td>
<td>Yes, first words at 2 y</td>
<td>Yes, no complete sentences at 6 y</td>
</tr>
<tr>
<td>Cognitive delay</td>
<td>Yes (IQ 73 at 3 7/12 y)</td>
<td>Yes, IQ of 64</td>
<td>Yes, although not formally assessed</td>
<td>Yes, IQ 65 and 58 at 1 y and 2 7/12 y</td>
<td>IQ of 51</td>
</tr>
<tr>
<td>Neurological and neuromuscular findings</td>
<td>Mild ventriculomegaly</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>None</td>
</tr>
<tr>
<td>Autism spectrum disorder/Autistic features</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>Other behavioral/psychiatric features</td>
<td>None</td>
<td>Short attention span and anxiety</td>
<td>Anxiety and ADD</td>
<td>None</td>
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<tr>
<td>Seizures</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Complex partial seizures</td>
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</tbody>
</table>

(Continues)
<table>
<thead>
<tr>
<th>TABLE 1 (Continued)</th>
<th>Patient 1 (this report)</th>
<th>Patient 2 (this report)</th>
<th>Patient 3 (this report)</th>
<th>Patient 4 (this report)</th>
<th>Courtens et al⁹</th>
<th>Brandau et al¹⁰</th>
<th>Rosenfeld et al¹¹</th>
<th>Jang et al¹²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unsteady gait</strong></td>
<td>Wide-based gait</td>
<td>Abnormal gait</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Hypotonia</strong></td>
<td>Yes, proximal</td>
<td>Yes, centrally</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Other neuromuscular symptoms</strong></td>
<td>Previous positive gower sign (resolved before age 4), muscle strength in proximal muscles normal at age 4</td>
<td>Increased lower tone, contractures, mild positional scoliosis, cerebral palsy, decreased fetal movements</td>
<td>Decreased tone in upper extremities, increased tone in lower extremities</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NR</td>
<td>None</td>
</tr>
<tr>
<td><strong>Craniofacial dysmorphism</strong></td>
<td>None</td>
<td>Sloping forehead, midface hypoplasia</td>
<td>None</td>
<td>None</td>
<td>High forehead, prominent beaked nose, absent earlobes</td>
<td>2 posterior hair whorls, epicanthal folds, high forehead, ptosis, prominent nasal tip and dental anomalies</td>
<td>NR</td>
<td>Down-slanting palpebral fissures, high forehead, small mouth, high palate</td>
</tr>
<tr>
<td><strong>Other dysmorphic feature</strong></td>
<td>Hypospadius</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Fifth finger clinodactyly, wide gap between first and second toes</td>
<td>Fifth finger clinodactyly, 2,3 syndactyly, undescended testes, hypospadius</td>
<td>NR</td>
<td>None</td>
</tr>
<tr>
<td><strong>Feeding issues</strong></td>
<td>None</td>
<td>Yes</td>
<td>Yes, continue at age 16 with G-tube and Nissen fundoplication</td>
<td>Yes, as a neonate</td>
<td>None</td>
<td>Yes, with Failure to Thrive reported</td>
<td>Yes</td>
<td>NR</td>
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<tr>
<td><strong>Other anomalies</strong></td>
<td>Hyperextensibility</td>
<td>None</td>
<td>Prolonged dysautonomia, asthma, strabismus requiring surgery</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviation: NR, not reported.
was collected during the routine clinical care of a patient and this study is exempt from Institutional Research Board approval. All subjects participating in this study provided signed, written consent allowing for the publication of their clinical photographs and/or data. In the case of minors, signed written consent was provided by their parents or legal guardians. Individuals were identified through GeneMatcher.¹⁷

### 2.2 Structural analysis

The structure of KLF7 with bound DNA was modeled based on the crystal structure of KLF4 bound to DNA (PDB: 4M9E).¹⁸ The zinc finger domains of KLF4 and KLF7 share 73% identity, with 100% conservation observed at DNA interacting residues. The program Coot¹⁹ was used to change the amino acids that differed between the sequences of KLF4 and KLF7, most of which were solvent-exposed, and reasonable sidechain rotamers were chosen manually. In silico mutagenesis of Asp 264 to Asn was carried out using FoldX.²⁰ Model graphics were generated using PyMol (The PyMOL Molecular Graphics System, Version 1.7.1 Schrödinger, LLC.). Motifs were identified using the web server ELM.²¹ Disorder was predicted by IUPRED and GlobPlot.²² Low sequence complexity was predicted by SMART.²³,²⁴

### 3 RESULTS

We report patients from 4 unrelated families with a similar phenotype found to have probably gene damaging de novo variants in KLF7. The genotypes and analysis of the variants are presented in Figure 1. All sequence variants are described in reference to RefSeq transcript NM_003709. In patients 1 and 4, DES independently identified the heterozygous de novo missense variant KLF7 c.410C>T (p.T137M). In patients 2 and 3, DES identified the heterozygous de novo missense variants KLF7 c.790G>A (p.D264N) and c.415C>T (p.P139S), respectively.

#### 3.1 Structural interpretation of the variants

The KLF7 c.410C>T (p.T137M) and c.415C>T (p.P139S) variants lie in a flexible, unstructured sequence, in the N-terminal regulatory region of KLF7, whereas the c.790G>A (p.D264N) variant is located in the C-terminal zinc finger-containing DNA-binding domain (Figure 2A). The 3 KLF7 variants are all in highly conserved amino acid positions and are not present in the ExAC database.²⁵ The 2 N-terminal variants are contained in a span containing 2 noteworthy linear motifs—“Skp1–Cullin–Fbox-WD40 (SCF) ubiquitin ligase target” motif and a “glycogen synthase kinase (GSK) target” motif (Figure 2B). The “GSK target” motif—defined as the GSK motif where the positions immediately after each S/T are proline ([S/T]-P-X-X-[S/T]-P). Upon phosphorylation at both S/T positions by the kinase, this site would be recognized by the SCF ubiquitination complex, which promotes degradation. In the paralog KLF2, which has the same motif, direct variant of these motifs negatively impacted its SCF-mediated degradation in vitro and in vivo indicating a key biological role for this sequence.²⁶ In KLF7, the variant c.410C>T (p.T137M) sits within the motifs and is anticipated to disrupt this motif by eliminating a phosphorylation site; on the other hand there is no evidence that,
c.415C>T (p.P139S) would impact the motif and probably mechanism of disruption of this variant cannot be elucidated from available information, suggesting that this region warrants deeper functional investigation. The third variant, c.790G>A (p.D264N), is located in the C-terminal zinc finger-containing DNA-binding domain, shown to bind to the minimal enhancer of the TrkA gene, which encodes the high affinity receptor for nerve growth factor. This variant involves one of the specificity residues of the zinc-finger domain involved in
interactions with DNA. An variant of this residue is probably to shift DNA binding selectivity due to altered interaction possibilities, which may result in impaired activation of target genes which has been observed in the zinc-finger proteins (Figure 3).

4 | DISCUSSION

We report patients from 4 unrelated families with a similar phenotype found to have probably gene damaging de novo variants in KLF7. Among the 3 different de novo missense variants identified in our cohort, there is one heterozygote with the p.T137M in GnomAD, and one heterozygote with p.P139L (a different substitution at same residue) in GnomAD. An additional female patient with ID was reported through ClinVar, carrying the same de novo variant as our patient 2 - (ClinVar submission accession number SCV000493088.1). The variant was reported as of uncertain clinical significance; no other details were available. While the individuals reported in GnomAD are not expected to have early-onset severe disorder, due to the variability present in patients 1 and 4, it is possible that this individual might be mildly affected. Without further analysis of this variant in additional individuals, a potential a female protective model cannot be ruled out.

4.1 | Phenotype of patients with KLF7 variants and 2q33.3q34 deletion

DD/ID, hypotonia, feeding/swallowing issues, psychiatric features and neuromuscular symptoms were present in 3 patients in our cohort, with the additional patient showing mild neuromuscular symptoms, DD, but being too young for formal psychiatric testing. The additional individual present in ClinVar was reported with at least ID. These characteristics are similar to the individuals reported with the 2q33.3q34 deletion syndrome, although the phenotype is also highly variable (reviewed in Table 1). Of the 4 previously reported patients in the literature with the deletion, varying degrees of DD/ID, hypotonia, feeding/swallowing issues, psychiatric features and neuromuscular issues were reported along with dysmorphic features, seizures and microcephaly. None of the cases in our cohort had seizures. Dysmorphic features were reported in 3 individuals with the overlapping deletion and in one affected individual of our cohort. Growth retardation and microcephaly were reported in 2 individuals with the 2q deletion and one individual in our cohort. In contrast, one individual in our cohort was reported with tall stature and macrocephaly. Ronzoni et al also reported an additional patient with a KLF7 deletion. This deletion also included more than 50 RefSeq genes; 17 listed as human disease genes in OMIM. Therefore, it is difficult to establish correlation to individuals with KLF7 only variants especially due to the cumulative nature of these genes, but suggests that KLF7 does have a role in neurodevelopmental features.

The variability between the 2 patients within our cohort with the same variant may either be explained by the lack of phenotype/genotype connection within KLF7 or the young age of the patient. Further chronological study of the patients within this cohort and the discovery of additional patients may be helpful in elucidating this construct.
4.2 | Pathogenic mechanisms of KLF7 variants

Homozygous Klf7 null (Klf7<sup>−/−</sup>) mice were born at expected Mendelian frequencies but a majority of them (98.5%) died within the first 3 days of life with very little or no milk in their stomachs, hypopnea and cyanosis.29 Additionally, these mice showed defects in neurite outgrowth, axonal misprojection at specific locations within the nervous system including the olfactory and visual systems, cerebral cortex and hippocampus and reduced dendritic branching in the hippocampus.29 Abnormal corpus callosum (CC) in the null mouse was seen due to failure of the fibers of CC to cross the midline, a missing or severely disrupted anterior commissure and a reduction in the size of the fimbria.29,30 Lei et al<sup>30</sup> reported that sensory nociceptive neurons in Klf7<sup>−/−</sup> newborn mice are significantly reduced due to increased apoptosis. The authors find that TrkA expression is reduced in the DRG neurons of Klf7<sup>−/−</sup> mice because Klf7 has been shown to bind to a minimal enhancer in the TrkA upstream promoter region.

Using an RNAi-mediated knockdown approach in the neuroepithelial cell line PC12, Caiazza et al<sup>31</sup> observed that downregulation of KLF7 gene expression caused silencing of genes crucial for neuronal differentiation, namely MAP2 and the high affinity receptor for NGF, TrkA. Downregulation of TrkA caused a failure of shKlf7-PC12 stable clones to differentiate into neurons. KLF7 role in cellular differentiation was not limited to the nervous system. Klf7-silenced embryonic stem cells fail to differentiate into cardiomyocytes, and Klf7<sup>−/−</sup> mouse embryonic fibroblasts display impaired adipogenesis and enhanced osteogenesis ability.

The roles of proteins in the KLF family have been reviewed extensively and although a few of them have nervous system specific roles, none of them have been implicated in human disease to date.32 However, mutations affecting KLF7-interacting proteins have been shown to cause genetic diseases in humans. For example, a dominant mutation in the FBXO38 gene, encoding the F-box protein 38, has been reported in members of 2 unrelated families affected by a distinct form of distal spinal muscular atrophy which initially manifests as calf weakness.33 FBXO38 is a transcriptional coactivator of KLF7, and the authors report that this mutation led to dysregulation of KLF7 target genes and impairment of neurite outgrowth in primary motor neurons indicating a critical role for FBXO38 and KLF7 in axonal development and neuronal maintenance. One of the KLF7 target genes is L1CAM, encoding the neural cell adhesion molecule L1 that plays essential roles in neuronal migration, axon growth and guidance and synaptic plasticity in both the central and peripheral nervous systems.34 X-linked recessive mutations in the L1CAM gene have been shown to cause hydrocephalus, a phenotype associated with enlarged cerebral ventricles, mental retardation and often with spastic paraparesis and adducted thumbs (OMIM: 307000).

5 | CONCLUSION

In summary, we present the clinical phenotype information of 4 patients with de novo KLF7 variants, supporting the relationship of the gene with neurodevelopmental features. We hypothesize that the core phenotype of DD/ID, neuromuscular and psychiatric symptoms is present in individuals with KLF7 variants along with potential additional features. We report the varying phenotype of the individuals with these variants. It is anticipated that additional individuals with KLF7 variants will be identified, adding to the knowledge of the clinical spectrum and pathogenicity. Additional studies on patients with KLF7 variants and functional studies of these variants are necessary to further elucidate the correlation between genotype and phenotype, preferably supported by further investigations of the molecular mechanism of KLF7 during neurological development and function.

ACKNOWLEDGEMENTS

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Conflict of interest

Zoe Powis, Igor Petrlik, Robert Huether, Sha Tang and Deepali N. Shinde are employed and receive a salary from Ambry Genetics. Rashmi Chikarmane and Amber Begtrup are employed and receive a salary from GeneDx. Julie S. Cohen is a consultant to Invitae. Exome sequencing is a commercially available test.

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REFERENCES


