Mutations in TBCK, Encoding TBC1-Domain-Containing Kinase, Lead to a Recognizable Syndrome of Intellectual Disability and Hypotonia

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Through an international multi-center collaboration, 13 individuals from nine unrelated families and affected by likely pathogenic biallelic variants in TBC1-domain-containing kinase (TBCK) were identified through whole-exome sequencing. All affected individuals were found to share a core phenotype of intellectual disability and hypotonia, and many had seizures and showed brain atrophy and white-matter changes on neuroimaging. Minor non-specific facial dysmorphism was also noted in some individuals, including multiple older children who developed coarse features similar to those of storage disorders. TBCK has been shown to regulate the mammalian target of rapamycin (mTOR) signaling pathway, which is also stimulated by exogenous leucine supplementation. TBCK was absent in cells from affected individuals, and decreased phosphorylation of phospho-ribosomal protein S6 was also observed, a finding suggestive of downregulation of mTOR signaling. Lastly, we demonstrated that activation of the mTOR pathway in response to L-leucine supplementation was retained, suggesting a possible avenue for directed therapies for this condition.

Intellectual disability (ID) is a common diagnosis with few specific disease-directed therapeutic options.1 In some specific cases when a molecular etiology for ID has been discovered, especially in the setting of inborn errors of metabolism, there has been vast improvement in the outcomes of those individuals.2 The genetic heterogeneity of ID has traditionally complicated both diagnosis and gene discovery, leading to a large proportion of affected individuals who never receive a molecular diagnosis. The introduction of agnostic testing, such as genome-wide arrays and whole-exome sequencing (WES), has accelerated both discovery of and diagnosis for individuals with ID. However, such acceleration has also highlighted the need to identify independent pathogenic variants in the same gene before a causal link to ID can be established. Using WES, in nine unrelated families we were able to identify 13 individuals who are affected by ID and hypotonia and harbor biallelic pathogenic variants in TBC1-domain-containing kinase (TBCK), a gene recently implicated in the etiology of ID in a single family.3

All affected individuals were recruited under research protocols approved by the institutional review boards (IRBs) of their respective institutions after informed consent was obtained. We sequenced DNA from 13 affected individuals from nine families and their healthy parents to identify the etiology of their ID and hypotonia. We performed WES if the variant was unknown in the family or Sanger sequencing if the variant had been identified in another family member (Table S1). After identifying TBCK variants in each family, we assembled the cohort through personal collaborations and the assistance of GeneMatcher. Each subject sequenced provided written consent, and all work was performed either in a clinical laboratory or under an IRB-approved protocol. Variants previously reported in dbSNP, the 1000 Genomes Project, and the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP) Exome Variant Server with a minor allele frequency > 1% were excluded. Anticipating that synonymous variants are far less likely to be pathogenic, we focused our variant analysis primarily on nonsynonymous variants, splice-acceptor and donor-site mutations, and coding insertions/deletions (indels). When applicable, emphasis was placed on identifying homozygous or compound-heterozygous variants

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shared between the two affected siblings. In all cases, filtering WES data for compound-heterozygous, X-linked, or heterozygous de novo variants did not provide any additional candidate variants. Pathogenic homozygous or compound-heterozygous variants in TBCK were confirmed by Sanger sequencing. Parents were found to be

Table 1. Characteristics of Individuals with Biallelic Variants in TBCK

<table>
<thead>
<tr>
<th>Affected Individuals</th>
<th>1-1*</th>
<th>1-2*</th>
<th>2-1</th>
<th>3-1</th>
<th>4-1</th>
<th>4-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variants (GenBank: NM_001163435.2)</td>
<td>Hom c.1897+1G&gt;TA</td>
<td>Hom c.1897+1G&gt;TA</td>
<td>Hom c.831_832insTA (p.Pro278Tyrfs*18)</td>
<td>Hom c.1652T&gt;C (p.Leu551Pro)</td>
<td>Het c.[2060–2A&gt;G]; [803_806delTGAA], p.[=];[Met268fsArg*26];</td>
<td>Het c.[2060–2A&gt;G]; [803_806delTGAA], p.[=];[Met268fsArg*26];</td>
</tr>
<tr>
<td>Mutation type</td>
<td>splice site and frameshift</td>
<td>splice site and frameshift</td>
<td>insertion and frameshift</td>
<td>missense</td>
<td>splice site and frameshift; frameshift</td>
<td>splice site and frameshift; frameshift</td>
</tr>
<tr>
<td>Consanguinity reported</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age at last exam</td>
<td>died at 5 years</td>
<td>11 years</td>
<td>5 years</td>
<td>11 years</td>
<td>4 years</td>
<td>2 years</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>female</td>
<td>male</td>
<td>male</td>
<td>female</td>
<td>female</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Saudi</td>
<td>Saudi</td>
<td>Syrian</td>
<td>Pakistani</td>
<td>mixed European</td>
<td>mixed European</td>
</tr>
<tr>
<td>Prenatal findings</td>
<td>decreased fetal movement, oligohydramnios</td>
<td>none reported</td>
<td>premature contractions</td>
<td>none reported</td>
<td>ventriculomegaly</td>
<td>none reported</td>
</tr>
<tr>
<td>Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor</td>
<td>no sitting</td>
<td>rolling at 11 years</td>
<td>no sitting</td>
<td>mild delay</td>
<td>independent walking</td>
<td>sitting</td>
</tr>
<tr>
<td>Speech</td>
<td>few words</td>
<td>babbling, no words</td>
<td>non-verbal</td>
<td>mild delay</td>
<td>few single words</td>
<td>many words</td>
</tr>
<tr>
<td>Cognitive</td>
<td>severe delay</td>
<td>severe delay</td>
<td>severe delay</td>
<td>mild delay</td>
<td>severe delay</td>
<td>mild delay</td>
</tr>
<tr>
<td>Regression</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ventilator dependence</td>
<td>–</td>
<td>–</td>
<td>tracheostomy only</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MRI findings</td>
<td>diffuse brain atrophy, abnormal white-matter signal intensity</td>
<td>diffuse brain atrophy</td>
<td>posterior thinning of the corpus callosum and brainstem, ex vacuo dilation of the ventricles, periventricular leukomalacia</td>
<td>mild prominence of the lateral ventricles</td>
<td>none reported</td>
<td>none reported</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>severe</td>
<td>severe</td>
<td>severe</td>
<td>mild to moderate</td>
<td>moderate</td>
<td>severe</td>
</tr>
<tr>
<td>Reflexes</td>
<td>delayed</td>
<td>reduced</td>
<td>absent in lower extremities</td>
<td>normal</td>
<td>reduced</td>
<td>reduced</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unusual facial features</td>
<td>sloped forehead, bulbous nose, tented upper lip, upward slant of palpebral fissures</td>
<td>tented upper lip, sloped forehead, bulbous nose</td>
<td>macrocephaly</td>
<td>epicantual folds, broad nasal bridge, deep-set eyes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other findings</td>
<td>abnormal eye movements, asthma, eczema</td>
<td>hypothryoidism, pernicious anemia, recurrent candidiasis</td>
<td>strabismus, nystagmus</td>
<td>autism, bipolar disorder, high-arched palate, broad fingers and toes, mild scoliosis</td>
<td>mild macrocephaly</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations are as follows: Hom, homozygous; and Het, heterozygous.

* These individuals were previously reported by Alazami et al.3

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heterozygous for each of the variants found in their children, consistent with an autosomal-recessive model. The one homozygous missense variant was computationally predicted to have a functional effect, given that it contains a highly conserved nucleotide (phyloP score = 8.35 [−20.0;10.0]), encodes an amino acid highly
conserved up to C. elegans (considering 13 species), has a moderate physicochemical difference between Leu and Pro (Grantham distance = 98 [0–215]), is found in the Rab-GTPase-TBC domain, and is predicted to be deleterious by SIFT (score = 0.01, median = 3.35) and disease causing by MutationTaster (p value = 1).

These 13 affected individuals all share a core phenotype of ID and hypotonia in addition to variable expressivity of other features as summarized in Table 1 (see also Figure 1A). A few individuals demonstrated facial features reminiscent of a storage disease (e.g., macroglossia and coarse facies), but it was not a universal feature and might develop as the children age. Several individuals demonstrated other mild facial anomalies, but none that appeared diagnostic (Figures 1B–1D and 1G–1I). Seven individuals from five families had seizures, which were often refractory to pharmacologic management. Development ranged from profound to mild developmental delay, and only one individual demonstrated regression. Brain MRI showed a thin corpus callosum and brainstem, ex vacuo dilation of the lateral ventricles, periventricular leukomalacia, and white-matter changes ranging from non-specific to a picture of leukodystrophy (Figures 1E, 1F, 1J, and 1K). The following features were found in a minority of individuals, so their relevance to the phenotype remains unclear: hyperthyroidism, hypothyroidism, pernicious anemia, suspected immune deficiency, corneal clouding, scoliosis, central adrenal insufficiency, growth hormone deficiency, and profound bilateral hearing loss.

Although all 13 individuals demonstrated developmental delays, the least-affected individual (individual 3-1) had the only missense variant in our cohort and showed only mild delays and minimal MRI abnormalities. The other individuals all had variants suspected to lead to significant protein truncation from splice-site substitutions, small insertions, small deletions, or multi-exonic deletions and subsequently appeared to have a more severe phenotype. It is likely that additional individuals with
pathogenic missense variants in the population have yet to be identified given that they would have a milder phenotype, and their variant would be less easily recognized as disease causing. Additionally, in one family (individuals 9-1 and 9-2), the combination of a nonsense variant and a multi-exon deletion required both exome sequencing and a genome-wide array to confirm the diagnosis.

Clinical diagnosis was also complicated by the significant variability in presentation, even within the sibling pairs we identified, and the lack of a clear environmental or therapeutic intervention as an explanation. Also, although multiple individuals in this series showed dysmorphic features, there does not appear to be a clearly identifiable facial gestalt associated with this syndrome. Coarse facial features did appear to develop in middle childhood in some individuals, but this was also non-specific. Similarly, brain MRI appeared non-specific and might have mimicked a leukodystrophy process in the more severely affected individuals. These features of the syndrome highlight the need for a clinically agnostic diagnostic tool, such as exome sequencing or gene panels, to confirm the diagnosis. Previous diagnoses considered for these individuals included static encephalopathy, lysosomal-storage disease, leukodystrophy, and mitochondrial disorder, but no single alternate differential diagnosis was clearly favored throughout the cohort. Also complicating diagnosis, this disease is not restricted to one geographic region, given that these affected individuals have diverse ethnic backgrounds. However, there does appear to be a common variant, c.376C>T (p.Arg126*) (GenBank: NM_001163435.2), reported in the three Hispanic families, suggesting a founder effect in this population.

**TBCK** was named for the Tre-2, Bub2p, and Cdc16p (TBC) 1 domain and the protein kinase domain found within the encoded protein. Interestingly, **TBCK** has recently been implicated in the regulation of mammalian target of rapamycin (mTOR) signaling, given that knockdown of **TBCK** decreases the transcription of mTOR complex (mTORC) proteins and downregulates mTOR signaling. mTOR is a proline-directed serine threonine protein kinase; activation of the mTOR pathway leads to phosphorylation of ribosomal protein S6. Many developmental disorders, including tuberous sclerosis (MIM: 191100) and PTEN-related disorders (MIM: 158350), are associated with aberrant mTOR signaling, and mTOR signaling has been shown to play a role in structural brain malformations, epilepsy, autism, and ID. One of the many activators of the mTOR pathway has been shown to be leucine, one of the essential amino acids, through mTORC1. The effects of leucine supplementation on mTOR activation have been studied in the context of adipogenesis, increased muscle mass, and diabetes control.

To evaluate the functional consequences of the **TBCK** variants, we used western blotting to assess the amount of **TBCK** in immortalized lymphoblastoid cell lines (LCLs) from one healthy control subject and two individuals (1-1 and 1-2) with homozygous frameshift variants predicted to lead to a frameshift. Blots were probed with a polyclonal anti-**TBCK** antibody raised against the C-terminal region within amino acids 457–694. Whereas **TBCK** was clearly detectable as a single band in control LCLs, no protein was found in cell extracts from the affected individuals’ LCLs (Figure 2A). Western blotting demonstrated mTOR-pathway signaling in LCLs and in cultured fibroblasts from one other individual (2-1) with a different predicted frameshift **TBCK** variant. Levels of non-phosphorylated and phosphorylated S6 (PS6) isoform Ser235/236 were assessed in triplicate. Densitometry of the blots revealed that PS6 levels were, on average, 78% (range = 76%–82%, SEM ± 6) lower in LCLs harboring **TBCK** mutations than in control LCLs (p < 0.05; Figure 2B) and that PS6 Ser235/236 levels were, on average, 36% (range = 31%–41%, SEM ± 5) lower in fibroblasts harboring mutant **TBCK** than in control fibroblasts (p < 0.05; Figure 3A); a decrease in PS6 (Ser240/244) was observed as well (Figure 3B). There was no change in the level of non-phosphorylated S6 or mTOR proteins in either LCLs or fibroblasts containing **TBCK** mutations. The addition of leucine...
(600 µg/ml), a known nutrient stimulator of mTOR activation, to the culture media of fibroblasts from individual 2-1 induced an upregulation of basal mTOR signaling, as evidenced by increased levels of PS6 (24% ± 4%; p < 0.05; Figure 4).

The precise function of TBCK has not been fully elucidated, but previous studies have shown that TBCK is downregulated by rapamycin and that RNAi directed against TBCK increases STAT3 phosphorylation and decreases ERK1/2 phosphorylation.\textsuperscript{11,12} In addition, depleting TBCK at the cellular level leads to decreased cell proliferation, smaller cells, and aberrant actin organization.\textsuperscript{5} Given that we have demonstrated the absence of TBCK in multiple affected individuals’ cells, it is likely that disruption of these processes causes their phenotypes. We were able to show that cells from these individuals demonstrated reduced phosphorylation of the Ser235/236 isoform of PS6, suggesting that reduced mTOR signaling is a pathogenic mechanism. This would add TBCK-related ID syndrome to the list of other neurodevelopmental disorders that have been associated with aberrant mTOR signaling: neurofibromatosis type 1 (MIM: 162200), tuberous sclerosis, PTEN-related disorders, and fragile X syndrome (MIM: 300624).\textsuperscript{6,13}

There are few targeted therapies for neurodevelopmental disorders, but by elucidating the molecular and cellular pathology in these individuals, we were able to hypothesize a targeted therapeutic for restoring mTOR signaling. When leucine was added to the cells of the affected individual, it was able to increase the mTOR signaling by using increased phosphorylation of PS6 Ser235/236 as a proxy. This shows that there is a TBCK-independent pathway for leucine to stimulate the mTOR pathway, which could be utilized for therapeutic effect in these individuals. Leucine supplementation is already clinically available for certain metabolic disorders and has a wide therapeutic range (as reviewed in Pedroso et al.\textsuperscript{14}).

In conclusion, we have reported a series of 13 individuals who are from nine unrelated families, harbor biallelic mutations in \textit{TBCK}, and display overlapping features of ID and hypotonia. We propose that this condition be named TBCK-related ID syndrome. A potential avenue for therapeutic intervention was demonstrated by TBCK-independent upregulation of mTOR signaling with the addition of leucine to the culture media in affected cells. Future work will include initiating a registry for individuals with TBCK-related ID syndrome to further delineate the natural history of the syndrome and test targeted therapies for the condition.

**Supplemental Data**

Supplemental Data include one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.03.016.

**Conflicts of Interest**

P.C. is a shareholder and a member of the scientific advisory board of Evogen. J.J., M.G., and G.D. are employees of GeneDx.
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Web Resources

The URLs for data presented herein are as follows:
1. 1000 Genomes, http://browser.1000genomes.org/index.html
5. OMIM, http://OMIM.org

References