Letter to the Editor

Parent–child exome sequencing identifies a *de novo* truncating mutation in TCF4 in non-syndromic intellectual disability

To the Editor:

Autosomal dominant mutations in the transcription factor TCF4 cause Pitt–Hopkins syndrome (PTHS; MIM#610954), which is characterized by severe intellectual disability (ID), facial dysmorphism, intermittent hyperventilation, epilepsy, and microcephaly (1–5). Here, we report a *de novo* nonsense mutation in TCF4 in a patient with mild-to-moderate non-syndromic ID (NSID) without the typical features of PTHS.

In the context of a project that aims to identify *de novo* mutations in neurodevelopmental disorders, we sequenced the exomes of a patient with NSID and her parents. The exomes were captured from blood genomic DNA using the Agilent SureSelect 50Mb library (Agilent Technologies, Santa Clara, CA), and sequenced on the ABI-SOLiD4 system (Life Technologies Applied Biosystems, Foster City, CA) (single-end 50 bp reads, four exomes/slide). Read mapping, variant calling and annotation were performed as described (6). We achieved an average per-target base coverage of 20-fold for the proband and her mother, and 22-fold for the father. A total of 11,241 coding/splicing variants were found in the proband. After removing synonymous, inherited, and common variants, and after visual inspection of the reads, we identified three variants, only one of which, c.C469T/p.R157X in TCF4, was confirmed as *de novo* by Sanger sequencing (Table 1). p.R157X was also identified in genomic DNA from the patient’s hair bulb and buccal cells as well as in total RNA from lymphoblastoid cells established using her blood (Fig. 1).

Table 1. Variants detected in the proband’s exome

<table>
<thead>
<tr>
<th>Total coding and splicing variants</th>
<th>11,241</th>
</tr>
</thead>
<tbody>
<tr>
<td>After removing synonymous variants</td>
<td>5796</td>
</tr>
<tr>
<td>After removing inherited variants</td>
<td>378</td>
</tr>
<tr>
<td>After removing known variants*</td>
<td>30</td>
</tr>
<tr>
<td>After manual inspection with IGV</td>
<td>3</td>
</tr>
<tr>
<td><em>De novo</em> mutations confirmed by Sanger sequencing</td>
<td>1</td>
</tr>
</tbody>
</table>

IGV, Integrative Genomic viewer.

*Present in our in-house exomes (n = 150) or in public single-nucleotide polymorphism databases (dbSNP, 1000 Genomes).

Reads were manually viewed by IGV (Broad Institute, Cambridge, MA) to exclude false-positive or missed-inherited variants.

The individual with p.R157X is the 11-year-old daughter of unrelated parents with no family history of developmental disabilities. Her psychomotor development was initially characterized by global delay with some hypotonia. She first walked at 20 months of age. A formal psychological evaluation was performed at 8 years and 8 months of age. Assessment with the Wechsler Intelligence Scale for Children (global score: <1st centile; homogeneous scores), the Leiter International Performance Scale Brief IQ (global score: <1st centile) and the Vineland Adaptive Behavior Scale showed mild-to-moderate ID. At 11 years of age, she is able to speak in French and English using complete sentences. She can tell an event and carry out short conversations. She can recognize all the letters of the alphabet and is able to read a few syllables. She is a good swimmer but cannot ride a bicycle. There is no history of seizures or breathing abnormalities. At 11 years of age, her weight is 36 kg (25–50th centile), height is 146.4 cm (50th centile) and head circumference is 52.7 cm (25–50th centile). Physical examination did not reveal any specific dysmorphic features. Neurological examination was unremarkable. The following investigations did not show any abnormalities: Fragile X syndrome molecular testing, karyotyping (510 bands), and brain magnetic resonance imaging.

Zweier et al. previously found the same p.R157X mutation in an individual with PTHS (5). Our patient, however, does not show the characteristic PTHS dysmorphic features, which includes deep-set eyes, large nasal bridge, wide mouth, and cup-shaped ear. Moreover, unlike all PTHS patients reported so far, who show severe ID with limited speech and mobility, our patient shows mild-to-moderate ID and is able to speak in full sentences. Finally, although breathing abnormalities, epilepsy, or acquired microcephaly are found in the majority of PTHS cases, our patient did not show any of these features (3, 5). The same mutation in TCF4 can thus result in variable phenotypes.

Kalscheuer et al. previously reported a girl with mild-to-moderate ID who carries a *de novo* balanced translocation disrupting TCF4 (7). This translocation resulted in the production of a fusion transcript that contains the bHLH domain. It is possible that this...
Fig. 1. Pathogenic mutations identified in TCF4. (a) Scheme showing the exons of the TCF4 gene (2–19 are coding) and the localization of previously published TCF4 mutations, including p.R157X, identified in this study and by Zweier et al. (5). (b) Integrative Genomic viewer tracks showing the presence of the p.R157X mutation in TCF4 [chr18:51169133 G>A (negative strand; hg18); NM_001083962:exon7:c.C469T] in a subset of the reads in the proband but not in her parents. (c) Sanger sequencing of TCF4 exon 7 in genomic DNA from the patient and her parents. The c.C469T/p.R157X mutation was detected in the patient but not in her mother and father, indicating that the mutation is de novo. The chromatogram’s signals corresponding to the mutant (MT) and wild-type (WT) alleles were represented at similar levels in the different tissues from the patient: blood (MT/WT = 1.08), buccal scraping (MT/WT = 0.95) and hair bulb (MT/WT = 1.15) (Mutation Surveyor allele ratio quantification tool, SoftGenetics). (d) Reverse transcription polymerase chain reaction (RT-PCR) done on total RNA extracted from the proband’s (Pb) lymphoblastoid cells and from a control individual (Ctrl) with no TCF4 mutations, using TCF4-specific primers flanking exon 7 with a forward primer at the junction of exon 4/5 and a reverse primer in exon 9. RT-PCR products were Sanger sequenced and the corresponding chromatograms showed the presence of the MT (c.C469T/p.R157X) and WT alleles at similar levels (MT/WT peak signals = 0.93), suggesting that this nonsense mutation does not cause detectable nonsense mediated decay. (e) Pictures of the proband described in this study showing no specific dysmorphism.

transcript display some TCF4 activity, providing an explanation for the patient’s milder phenotype. Our study, however, indicates that truncating mutations in TCF4 can cause milder forms of ID.

TCF4 mutations are probably undiagnosed in NSID, as clinicians are unlikely to screen this gene in individuals with this condition. More of such cases, however, are likely to be identified due to the wide use of exome sequencing which explores all the genes and not only those that have already been associated with a phenotype of interest.

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