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Autosomal recessive progeroid syndrome due to homozygosity for a TOMM7 variant

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Multiple genetic loci have been reported for progeroid syndromes. However, the molecular defects in some extremely rare forms of progeria have yet to be elucidated. Here, we report a 21-year-old man of Chinese ancestry who has an autosomal recessive form of progeria, characterized by severe dwarfism, mandibular hypoplasia, hyperopia, and partial lipodystrophy. Analyses of exome sequencing data from the entire family revealed only 1 rare homozygous missense variant (c.86C>T; p.Pro29Leu) in TOMM7 in the proband, while the parents and 2 unaffected siblings were heterozygous for the variant. TOMM7, a nuclear gene, encodes a translocase in the outer mitochondrial membrane. The TOMM complex makes up the outer membrane pore, which is responsible for importing many preproteins into the mitochondria. A proteomic comparison of mitochondria from control and proband-derived cultured fibroblasts revealed an increase in abundance of several proteins involved in oxidative phosphorylation, as well as a reduction in abundance of proteins involved in phospholipid metabolism. We also observed elevated basal and maximal oxygen consumption rates in the fibroblasts from the proband as compared with control fibroblasts. We concluded that altered mitochondrial protein import due to biallelic loss-of-function TOMM7 can cause severe growth retardation and progeroid features.

Introduction

In the last 2 decades, considerable progress has been made in identifying the molecular genetic basis of several progeroid syndromes, including mandibuloacral dysplasia (MAD), mandibular hypoplasia, deafness and progeroid syndrome (MDPS), Hutchinson-Gilford progeria syndrome (HGPS), and atypical progeroid syndrome (APS). Disease-causing variants in LMNA, ZMPSTE24, and MTX2 have been linked to MAD, HGPS, and APS, and variants in POLD1 have been linked to MDPS (1). However, the molecular defects in some other extremely rare forms of progeria have yet to be identified. Here, we elucidated the genetic basis of a rare, autosomal recessive form of progeroid syndrome that presented with severe dwarfism, mandibular hypoplasia, hyperopia, micro-opthalmia, and partial lipodystrophy. We identified the causative mutation of this disorder, a homozygous, rare missense variant in TOMM7, a gene that encodes a translocase of the outer mitochondrial membrane.

Results and Discussion

A 21-year-old man of Chinese ancestry living in Malaysia was referred to UT Southwestern with a presumptive diagnosis of MAD. He was born full term and weighed 2.8 kg at birth. He had severe postnatal growth retardation and presented at age 6 with short stature and dysmorphism. His hearing was normal but his vision was poor. Although he communicated well verbally, he had significant learning disabilities.

On physical examination, his height was 116 cm, with a weight of 20.9 kg (Figure 1, A and B), and a head circumference of 53.5 cm, which were all below the third percentile when compared with age- and sex-matched controls. He had proportionate short stature with relative macrocephaly (Figure 1, C–G). He had triangular facies with broad forehead, prominent nasal bridge, bulbous nose, severe mandibular hypoplasia, and dental crowding (Figure 1, C–G). He had pendular nystagmus and high hyperopia with visual acuity of 3/60 in both eyes (right eye: +10.75 Diopter Sphere and left eye: +6.00 Diopter Sphere). He also had bilateral microphthalmia with axial lengths of only 16.8 mm (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI156864DS1). He had some café au lait spots on his limbs, coarse eyebrows, and sparse hair. His muscles were well defined, especially on the hips and lower extremities (Figure 1G). Most of the skinfold thickness measurements over the trunk and thighs were below the tenth percentile for normal males (Supplemental Figure 2) (2). At the age of 17 years, he reached Tanner pubertal stage V, but had sparse moustache and axillary hair.

His complete blood counts, serum sodium, potassium, chloride, calcium, phosphorus, magnesium, urea, creatinine, total protein, albumin, globulin, bilirubin, creatine kinase, and lactate
The proband was a 17-year-old male with severe growth retardation, proportionate short stature, and mandibular hypoplasia (Figure 1). His serum dehydrogenase were within normal range. His serum alkaline phosphatase concentrations were slightly high, but serum fasting glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, TSH, total thyroxine, insulin-like growth factor 1 (IGF-1), LH, FSH, and testosterone concentrations were normal (Table 1).

At age 11, echocardiography showed mild mitral regurgitation with normal chamber sizes and a left ventricular ejection fraction of 73%. At age 17, echocardiography revealed mild dilatation of the left ventricle. No ventricular or atrial septal defects or patent ductus arteriosus was observed. Ultrasound of the abdomen and magnetic resonance imaging of the brain revealed no abnormalities. Skeletal surveys conducted at 11 and 17 years of age revealed no significant delay in his bone age (Supplemental Figure 3).

His parents denied any consanguinity. His eldest sister also had dwarfism, sparse hair, high hyperopia and died at age 10 during a febrile illness associated with cough and shortness of breath. He has 2 older sisters who are healthy (Figure 1H).

During a febrile illness associated with cough and shortness of breath, we performed whole exome sequencing (WES) on the proband, unaffected sisters, and their parents (Figure 2A). We hypothesized an autosomal-recessive inheritance and filtered for rare missense, nonsense, splicing, or frame shift variants — either homozygous or compound heterozygous — in the proband, but not in the 2 unaffected siblings or their parents. We used a liberal cutoff for variants using the minor allele frequency (MAF) < 0.01 in the genome aggregation database (gnomAD; http://gnomad.broadinstitute.org), even though this is a rare syndrome. Other criteria included a Genomic Evolutionary Rate Profiling ++ (GERP++) score (3) greater than 2.0, and a Combined Annotation Dependent Depletion (CADD) score (4) greater than 15. There was only 1 variant that passed the filtering strategy. This was a homozygous variant, (rs778567973) in TOMM7 (NC_000007.13:g.22862313G>A;
The TOMM7 variant, interacted poorly with the core TOMM complex — TOMM40 and TOMM22 — in contrast to TOMM7 in the patient's fibroblasts (Figure 3A). We replicated these results in CRISPR-Cas9 generated TOMM7+/- HeLa cells reconstituted with either HA-TOMM7 or HA-TOMM7 (Supplemental Figure 4, A–C) and observed reduced interactions between TOMM7 and TOMM40/TOMM22 (Figure 3B). This effect was not due to mis-localization, as GFP-tagged TOMM7 and TOMM7 both localized to mitochondria (Figure 3C).

To assess the effects of the TOMM7 variant, we turned our attention to dermal fibroblasts cultured from the proband and age-matched controls (see Supplemental Methods). The levels of TOMM7 protein and the morphology of the mitochondria were similar in cultured dermal fibroblasts from the proband and controls (Supplemental Figure 5A and Figure 3G). TOMM7 has been previously implicated in Parkin-induced mitophagy (13, 14). However, in the presence of the mitochondrial uncoupler carbonyl cyanide m-chlorophenylhydrazone, Parkin translocation to mitochondria and mitochondrial clearance were not impaired in the patient’s fibroblasts (Supplemental Figure 5, B and C), indicating that the P29L variant may not interfere with mitophagy.

We assessed mitochondrial respiration in the proband’s fibroblasts, making use of extracellular flux assays, which indicated elevated basal and maximal oxygen consumption rates compared with a panel of control cell lines (Figure 3D). Given that the TOMM complex facilitates the import of proteins into mitochondria, we compared the proteome of mitochondria purified from the control and patient-derived fibroblasts via label-free proteomics. Of the 587 detected mitochondrial proteins, 91 were differentially expressed (P < 0.05), indicating that a subset of the mitochondrial proteome is affected by the presence of the TOMM7 variant (Supplemental Figure 5D and Supplemental Table 1). Gene set enrichment analysis (GSEA) of significantly altered proteins (P < 0.05) indicated a higher abundance of proteins related to ATP-production and proton transport, and reduced expression of proteins related to phospholipid metabolic pathways in the patient’s mitochondria (Supplemental Figure 5E and Supplemental Table 1). We therefore assessed the levels of mitochondrial electron transport chain (ETC) proteins in our proteomics data set. These were often upregulated in the patient’s mitochondria (Figure 3E), which we verified through GSEA (Figure 3F) and Western blot (Figure 3G), specifically TFAM, ATP5A, UQCRCC2, SDHB, and NDUF8. We corroborated these results in primary tail fibroblasts from 2-week-old genetically engineered Tomm7 mice (Supplemental Figure 6, A and B), which also revealed increased oxygen consumption rates (Supplemental Figure 6C). Proteomic analysis from mouse fibroblasts also revealed a tendency toward upregulation of mitochondrial ETC components in Tomm7 cells (Supplemental Figure 6D and Supplemental Table 1), which was verified by GSEA (Supplemental Figure 6E).

In mammals, TOMM7 is part of the TOMM complex, which contains a central pore-forming protein (TOMM40) surrounded by additional proteins (TOMM5, TOMM6, TOMM7, and TOMM22), and accessory subunits (TOMM20, TOMM70). TOMM7 facilitates transport of preproteins into mitochondria (15). Interestingly, while several genetic syndromes are caused by mutations in proteins involved in translocase of the inner mitochondrial mem-
Figure 2. Identification of a Pro29Leu variant in the TOMM7 gene. (A) Schematic of segments on chromosome 7 of the proband (MAD5700.25) and his parents (MAD5700.12, 13) and sisters (MAD5700.23, 24), based on GRCh37/hg19 coordinates. For each individual, the top line displays markers with homozygous genotypes and the bottom line displays markers with heterozygous genotypes. The homozygous regions inferred from WES data are shown in gray (~1 Mb) and those derived from WGS (~0.8 Mb, chr7:22,506,999-23,366,145) shown with red. The two siblings of the proband and the parents did not share the homozygous region. (B) The location of various genes in the homozygous region on chromosome 7, the gene structure of TOMM7, and the location of the mutation in the proband. Human TOMM7 contains three exons (shown in black rectangles) and two introns (shown as a line). The pathogenic variant c.86C>T in TOMM7 is located in exon 1 (red arrow). (C) Sequence electropherogram for WT (top) sequence in exon 1 of TOMM7, MAD5700.12 (middle) showing heterozygous (het) c.86C>T variant, and MAD5700.25 (bottom) showing the homozygous (hom) c.86C>T variant. (D) An overview of the cryo-electron microscope structure for human TOM complex (PDB ID 7CK6). TOMM7 is shown in red, TOMM5 in gray, TOMM6 in orange, and TOMM40 in green. The expanded view shows the region surrounding residue proline (Pro) 29 in TOMM7, with nearby amino acids phenylalanine (Phe) 27, isoleucine (Ile) 28, and valine (Val) 31 forming interactions with TOMM40 (green). (E) Protein alignment of TOMM7 from the indicated organisms. Note that Proline 29 (highlighted in yellow) is conserved amongst all the phyla aligned. The three α-helices are indicated by gray bars.
Like what has been previously reported (18), we found that inactivation of TOMM7 was associated with changes in the concentration of selected proteins in the mitochondria of fibroblasts, including increases in several proteins involved in oxidative phos-
phorylation. Correspondingly, we observed elevated basal as well as maximal oxygen consumption rates in the presence of the TOMM7<sup>22–14</sup> variant, suggesting that TOMM7<sup>22–14</sup> may constitute a negative regulator of import for a subset of the mitochondrial proteome. We also observed reduced abundance of proteins involved in phospholipid metabolism. Given that defects in enzymes involved in phospholipid biosynthetic pathways, such as AGPAT2, PIK3R1, and PCYT1A, cause human lipodystrophies (19–21), it is possible that the lipodystrophy in our patient may be related to downregulation of these pathways. Future work to assess the precise consequences of this variant on the efficiency of mitochondrial protein import may further pinpoint the specific role of TOMM7 in human physiology.

Mice with homozygous deletion of Tomm7 also recapitulate some phenotypic features of our patient and have small body size and low body weight (22). Selective inactivation of tomm7 in zebrafish resulted in impaired cerebrovascular network formation and cerebral hemorrhages (22), and Tomm7 KO mice also experienced cerebrovascular abnormalities and premature death by day 21 (22). While the affected elder sister of our proband died at an early age of 10 years, the proband is doing well at 21 years of age and, based on MRI, has no structural abnormality of the brain.

TOMM7 is required for PINK1 stabilization and has been recently implicated as a positive regulator of Parkin translocation in the mitophagic response to uncoupled mitochondria (13, 14). Although the TOMM7<sup>22–14</sup> variant disrupts TOMM7’s interaction with the TOMM complex, Parkin-dependent mitophagy is not significantly impaired. Consistent with this, our patient has not developed any signs of Parkinson’s disease thus far. Therefore, the role of TOMM7 in Parkin-dependent mitophagy seems to be independent of its interaction with the outer mitochondrial import pore proteins.

In summary, we report a patient who presents with severe dwarfism, mandibular hypoplasia, hyperopia and partial lipodystrophy with a biallelic inactivating missense variant in TOMM7 that is associated with increased mitochondrial oxygen consumption.

**Methods**

Details are described in the Supplemental Methods.

**Study approval.** The protocol was approved by the IRB of UT Southwestern and all participants provided written, informed consent including that for patient pictures appearing in the manuscript. Animal studies were approved by the UT Southwestern IACUC.

**Author contributions**

AG, AKA, CX, and PM conceived the research, designed and supervised experiments, acquired and interpreted data and wrote the manuscript; WTK and PDK helped in phenotyping the patient; ZC, NPL, and CBL conducted experiments and acquired data; CX, AAS, and YS analyzed whole exome sequencing, whole genome sequencing, and proteomics data.

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