De novo Mutations in TOMM70, a central receptor of the main mitochondrial import translocase, Causes Developmental Delay and Neurological Phenotypes

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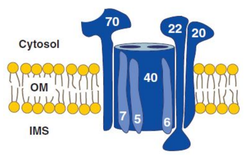
Abstract

More than 1000 mitochondrial proteins are imported into mitochondria. The Translocase of Outer Mitochondrial Membrane (TOMM) complex is the entry gate for virtually all mitochondrial proteins and essential to build the mitochondrial proteome. TOMM70 as a central receptor assists mainly in the import of proteins with internal mitochondrial targeting signals. To date, there have been no reports of disease related to any of the TOMM proteins. Here, we identified two individuals with a childhood onset neurological disorder that displays behavioral symptoms, physical signs and medical imaging evidence of ongoing neurodegenerative brain disease that had no explanation prompting their evaluation and genomic sequencing in the Undiagnosed Diseases Network or at Children's Hospital of Philadelphia. Both patients were ultimately found to have de novo (i.e. mutations in their germline not identified in their biological parents) missense changes in highly conserved residues of the human TOMM70 gene (p.Thr607Ile and p.Ile554Phe) raising the possibility that they have a novel syndrome related to TOMM70 mutations. To functionally assess TOMM70 variants, we used a humanization strategy in the fruit-fly (Drosophila melanogaster). TOMM70 is highly conserved in fruit-flies, and fly Tom70 is expressed in the larval and adult brain of fruit-fly. We replaced the entire Tom70 ORF with a Kozak-MiniGAL4 transgene using CRISPR. These animals die as pupae, but the lethality is rescued by the MiniGAL4 driven expression of human reference UAS-TOMM70 cDNA. However, both patient variants lead to significantly less rescue indicating that they are loss-of-function alleles. RNAi-mediated knock-down of Tom70 in the developing eye causes smoothened regions and necrotic spots. Electroretinograms (ERGs) of flies with RNAi-mediated knock-down also revealed a severe synaptic transmission defect. Co-expression of human reference TOMM70 in the fly Tom70 RNAi background, partially rescues the eye morphology defect and fully rescues the ERG defects. However, the patient variants fail to rescue these defects, further supporting the notion that these variants are indeed loss-of-function alleles. Altogether, our data indicates that loss-of-function mutations in TOMM70 results in variable white matter disease and neurological phenotypes in patients. Hence, Drosophila can be used as a valuable model to study the human disease(s) associated with the TOMM complex.

Background



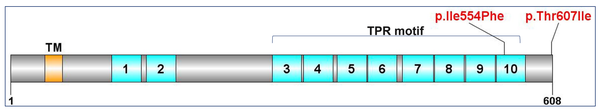
TOM complex

Tom70	Surface receptor
Tom40	Translocation pore
Tom22	Surface receptor
Tom20	Surface receptor
Tom7	Translocation pore
Tom6	Translocation pore
Tom5	Translocation pore

Neupert and Herrmann, 2007

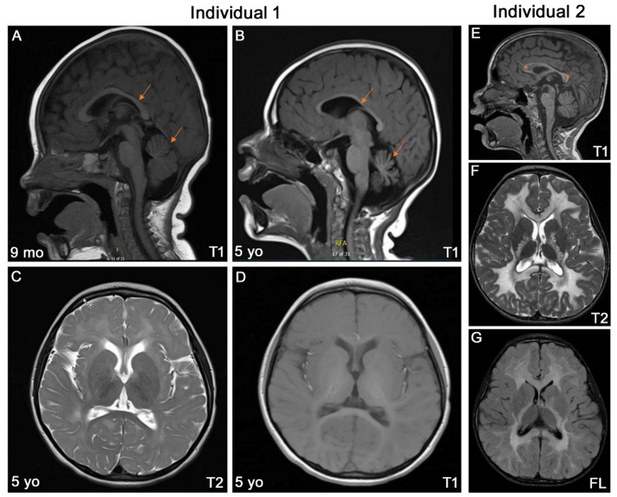
- Mitochondrial proteome consists of ~1100-1500 proteins. Only 13 proteins are encoded in mitoDNA and synthesized in mitochondria. Rest 99% of the proteins are synthesized in the cytoplasm and are transported into the mitochondria via Transporter Complex
- TOM (Translocase of the Outer Mitochondrial membrane) complex is required for import of these mitochondrial proteins. Tom70 is a part of TOM complex
- Tom70 remains anchored to the outer mitochondrial membrane by a N-terminal hydrophobic sequence; a major portion of the proteins is cytosolic
- Tom70 contains multiple TPR motif, which form scaffolds to mediate protein–protein interactions and often the assembly of multiprotein complexes

TOMM70 variants & patient description



		Subject 1	Subject 2
Variant	Sex	Female	Male
	Age at last evaluation	6y	11y
	Zygosity	Heterozygous	Heterozygous
	Inheritance	de novo	de novo
	Variant (NM_014820.3)	c.1820C>T (p.T670I)	c. 1660A>T (p.I554F)
	gnomAD	Absent	Absent
	SIFT	Deleterious	Deleterious
	PolyPhen2	Probably damaging	Probably damaging
	CADD	28.4	26.3
Phenotype	Developmental delay	No	No
	Regression	Possibly	Yes
	Dystonia	Yes	Yes
	Hypotonia	Yes	Yes
	Hyperreflexia	Yes	Yes
	Ankle clonus	Yes	No
	Cogwheeling	No	Yes
	Tremor	No	Yes
	Ataxia	Yes	Yes
	Dysarthria	No (non-verbal)	Yes
	Ptosis	No	Yes
	Brain MRI	Diffuse hypomyelination Moderate cerebellar atrophy Mild cerebral atrophy Diffusely thin corpus callosum	Diffuse white matter abnormalities with superimposed rarefaction

TOMM70 variants cause neurological phenotypes

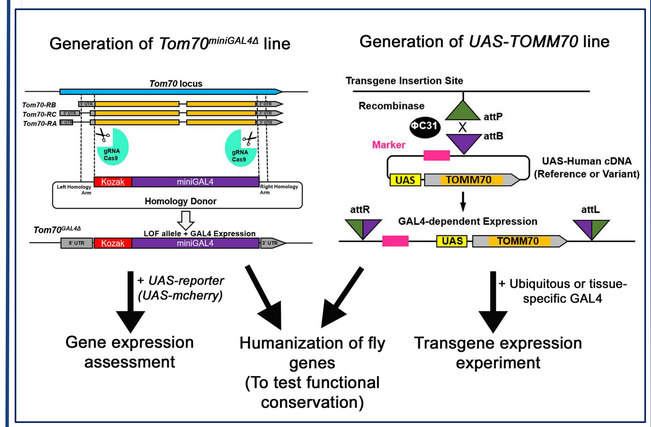


Brain MRI revealed abnormalities in both individuals. For example, individual 1 has diffuse hypomyelination and individual 2 has diffuse white matter abnormalities with superimposed rarefaction. Additionally, progressive cerebellar atrophy and diffusely thin corpus callosum were observed in individual 1.

TOMM70 is highly conserved

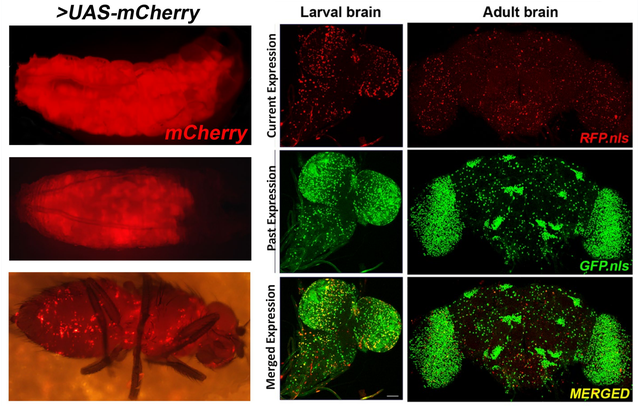
- Drosophila Tom70 is the ortholog of human TOMM70
- Tom70 has a very high DIOPT score (14/15) indicative of strong conservation between human and Fly
- Amino acid identity between human TOMM70 and Fly Tom70 is 40%
- Amino acid similarity between human TOMM70 and Fly Tom70 is 60%

Humanization strategy



In short, the *Tom70* locus in fly was replaced with a Kozak-mini-GAL4 construct (Kanca et al., 2019). This GAL4 knock-in line was a null allele. Due to the presence of Kozak-miniGAL4, this line was used to express human *TOMM70* reference and the variant transgenes in Tom70 null background in fly. Additionally, the GAL4 knock-in line was used to determine the *Tom70* expression. The UAS-transgenic lines expressing human TOMM70 and the variants were used to test if the variants are dominant in nature.

Tom70 expression in Drosophila

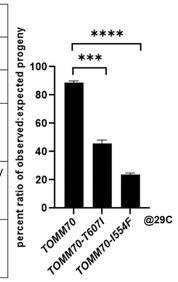


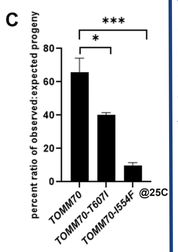
(A)*Tom70* is expressed in the larval, pupal, and adult stages in Drosophila. (B) G-Trace analysis was performed to see the *Tom70* expression in larval brain and adult brain. The red channel indicates the cell, where the gene is expressed in the real-time. The green channel indicates the cells, where the gene was active in the past. A limited expression pattern of Tom70 was observed in both larval and adult brains. It indicates that the gene was actively expressed during in the early stage of the larva and adulthood and the expression becomes limited at the later stage. Scale bar: 50 µm.

TOMM70 variants are partial loss-of-function in nature

A

Genotype	Lethality?
Tom70$^{miniGAL4\Delta}$/Df(2L); +/+	Pupal lethal
Tom70$^{miniGAL4\Delta}$/*Tom70*$^{miniGAL4\Delta}$; +/+	Pupal lethal
Tom70$^{miniGAL4\Delta}$/*Tom70*$^{miniGAL4\Delta}$; UAS-TOMM70/+	Lethality rescued
Tom70$^{miniGAL4\Delta}$/*Tom70*$^{miniGAL4\Delta}$; UAS-TOMM70^{T607I}/+	Lethality partially rescued
Tom70$^{miniGAL4\Delta}$/*Tom70*$^{miniGAL4\Delta}$; UAS-TOMM70^{I554F}/+	Lethality mildly rescued

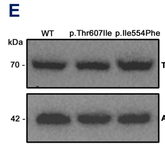




D

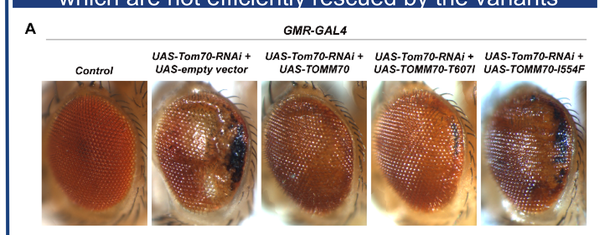
	UAS-TOMM70	UAS-TOMM70-T607I	UAS-TOMM70-I554F
Actin-GAL4	No Phenotype	No Phenotype	No Phenotype
Ey-GAL4	No eye Phenotype	No eye Phenotype	No eye Phenotype
GMR-GAL4	No eye Phenotype	No eye Phenotype	No eye Phenotype

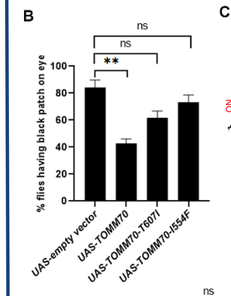
E

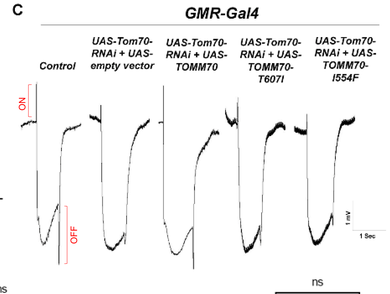


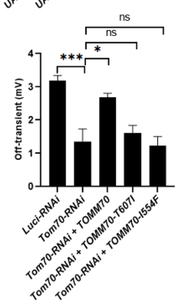
(A-C) Tom70miniGAL4Δ homozygous and Tom70miniGAL4Δ/Df(2L) causes pupal lethality that was rescued by human *TOMM70*. Both of the variants partially rescued the lethality, which indicates that the variants are loss-of-function alleles. Error bar is indicative of ±SEM. One-way ANOVA with Tukey's posthoc test was performed to test the significance (*=p<0.05, **=p<0.01, ***=p<0.001, ****p<0.0001).
(D-E) Expression of *TOMM70* reference and variant transgenes using a ubiquitous GAL4 line and eye-specific GAL4 lines does not cause a morphological phenotypes. Additionally, GMR-GAL4 mediated expression of the reference and variants of TOMM70 does not affect the stability of the proteins. These observations suggest that the variants are not dominant-negative or gain-of-function in nature.

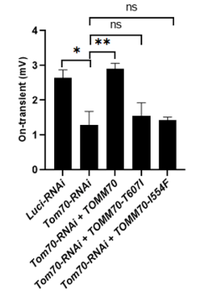
RNAi-mediated knockdown of *Tom70* causes eye morphology and synaptic transmission defects, which are not efficiently rescued by the variants











(A-B)Eye-specific knockdown of Tom70 results in eye morphology defects. The eye morphology defect was partially rescued by human *TOMM70* reference transgene. Both of the variants were failed to significantly rescue the eye phenotype. (C-E) Electroretinogram recording and quantification indicate a reduced ON and OFF transients indicative of synaptic transmission defect in *Tom70* knockdown condition. The synaptic transmission defect was rescued by human *TOMM70*, however, both of the variants could not rescue it. Error bar indicates of ±SEM. One-way ANOVA with Tukey's posthoc test was performed to test the significance (*=p<0.05, **=p<0.01, ***=p<0.001, ****p<0.0001).

Future directions

- Identification of Tom70 interacting partners
- Investigate how Tom70 regulates synaptic transmission and neuronal maintenance
- Determine the role of Tom70 in neurodegeneration

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