

Defects in tRNA Anticodon Loop 2'-O-Methylation Are Implicated in Nonsyndromic X-Linked Intellectual Disability due to Mutations in *FTSJ1*

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ABSTRACT: tRNA modifications are crucial for efficient and accurate protein synthesis, and modification defects are frequently associated with disease. Yeast *trm7Δ* mutants grow poorly due to lack of 2'-O-methylated C₃₂ (Cm₃₂) and Gm₃₄ on tRNA^{Phe}, catalyzed by Trm7-Trm732 and Trm7-Trm734, respectively, which in turn results in loss of wybutosine at G₃₇. Mutations in human *FTSJ1*, the likely TRM7 homolog, cause nonsyndromic X-linked intellectual disability (NSXLID), but the role of *FTSJ1* in tRNA modification is unknown. Here, we report that tRNA^{Phe} from two genetically independent cell lines of NSXLID patients with loss-of-function *FTSJ1* mutations nearly completely lacks Cm₃₂ and Gm₃₄, and has reduced peroxywybutosine (o₂yW₃₇). Additionally, tRNA^{Phe} from an NSXLID patient with a novel *FTSJ1*-p.A26P missense allele specifically lacks Gm₃₄, but has normal levels of Cm₃₂ and o₂yW₃₇. tRNA^{Phe} from the corresponding *Saccharomyces cerevisiae* *trm7-A26P* mutant also specifically lacks Gm₃₄, and the reduced Gm₃₄ is not due to weaker Trm734 binding. These results directly link defective 2'-O-methylation of the tRNA anticodon loop to *FTSJ1* mutations, suggest that the modification defects cause NSXLID, and may implicate Gm₃₄ of tRNA^{Phe} as the critical modification. These results also

underscore the widespread conservation of the circuitry for Trm7-dependent anticodon loop modification of eukaryotic tRNA^{Phe}.

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KEY WORDS: *FTSJ1*; intellectual disability; NSXLID; tRNA; 2'-O-methylation; TRM7

Introduction

The numerous post-transcriptional modifications of tRNA are crucial for accurate and efficient translation of the genetic code. In the yeast *Saccharomyces cerevisiae*, mutations affecting 16 of the 25 tRNA modifications lead to distinct phenotypes, including lethality for three mutants, and poor growth or temperature sensitivity for six other mutants [Hopper, 2013]. Modifications in and around the tRNA anticodon loop (residues 31–39) are particularly important in all organisms [de Crecy-Lagard et al., 2012], often affecting decoding [Murphy et al., 2004; Agris et al., 2007], charging by the cognate tRNA aminoacyl synthetase [Muramatsu et al., 1988; Putz et al., 1994], and/or frame maintenance [Urbanavicius et al., 2001; Bekaert and Rousset, 2005; Waas et al., 2007], whereas modifications in the body of the tRNA often contribute to folding or stability [Hall et al., 1989; Yue et al., 1994; Helm et al., 1999; Whipple et al., 2011] and are required to avoid decay by two known degradation pathways [Kadaba et al., 2004; LaCava et al., 2005; Vanacova et al., 2005; Alexandrov et al., 2006; Kadaba et al., 2006; Schneider et al., 2007; Chernyakov et al., 2008].

Emerging evidence shows that tRNA modifications have important roles in human health. Mutations in nine predicted human homologs of tRNA modification genes have been strongly linked to specific diseases, seven of which are linked to neurological disorders, including six linked to intellectual disability (ID) [Anderson et al., 2001; Slaugenhaupt et al., 2001; Cuajungco et al., 2003; Freude et al., 2004; Ramser et al., 2004; Froyen et al., 2007; Takano et al., 2008; Zeharia et al., 2009; Najmabadi et al., 2011; Abbasi-Moheb et al., 2012; Khan et al., 2012; Martinez et al., 2012; Alazami et al., 2013; Igoillo-Esteve et al., 2013; Fahiminiya et al., 2014; Gillis et al., 2014; Yarham et al., 2014]. However, the molecular basis of the link

Additional Supporting Information may be found in the online version of this article.

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Table 1. *FTSJ1* Mutations Associated with NSXLID Analyzed for tRNA Modifications in This Study

<i>FTSJ1</i> allele	Family	Mutation	Effect	Reference
<i>FTSJ1</i> Δ	6	Deletion of <i>FTSJ1</i> and <i>SLC38A5</i>	Loss of <i>FTSJ1</i>	Froyen et al. (2007)
<i>FTSJ1</i> splice site (<i>FTSJ1</i> -ss)	3	c.121+1delG	Significant reduction of <i>FTSJ1</i> mRNA levels	Freude et al. (2004)
p.A26P	7	c.76G>C; p.A26P	Altered <i>FTSJ1</i> protein function	This study, Hu et al. (2015)

between the neurological disorders and mutations in predicted tRNA modification genes is not clear. Indeed, decreased levels of tRNA modifications have only been demonstrated in cells derived from patients with two neurological disorders. First, bulk tRNA from brain tissue and cell lines derived from familial dysautonomia (FD) patients, which is due to mutations in *IKBKAP* in numerous cases [Anderson et al., 2001; Slangen et al., 2001; Dong et al., 2002; Cuajungco et al., 2003], had reduced levels of the mcm⁵s²U₃₄ modification and the IKAP protein [Karlsborn et al., 2014]. This result is consistent with the homology between IKAP and the Elp1 subunit of the *S. cerevisiae* elongator complex [Hawkes et al., 2002], which is required for cm⁵U formation [Huang et al., 2005]. Second, tRNA from cells derived from patients with a Dubowitz-like syndrome (characterized by phenotypes including microcephaly and mental and speech delays) linked to an *NSUN2* splice-site mutation lacked m⁵C at residues 34, 48, 49, and 50 [Martinez et al., 2012; Blanco et al., 2014], consistent with the activity of the yeast and mammalian homologs [Motorin and Grosjean, 1999; Brzezicha et al., 2006; Blanco et al., 2011; Tuorto et al., 2012]. *NSUN2* mutations are also linked to other neurological disorders including a Noonan-like syndrome similar to the *NSUN2*-linked Dubowitz-like syndrome [Fahiminiya et al., 2014] and autosomal-recessive ID (ARID) in four independent families [Abbasi-Moheb et al., 2012; Khan et al., 2012].

One of the strongest links between ID and a putative tRNA modification gene is that linking nonsyndromic X-linked ID (NSXLID) to mutations in *FTSJ1* (MIM #300499), a homolog of yeast Trm7, which catalyzes formation of Cm₃₂ and Nm₃₄ on substrate tRNAs [Pintard et al., 2002] and is critical for normal function of tRNA^{Phe} [Guy et al., 2012]. Distinct alleles of *FTSJ1* from five independent families are linked to NSXLID and each allele results in reduced levels of mRNA or reduced predicted protein function [Freude et al., 2004; Ramser et al., 2004; Takano et al., 2008]. In addition, NSXLID is linked to a microdeletion that removes *FTSJ1* and *SLC38A5* [Froyen et al., 2007] (Table 1; Supp. Table S1). Moreover, *FTSJ1* is the likely human ortholog of the yeast *TRM7* gene, since expression of human *FTSJ1* under control of the strong P_{GAL} promoter (P_{GAL}-*FTSJ1*) in a high copy (2μ) plasmid suppresses the severe growth defect of *S. cerevisiae* *trm7*Δ mutants [Guy and Phizicky, 2015].

In *S. cerevisiae*, Trm7 is the central component of a complex modification circuitry required for anticodon loop modification of target tRNAs, wherein Trm7 separately interacts with Trm732 and Trm734 to form Cm₃₂, and Nm₃₄, respectively, both of which are required on tRNA^{Phe} for efficient formation of wybutosine (yW) at m¹G₃₇ by other proteins (Fig. 1A) [Noma et al., 2006; Guy et al., 2012]. Moreover, the same circuitry appears to be conserved in the phylogenetically distant yeast *Schizosaccharomyces pombe*, since the nearly lethal phenotype of *trm7*Δ mutants is suppressed by overproduction of tRNA^{Phe}, since tRNA^{Phe} of *trm7*Δ mutants likewise lacks Cm₃₂, Gm₃₄, and yW₃₇, and since the homologous *S. pombe* Trm732 and Trm734 proteins are required for the respective Cm₃₂ and Gm₃₄ modifications [Guy and Phizicky, 2015]. Indeed, this conserved circuitry might be further extended in eukaryotes since suppression of the growth defect of *S. cerevisiae* *trm7*Δ mutants by *FTSJ1* expression requires the function of Trm732 or its human homolog THADA to form Cm₃₂ on tRNA^{Phe} [Guy and Phizicky, 2015].

Although it is well established that human *FTSJ1* mutations cause NSXLID, the connection between *FTSJ1* and tRNA modifications is not known in human cells, or in any metazoan. Indeed, it is not necessarily true that *FTSJ1* modifies human tRNAs based on complementation of a yeast mutant when *FTSJ1* is expressed at high levels.

Here, we show that cell lines derived from NSXLID patients bearing genetically distinct disease-causing *FTSJ1* mutations have pronounced defects in 2'-O-methylation of the anticodon loop of tRNAs, with reduced levels of peroxywybutosine (o₂yW₃₇) on tRNA^{Phe}. These findings provide strong evidence that *FTSJ1* catalyzes Cm₃₂ and Nm₃₄ modification in humans, and further support the conserved circuitry for tRNA^{Phe} anticodon loop modification. Intriguingly, tRNA^{Phe} from human cell lines with a novel missense *FTSJ1*-p.A26P mutation (NM_012280.2, c.76G>C, p.A26P; nucleotide numbering is based on cDNA sequence) or from the corresponding yeast *trm7*-A26P mutant lacks Gm₃₄ but retains Cm₃₂, apparently due to selective loss of substrate recognition and/or catalysis, rather than to reduced expression or protein interactions. These results strongly suggest that *FTSJ1*-associated NSXLID is due to lack of modifications of the anticodon loop of substrate tRNAs, and suggest the possibility that if tRNA^{Phe} is the important human *FTSJ1* target (as is true in two phylogenetically distant model yeast organisms), that lack of only Gm₃₄ of tRNA^{Phe} might be sufficient to trigger NSXLID.

Materials and Methods

Sequencing and Identification of the *FTSJ1*-p.A26P Variant

DNA extracted from whole-blood (QIAamp DNA blood maxi kit; Qiagen, Limburg, The Netherlands) was part of a large X-chromosome exome sequencing study [Hu et al., 2015], with one affected individual sequenced from this family (III-2, HiSeq; Illumina, San Diego, CA). Confirmation of the *FTSJ1* gene (NM_012280.2, c.76G>C, p.A26P; nucleotide numbering based on cDNA sequence) variant and segregation analysis by Sanger sequencing was carried out using standard methods. *FTSJ1* exon 2 DNA was amplified and sequenced using the following primers; F - 5'-GCA GTG GAG CCT GAG AGT TC-3' and R - 5'-CTA TCT TCC TGC CTG TCT CCC T-3'.

Generation of Lymphoblastoid Cell Lines

Lymphoblastoid cell lines (LCLs) derived from patients bearing a c.121+1delG mutation in *FTSJ1* (Table 1; Supp. Table S1; *FTSJ1*-ss; family 3), and from a patient bearing an *FTSJ1* deletion (*FTSJ1*Δ; family 6) have been described previously [Freude et al., 2004; Froyen et al., 2007]. Other LCLs were generated using standard methods.

Yeast Strains and Plasmids

Yeast strains are listed in Supp. Table S2. Plasmids used in this study are listed in Supp. Table S3. See Supp. Methods for detailed information on construction of strains and plasmids.

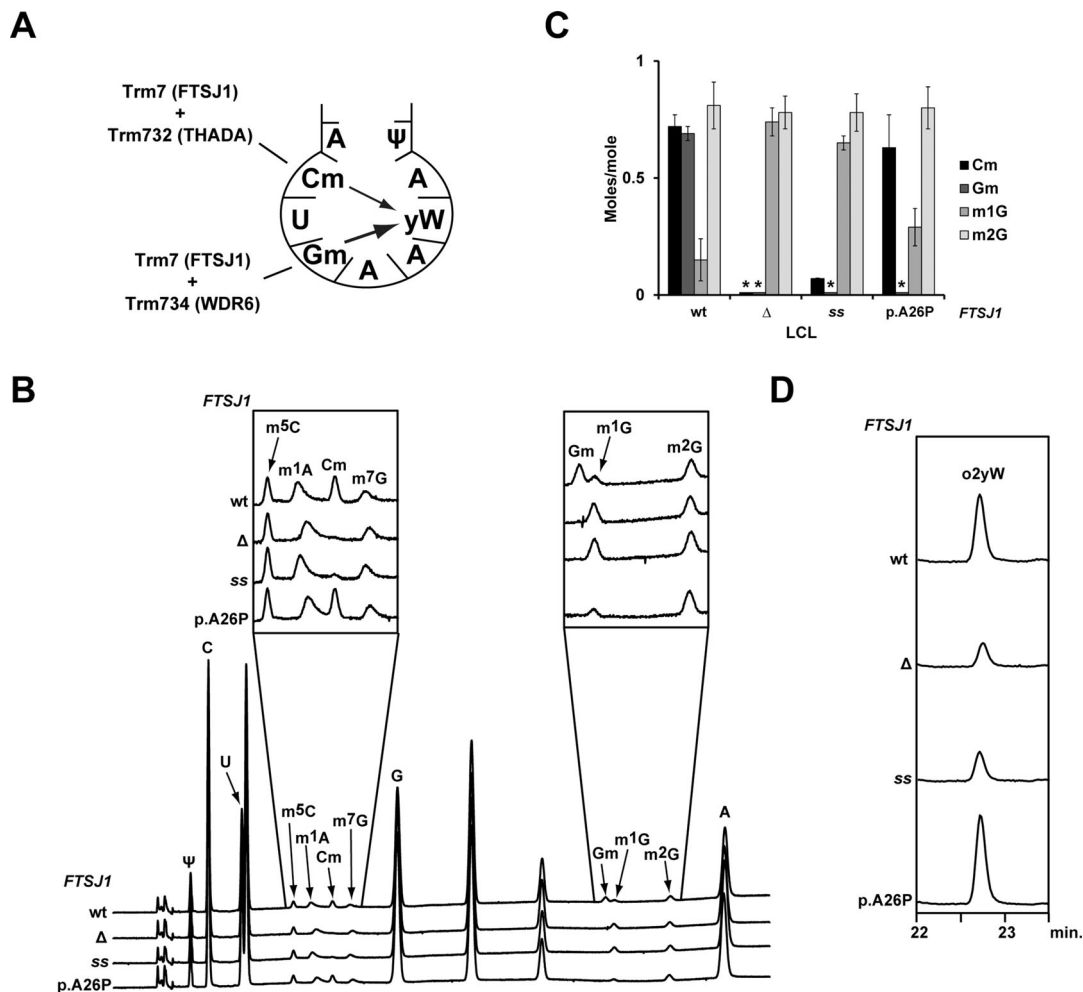


Figure 1. *FTSJ1* mutations associated with NSXLID are defective for 2'-*O*-methylation on tRNA^{Phe}. **A:** Schematic of the circuitry for tRNA^{Phe} anticodon loop modification in *S. cerevisiae* and *S. pombe*. Trm7 acts with Trm732 to form Cm₃₂ and separately with Trm734 to form Gm₃₄, and these modifications in turn drive yW formation at m¹G₃₇ on tRNA^{Phe}. Wider arrow from Gm₃₄ indicates that yW formation is more dependent on this modification than on Cm₃₂. Predicted human homologs of Trm7, Trm732, and Trm734 are in brackets. **B:** HPLC traces of nucleosides of tRNA^{Phe} purified from human LCLs derived from *FTSJ1*-associated NSXLID patients. tRNA^{Phe} isolated from LCLs derived from NSXLID patients with the indicated *FTSJ1* alleles, and from control LCLs, was digested to nucleosides and analyzed by HPLC as described in *Materials and Methods*. ss, splice-site mutation; wt, wild type. **C:** Quantification of tRNA^{Phe} nucleoside analysis from panel **B**. *, levels below threshold of detection. **D:** Analysis of o2yW levels on tRNA^{Phe} purified from LCLs of *FTSJ1*-associated NSXLID patients. Nucleosides of tRNA^{Phe} from indicated LCLs were analyzed as in panel **B** under conditions optimized for evaluating o2yW modification.

tRNA Purification and Northern Blot Analysis

For a detailed description of cell growth conditions and RNA preparation, see Supp. Methods. Briefly, RNA from LCLs was extracted using TRIzol (Life Technologies, Carlsbad, CA) according to manufacturer's instructions. Extraction of RNA using phenol for northern analysis under acidic conditions to preserve aminoacylation was previously described [Alexandrov et al., 2006]. Low molecular weight RNA was extracted from yeast cells as previously described [Jackman et al., 2003], and appropriate 5' biotinylated oligonucleotides were used to purify tRNA from yeast and human RNA preparations as previously described [Jackman et al., 2003].

HPLC Analysis of tRNA

Purified tRNA was digested with P1 nuclease and phosphatase as previously described [Jackman et al., 2003], and nucleosides were subjected to HPLC analysis essentially as previously described [Jack-

man et al., 2003]. Nucleosides from tRNA^{Phe} were separated by HPLC at pH 7.0 to maximize separation of Gm and m¹G, as previously described [Guy et al., 2012], and o2yW was separated by HPLC as previously described [Noma et al., 2006].

Immunoblot Analysis

Yeast crude extracts were subjected to SDS-PAGE and proteins were transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA). MORF-tagged and PT-tagged constructs were detected with rabbit polyclonal antipeptide A (1:5,000; Sigma-Aldrich, St. Louis, MO.), followed by incubation with goat antirabbit IgG-HRP (1:10,000; Bio-Rad), and visualization with Amersham ECL Plus (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The 9myc tag was detected with mouse monoclonal anti-[c-myc] (1:10,000; Roche, Basel, Switzerland), followed by incubation with goat antimouse IgG-HRP (1:10,000; Bio-Rad), and visualization.

Table 2. HPLC Analysis of tRNA^{Phe} Nucleoside Content from Human LCLs Derived from Patients with Mutations in *FTSJ1*

Cell line <i>FTSJ1</i>		(Control 1) wild type	(Control 2) wild type	(F6) Δ	(F3:1) ss	(F3:2) ss	(F7) p.A26P
Modification	Moles expected						
Cm	1	0.74 ± 0.14	0.72 ± 0.05	<0.03	0.07 ± 0.002	0.05 ± 0.01	0.63 ± 0.14
Gm	1	0.74 ± 0.04	0.69 ± 0.03	<0.03	<0.03	<0.03	<0.03
m ¹ G	0	0.42 ± 0.03	0.15 ± 0.09	0.74 ± 0.06	0.65 ± 0.03	0.65 ± 0.03	0.29 ± 0.08
Ψ	4	3.12 ± 0.29	3.21 ± 0.16	3.17 ± 0.31	3.16 ± 0.27	3.31 ± 0.33	3.32 ± 0.33
m ⁵ C	1	0.68 ± 0.14	0.70 ± 0.08	0.62 ± 0.13	0.72 ± 0.08	0.73 ± 0.05	0.58 ± 0.12
m ¹ A	2	0.84 ± 0.11	0.94 ± 0.13	0.78 ± 0.07	0.83 ± 0.13	0.93 ± 0.05	0.91 ± 0.17
m ⁷ G	1	0.48 ± 0.15	0.46 ± 0.11	0.39 ± 0.11	0.48 ± 0.16	0.44 ± 0.08	0.46 ± 0.12
m ² G	1	0.77 ± 0.10	0.81 ± 0.10	0.78 ± 0.07	0.78 ± 0.08	0.82 ± 0.07	0.80 ± 0.09
o2yW	1	0.60 ± 0.05	1.00 ± 0.12	0.16 ± 0.08	0.22 ± 0.06	0.23 ± 0.09	0.80 ± 0.011

Mean and standard deviation based on three individual growths and RNA preparations. o2yW values are relative to control 2.

Affinity Purification of Tagged Proteins

MORF-tagged and PT-tagged proteins were purified by affinity purification with IgG Sepharose followed by elution with GST-3C protease, and removal of the protease using glutathione Sepharose resin, essentially as previously described [Quartley et al., 2009].

Clinical Description

For a detailed clinical description, see Supp. Methods. This research was approved by the Women's and Children's Health Network Human Research Ethics Committee.

Results

FTSJ1 Is Required for Cm₃₂ and Gm₃₄ Modification of tRNA^{Phe}

To determine whether patients with *FTSJ1*-associated NSXLID have reduced 2'-O-methylation in their tRNAs, we analyzed tRNA^{Phe} purified from LCLs derived from a patient with an X-chromosome microdeletion of *FTSJ1* and *SLC38A5* (*FTSJ1Δ*, family 6; Table 1; Supp. Table S1) [Froyen et al., 2007] and from two brothers with a splice-site mutation in *FTSJ1* (c.121+1delG, *FTSJ1-ss*, family 3; Table 1; Supp. Table S1) [Freude et al., 2004], as well as from two control LCLs derived from healthy individuals. We found that tRNA^{Phe} purified from the *FTSJ1Δ* LCL had no detectable Cm or Gm modification (<0.03 moles/mole for each vs. 0.72–0.74 moles/mole for Cm and 0.69–0.74 moles/mole for Gm), and that tRNA^{Phe} from both of the *FTSJ1-ss* LCLs had undetectable Gm and a small, but detectable, amount of Cm (0.07 and 0.05 moles/mole, respectively), whereas levels of Ψ, m⁵C, m¹A, m⁷G, and m²G were similar to those from control LCLs [Table 2; Fig. 1B and C]. Thus, these data strongly indicate that *FTSJ1* is the homolog of yeast *TRM7* that is responsible for 2'-O-methylation of tRNA^{Phe} in humans, and link *FTSJ1*-associated NSXLID with tRNA modification defects.

We also found that *FTSJ1Δ* and *FTSJ1-ss* LCLs have reduced levels of the o2yW modification of m¹G₃₇ of tRNA^{Phe}. Thus, we observed an increase in m¹G on tRNA^{Phe} from LCLs with these *FTSJ1* mutations compared with control LCLs (0.65–0.74 moles/mole, compared with 0.15–0.42 moles/mole; Table 2; Fig. 1B and C), and a corresponding decrease in relative amounts of o2yW (to 0.16–0.23 compared with control values of 0.6 and 1.0; Table 2; Fig. 1D). This result implies that, as for yW₃₇ formation in both *S. cerevisiae* and *S. pombe* [Guy et al., 2012; Guy and Phizicky, 2015], o2yW₃₇ formation from m¹G is stimulated by Cm₃₂ and/or Gm₃₄ of tRNA^{Phe}.

We also analyzed Cm levels on tRNA^{Trp}, which has Cm₃₂ and Cm₃₄ in five of six characterized tRNA^{Trp} species from eukaryotes [Machnicka et al., 2013; Guy and Phizicky, 2015], including the mammal *Bos taurus* [Fournier et al., 1978] and is modified at both residues by Trm7 in *S. cerevisiae* [Pintard et al., 2002]. Since tRNA^{Trp} purified from the *FTSJ1Δ* LCL lacked Cm, whereas tRNA^{Trp} from a control LCL had substantial amounts of Cm (<0.03 vs. 1.06 moles/mole; Supp. Table S4), we infer that tRNA^{Trp} is modified by *FTSJ1* in humans.

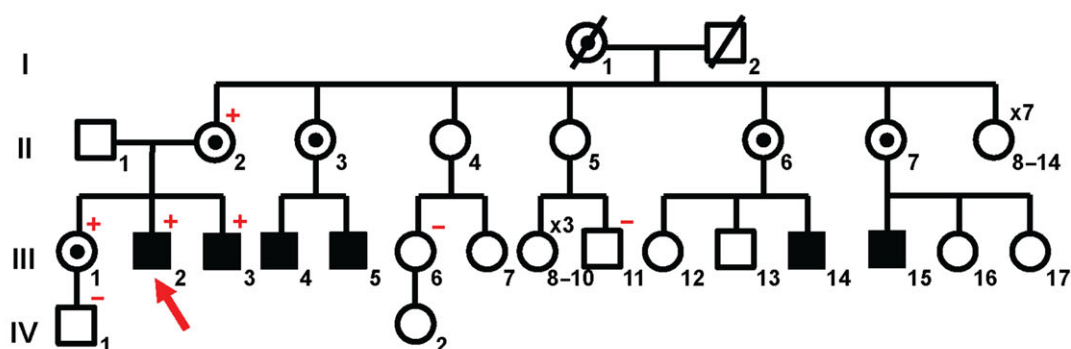
As in yeast [Guy et al., 2012], the lack of modification in LCL tRNAs that is due to *FTSJ1* mutations does not appear to affect the amounts or the charging levels of substrate tRNAs. Thus, levels of tRNA^{Phe} and tRNA^{Trp} from LCLs with *FTSJ1-ss* or *FTSJ1Δ* mutations appear to be normal relative to wild-type LCLs, as determined by Northern blot analysis (Supp. Fig. S1). Furthermore, lack of these modifications does not appear to affect tRNA charging in these cells, since there is as much or more charged tRNA^{Phe} and tRNA^{Trp} relative to uncharged tRNA in the mutant LCLs as in the wild-type LCLs (Supp. Fig. S1).

Identification of a Novel, Missense *FTSJ1* Variant c.76G>C; p.A26P in a Family with NSXLID

By investigation of the X-chromosome exome of a proband (III-2) from a large multigenerational family with NSXLID (Fig. 2A; Table 1; Supp. Table S1; family 7, see Materials and Methods and Hu et al., 2015), we identified a novel *FTSJ1* variant c.76G>C, p.A26P (RefSeq NM_012280.2). Subsequent sequencing analysis of six other available family members showed that the c.76G>C; p.A26P allele segregates with ID (Fig. 2A; Supp. Fig. S2). The c.76G>C; p.A26P allele is not currently present in the Exome Variant Server, ExAC Browser, or SNP database from the UCSC genome browser. This variant was submitted to the Leiden Open Variation Database (<http://www.lovd.nl/FTSJ1>).

Based on the fact that the *FTSJ1*-p.A26P allele was unique, segregated with ID in the pedigree, and is in a highly conserved region of the *FTSJ1*/Trm7 protein family (Fig. 2B), we predicted that this allele was deleterious to *FTSJ1* protein function, thus causing ID in this family, consistent with previous studies linking loss of function *FTSJ1* mutations and NSXLID [Freude et al., 2004; Ramser et al., 2004; Froyen et al., 2007; Takano et al., 2008]. Indeed, the *FTSJ1*-p.A26P variant might also be expected to have impaired methyltransferase activity because p.A26 of *FTSJ1* is only two residues from the predicted catalytic residue p.K28 in the same predicted α-helix, based on the structure of the FtsJ protein family member RrmJ (Fig. 2C) [Bugl et al., 2000; Pintard et al., 2002; Feder et al., 2003].

A



B

<i>H.sapiens</i>	1	---	MGRT	SKDKRD	VYYRL	LAKENG	WRARS	AFKLLQ	LDKEF	QLFQG
<i>S.cerevisiae</i>	1	---	MGKS	SKDKRD	IYYRK	AKEQGY	RARS	AFKLLQ	LNDFH	FLDD
<i>D.melanogaster</i>	1	---	MGKT	SKDKRD	IYYRQ	AKDEG	WRARS	AFKLLQ	HVDEA	YGILNG
<i>S.pombe</i>	1	---	MGRS	SKDKRD	AYYRL	AKEQGY	RARS	AFKLLQ	LNEQF	NLFEG
<i>A.thaliana</i>	1	---	MGKA	SRDKRD	IYYRK	AKEEG	WRARS	AFKLLQ	IDEEFN	IFEG
<i>T.thermophila</i>	1	---	MGKFT	KDKRD	IYYRK	AKEDC	FRARS	AYKLLQ	IDEVFG	IFEN
<i>D.discoideum</i>	1	---	MGKS	SKDKRD	IYYRK	AKEEG	WRARS	AFKLLQ	IDEEYQ	IFEG
<i>T.brucei</i>	1	---	MGTKS	KKAK	TRLDA	YYRLA	KDQGF	RARS	AFKLVQ	LNRYDFLSK

C

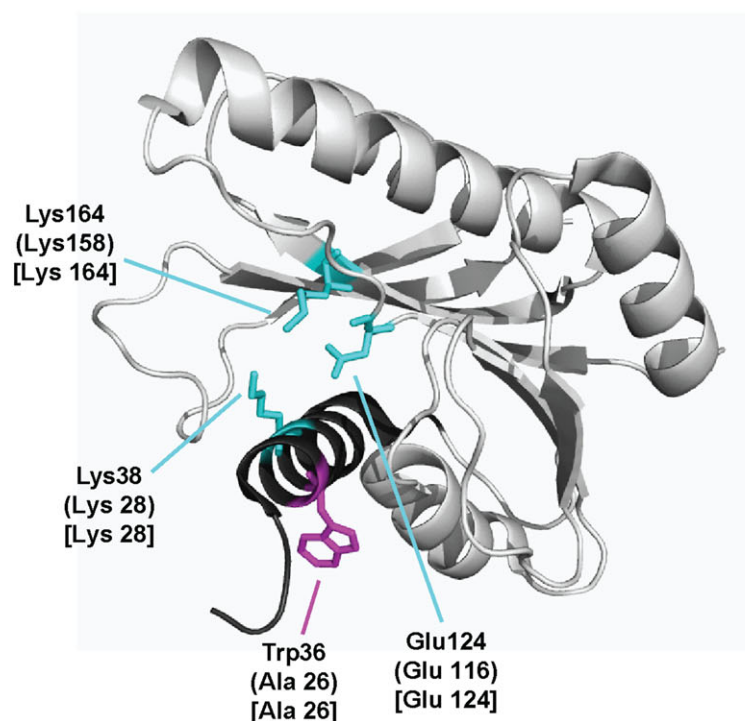


Figure 2. The FTSJ1-p.A26P variant is associated with NSXLID. **A:** Pedigree of family 7, with the FTSJ1-p.A26P allele. Filled square, affected male; circle with dot, known or obligate female carrier; open square, normal male; open circle, normal female. X-chromosome exome sequenced proband with the FTSJ1 NM_012280.2, c.76G>C, p.A26P variant is indicated by a red arrow. FTSJ1 genotype is indicated where DNA analysis was carried out. (+), c.76G>C; (–), wt. Nucleotide sequence is based on cDNA numbering. **B:** Amino acid sequence alignment of the N-terminal region of human FTSJ1 with known or predicted Trm7 proteins from diverse eukaryotes. Residue A26 is indicated by magenta arrow, and other residues analyzed in this study are indicated by black arrows. **C:** Predicted location of A26 residue of FTSJ1, based on the *Escherichia coli* RrmJ crystal structure. Representation of *E. coli* RrmJ (PDB 1EIZ), with catalytic triad residues in cyan, corresponding human FTSJ1 residues in round brackets, and corresponding *S. cerevisiae* residues in square brackets. Trp36 of RrmJ (corresponding to FTSJ1 p.A26) is magenta, and the predicted α -helix containing A26 (residues 33–46 of RrmJ) is dark gray.

The FTSJ1-p.A26P Variant Results in tRNA^{Phe} Lacking Only Gm₃₄

Consistent with a decrease in FTSJ1 activity, tRNA^{Phe} from an LCL derived from the proband III-2 (Fig. 2A) with the FTSJ1-p.A26P allele had undetectable Gm (Table 2; Fig. 1B and C); however, this tRNA^{Phe} had nearly normal Cm levels (0.63 moles/mole vs. 0.72–0.74 moles/mole in control LCLs with wild-type *FTSJ1*), and o2yW₃₇ levels (0.80 relative to 1.0 and 0.6 from two control LCLs; Table 2; Fig. 1D). The FTSJ1-p.A26P LCL was also defective for modification of tRNA^{Trp} with low, but detectable levels of Cm modification for the sample that was analyzed (0.15 vs. 1.06 moles/mole; Supp. Table S4), but comparable levels of the other measured tRNA^{Trp} modifications.

S. cerevisiae Trm7-A26P Forms Cm₃₂, but not Gm₃₄ on tRNA^{Phe} in Yeast Cells

To further examine the defect of the FTSJ1-p.A26P variant, we determined whether high-level expression of FTSJ1-p.A26P could complement the growth defect of an *S. cerevisiae* *trm7Δ* mutant, by evaluating growth of a *trm7Δ* [CEN *URA3* P_{TRM7}-*TRM7*] strain containing a [2μ *LEU2* P_{GAL}-*FTSJ1*-p.A26P] plasmid, after plating on medium containing 5-FOA and galactose to select against the [CEN *URA3* P_{TRM7}-*TRM7*] plasmid. Consistent with our earlier results, expression of wild-type *FTSJ1* from a strong promoter on a high copy plasmid complemented the slow growth phenotype of the *trm7Δ* mutant [Guy and Phizicky, 2015]; however, no complementation was observed upon expression of the FTSJ1-p.A26P variant, since growth on FOA was similar to that of the *trm7Δ* mutant bearing the vector or expressing the presumed catalytically dead variants FTSJ1-p.K28A or FTSJ1-p.K158A (Fig. 3A) [Feder et al., 2003]. We note, however, that levels of an affinity tagged FTSJ1-p.A26P-PT variant (for a description of the PT tag, see Supp. Methods) were reduced about 10-fold relative to those of FTSJ1-PT or the FTSJ1-p.K28A-PT variant (Supp. Fig. S3), suggesting that lack of complementation in yeast could be due, in part, to reduced protein levels.

However, we found that expression of *S. cerevisiae* *trm7-A26P* complements the growth defect of a *trm7Δ* mutant, with similar effects on tRNA^{Phe} modification as in the human FTSJ1-p.A26P LCL. Thus, a [CEN *LEU2* P_{TRM7}-*trm7-A26P*] plasmid fully complemented a *trm7Δ* mutant, whereas no complementation was observed with plasmids expressing the predicted catalytic dead variants *trm7-K28A* or *trm7-K164A* (Fig. 3A). Furthermore, tRNA^{Phe} from the *trm7Δ* [CEN *LEU2* P_{TRM7}-*trm7-A26P*] mutant had nearly normal levels of Cm₃₂ (0.82 moles/mole vs. 1.00 in *trm7Δ* [CEN *LEU2* P_{TRM7}-*TRM7*] cells; Table 3; Fig. 3B) but barely measurable Gm₃₄ (0.05 moles/mole vs. 0.81 in *trm7Δ* [CEN *LEU2* P_{TRM7}-*TRM7*] cells), almost exactly as observed for tRNA^{Phe} from the FTSJ1-p.A26P human cell line. As expected of catalytic dead mutants, tRNA^{Phe} from *trm7Δ* mutants expressing either *trm7-K28A* or *trm7-K164A* had no detectable Cm or Gm (Table 3; Fig. 3B); indeed, tRNA^{Phe} from a *trm7Δ* strain overproducing *trm7-K28A* on a [2μ P_{GAL}-*trm7-K28A*] plasmid still had no detectable Cm or Gm modifications. Consistent with the full modification of Cm₃₂ and the partial modification of Gm₃₄ of tRNA^{Phe} by Trm7-A26P, introduction of a [CEN *LEU2* P_{TRM7}-*trm7-A26P*] plasmid fully suppressed the slow growth of a *trm7Δ* *trm734Δ* mutant, but only partially suppressed the slow growth of a *trm7Δ* *trm732Δ* mutant over a range of temperatures from 18°C to 37°C (Fig. 3C).

To further characterize *in vivo* activity of the Trm7-A26P variant, we examined modifications of the other *S. cerevisiae* Trm7 sub-

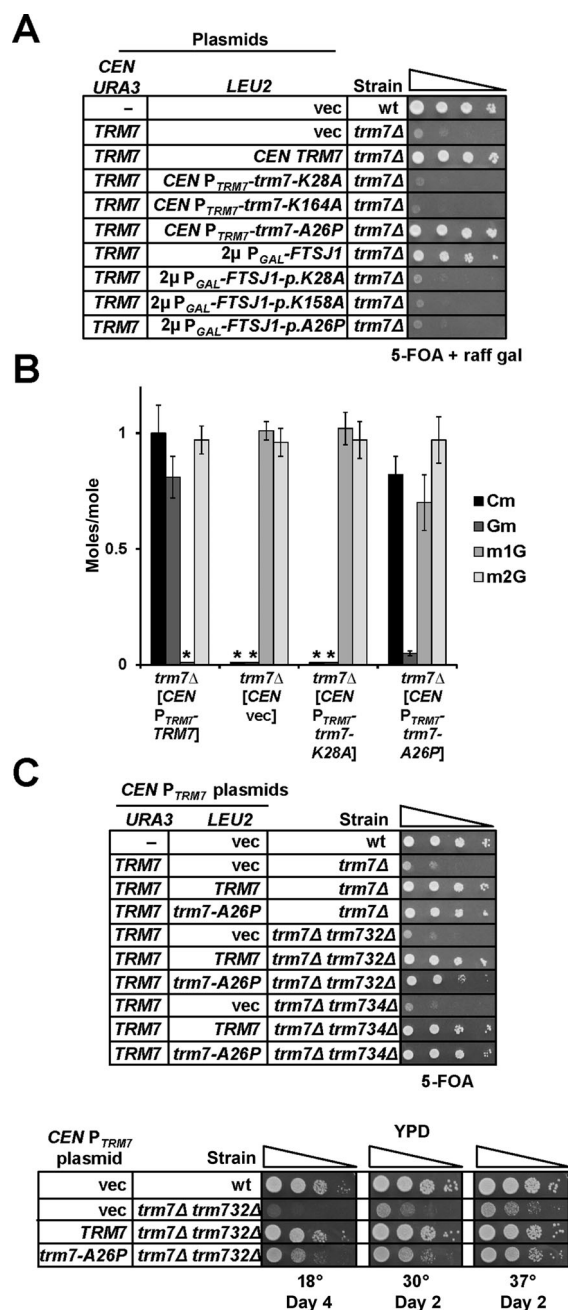


Figure 3. The *S. cerevisiae* Trm7-A26P variant is specifically defective for Gm₃₄ modification of tRNA^{Phe}. **A:** *trm7-A26P* suppresses the slow growth of *S. cerevisiae* *trm7Δ* mutants. Wild-type and *trm7Δ* [CEN *URA3* P_{TRM7}-*TRM7*] strains with [CEN *URA3* P_{TRM7}-*TRM7*] plasmids expressing *FTSJ1* or *TRM7* variants as indicated were grown overnight in S-Leu medium containing raffinose and galactose, diluted to OD₆₀₀ of ~0.5 in H₂O, and serially diluted 10-fold in H₂O, and then 2 μL was spotted onto S medium containing raffinose, galactose, and 5-FOA, followed by incubation for 3 days at 30°C. **B:** An *S. cerevisiae* *trm7Δ* mutant expressing *trm7-A26P* from a low copy (CEN) plasmid efficiently forms Cm₃₂, but not Gm₃₄ on tRNA^{Phe}. Quantification of nucleosides from tRNA^{Phe} isolated from indicated yeast strains. *, levels below threshold of detection. **C:** A CEN plasmid expressing *trm7-A26P* does not fully suppress the slow growth of *S. cerevisiae* *trm7Δ* *trm732Δ* mutants. Wild type, *trm7Δ*, *trm7Δ* *trm732Δ*, and *trm7Δ* *trm734Δ* strains with [CEN *URA3* P_{TRM7}-*TRM7*] and [CEN *URA3* P_{TRM7}-*trm7-A26P*] plasmids as indicated were grown overnight in SD-Leu, diluted, plated on SD-Leu medium containing 5-FOA, and incubated for 2 days at 30°C to select for loss of the *URA3* plasmid (top panel), followed by growth analysis on YPD at the indicated temperatures (bottom panel).

Table 3. HPLC Analysis of tRNA^{Phe} Nucleoside Content from an *S. Cerevisiae* *trm7*Δ Strain Expressing *TRM7* Variants

Modification		Cm	Gm	m ¹ G	Ψ	m ⁵ C	m ² G
Moles expected	Medium	1	1	0	2	2	1
Wild type [<i>CEN vec</i>]	SD-Leu	1.09 ± 0.23	0.85 ± 0.07	<0.03	2.08 ± 0.10	1.77 ± 0.06	0.95 ± 0.09
<i>trm7</i> Δ [<i>CEN P_{TRM7}-TRM7</i>]	SD-Leu	1.00 ± 0.12	0.81 ± 0.09	<0.03	2.04 ± 0.04	1.75 ± 0.06	0.97 ± 0.06
<i>trm7</i> Δ [<i>CEN vec</i>]	SD-Leu	<0.03	<0.03	1.01 ± 0.04	1.98 ± 0.10	1.78 ± 0.05	0.96 ± 0.06
<i>trm7</i> Δ [<i>CEN P_{TRM7}-trm7-K28A</i>]	SD-Leu	<0.03	<0.03	1.02 ± 0.07	2.1 ± 0.11	1.82 ± 0.03	0.97 ± 0.08
<i>trm7</i> Δ [<i>CEN P_{TRM7}-trm7-K164A</i>]	SD-Leu	<0.03	<0.03	1.04 ± 0.08	2.15 ± 0.08	1.83 ± 0.06	0.99 ± 0.09
<i>trm7</i> Δ [<i>CEN P_{TRM7}-trm7-A26P</i>]	SD-Leu	0.82 ± 0.08	0.05 ± 0.01	0.70 ± 0.12	2.01 ± 0.01	1.77 ± 0.04	0.97 ± 0.10
<i>trm7</i> Δ [<i>2μ P_{GAL}-trm7-K28A</i>]	S-Leu + raff gal	<0.03	<0.03	0.87 ± 0.04	2.13 ± 0.07	1.64 ± 0.07	0.83 ± 0.05
<i>trm7</i> Δ [<i>CEN P_{TRM7}-TRM7</i>]	S-Leu + raff gal	1.13 ± 0.06	0.88 ± 0.03	<0.03	2.1 ± 0.08	1.83 ± 0.04	1.0 ± 0.01
<i>trm7</i> Δ [<i>CEN P_{TRM7}-TRM7-MORF</i>]	S-Leu + raff gal	0.66 ± 0.13	0.50 ± 0.06	0.39 ± 0.06	2.06 ± 0.04	1.79 ± 0.03	0.96 ± 0.03
<i>trm7</i> Δ [<i>CEN P_{TRM7}-trm7-A26P</i>]	S-Leu + raff gal	0.75 ± 0.03	0.14 ± 0.04	0.49 ± 0.07	2.11 ± 0.02	1.79 ± 0.02	0.99 ± 0.02
<i>trm7</i> Δ [<i>CEN P_{TRM7}-trm7-A26P-MORF</i>]	S-Leu + raff gal	0.53 ± 0.10	<0.03	0.75 ± 0.05	2.19 ± 0.08	1.79 ± 0.03	1.01 ± 0.01
<i>trm7</i> Δ [<i>2μ P_{GAL}-TRM7</i>]	S-Leu + raff gal	0.98 ± 0.10	0.93 ± 0.05	<0.03	2.18 ± 0.02	1.85 ± 0.06	0.98 ± 0.02
<i>trm7</i> Δ [<i>2μ P_{GAL}-TRM7-PT</i>]	S-Leu + raff gal	0.89 ± 0.07	0.85 ± 0.04	<0.03	2.12 ± 0.05	1.86 ± 0.04	0.93 ± 0.03
<i>trm7</i> Δ [<i>2μ P_{GAL}-trm7-A26P</i>]	S-Leu + raff gal	0.93 ± 0.08	0.26 ± 0.01	0.38 ± 0.02	2.17 ± 0.03	1.85 ± 0.02	0.99 ± 0.02
<i>trm7</i> Δ [<i>2μ P_{GAL}-trm7-A26P-PT</i>]	S-Leu + raff gal	0.97 ± 0.12	<0.03	0.48 ± 0.08	2.16 ± 0.04	1.84 ± 0.02	1.01 ± 0.02

Mean and standard deviation based on three individual growths and RNA preparations.

strates, tRNA^{Trp} and tRNA^{Leu(UAA)} [Pintard et al., 2002]. Both Cm₃₂ and ncm⁵Um₃₄ of tRNA^{Leu(UAA)} were virtually undetectable in the *trm7*Δ [*CEN LEU2 P_{TRM7}-trm7-A26P*] mutant (Supp. Table S5). Similarly, both Cm₃₂ and Cm₃₄ of tRNA^{Trp} were severely reduced in the *trm7*Δ [*CEN LEU2 P_{TRM7}-trm7-A26P*] mutant (0.09 vs. 1.40 moles/mole in wild-type cells; Supp. Table S6), similar to the reduced Cm found on tRNA^{Trp} from FTSJ1-p.A26P LCLs (Supp. Table S4).

We note that the residual Cm found on tRNA^{Trp} in the *trm7*Δ [*CEN LEU2 P_{TRM7}-trm7-A26P*] mutant was likely at C₃₂, since similar Cm levels were found on tRNA^{Trp} from *trm7*Δ *trm734*Δ mutants, but no detectable Cm was found on tRNA^{Trp} from *trm7*Δ *trm732*Δ mutants (Supp. Table S6). This finding may suggest that the Trm7-A26P variant has retained some specificity for C₃₂ modification, while losing specificity for N₃₄ modification, regardless of base identity. As expected, the catalytic dead Trm7-K28A variant and the presumed catalytic dead Trm7-K164A variant lacked the ability to 2'-O-methylate tRNA^{Leu(UAA)} or tRNA^{Trp} (Supp. Tables S5 and S6). Our finding that the Trm7-A26P variant fully suppresses the growth defect of *trm7*Δ mutants, but only substantially modifies C₃₂ of tRNA^{Phe} (and not other tRNAs), is consistent with our previous findings that only Cm₃₂ or Gm₃₄ modification of tRNA^{Phe} is required for healthy growth in *S. cerevisiae* [Guy et al., 2012].

Overexpression of *S. Cerevisiae* Trm7-A26P Does Not Restore Full Gm₃₄ Modification Levels on tRNA^{Phe}

Although expression of Trm7-A26P on a [*CEN LEU2 P_{TRM7}-trm7-A26P*] plasmid fully complemented the growth defect of a *trm7*Δ mutant, expression levels of the corresponding C-terminally affinity-tagged Trm7-A26P-MORF variant (for a description of the MORF tag, see Materials and Methods) were reduced to ~1/3 the levels observed from corresponding strains expressing wild-type Trm7-MORF or the Trm7-K28A-MORF variant (Supp. Fig. S4). Thus, it was possible that the selective loss of Gm₃₄ on tRNA^{Phe} in the *trm7*Δ [*CEN LEU2 P_{TRM7}-trm7-A26P*] mutant was due to reduced Trm7-A26P. Alternatively, it might be due to reduced interaction of Trm7-A26P with Trm734, since Trm7 forms a distinct complex with Trm734, and both proteins are required for Nm₃₄ formation in *S. cerevisiae* [Guy et al., 2012].

To test whether there was a reduced interaction between Trm7-A26P and Trm734, we transformed *CEN* plasmids expressing *TRM7-MORF* or *trm7-A26P-MORF* into *TRM732-9myc* or

TRM734-9myc yeast strains, and then purified the Trm7 using the tag. Both Trm732-9myc and Trm734-9myc copurified with Trm7-MORF and with Trm7-A26P-MORF with comparable efficiency, after taking into account the reduced expression of Trm7-A26P (Fig. 4A and B).

To further probe the selective loss of Gm₃₄ on tRNA^{Phe} in the *trm7*Δ strain expressing *trm7-A26P*, we overexpressed Trm7-A26P and examined the interaction with Trm734 and Gm levels of tRNA^{Phe}. Immunoblot analysis against the ZZ domain of protein A demonstrated that expression of Trm7-A26P-PT (the PT tag has identical components as the MORF tag, see Supp. Methods) from a 2μ *P_{GAL}* plasmid ([2μ *URA3 P_{GAL}-trm7-A26P-PT*]) resulted in more than a 125-fold increase in Trm7-A26P levels, compared with wild-type Trm7-MORF expressed from a [*CEN URA3 P_{TRM7}-TRM7-MORF*] plasmid (Fig. 4C). Furthermore, purification of overexpressed Trm7-A26P-PT resulted in pulldown of ~10-fold more Trm734-9myc than was pulled down during purification of Trm7-MORF expressed from the [*CEN URA3 P_{TRM7}-TRM7-MORF*] plasmid. However, despite the >125-fold overexpression and the ~10-fold increase in Trm734 pull down, Gm₃₄ formation on tRNA^{Phe} was not fully restored by overexpression of either tagged Trm7-A26P from the [2μ *LEU2 P_{GAL}-TRM7-A26P-PT*] plasmid or untagged Trm7-A26P from the otherwise identical [2μ *LEU2 P_{GAL}-TRM7-A26P*] plasmid (Table 3). Indeed, under these conditions, overexpression of untagged Trm7-A26P in the *trm7*Δ strain resulted in only 0.26 moles/mole Gm on tRNA^{Phe}. Consistent with this finding, overexpression of *trm7-A26P* also failed to fully suppress the growth defect of a *trm7*Δ *trm732*Δ mutant (Fig. 4D).

We note that the C-terminal tag of Trm7-PT or Trm7-MORF appears to interfere with Gm formation, since Gm modification of tRNA^{Phe} was reduced from 0.88 to 0.50 moles/mole in a *trm7*Δ [*CEN LEU2 P_{TRM7}-TRM7-MORF*] strain relative to a *trm7*Δ [*CEN P_{TRM7}-LEU2 TRM7*] strain (Table 3), and similar results were observed when *TRM7* had a C-terminal c-myc tag (data not shown). This loss of Gm modification is even more extreme in the corresponding strains expressing Trm7-A26P-MORF or Trm7-A26P-PT. Nonetheless, we conclude that the loss of Gm₃₄ modification activity in the Trm7-A26P variant is not due to a Trm734 binding defect, since overexpression of Trm7-A26P-PT results in more pulldown of Trm734 than occurs with wild-type Trm7-MORF expressed from a *CEN* plasmid, whereas overexpression of even untagged Trm7-A26P (presumably resulting in the same or more Trm7 than with tagged Trm7) does not fully rescue Gm₃₄ modification of tRNA^{Phe} (Table 3).

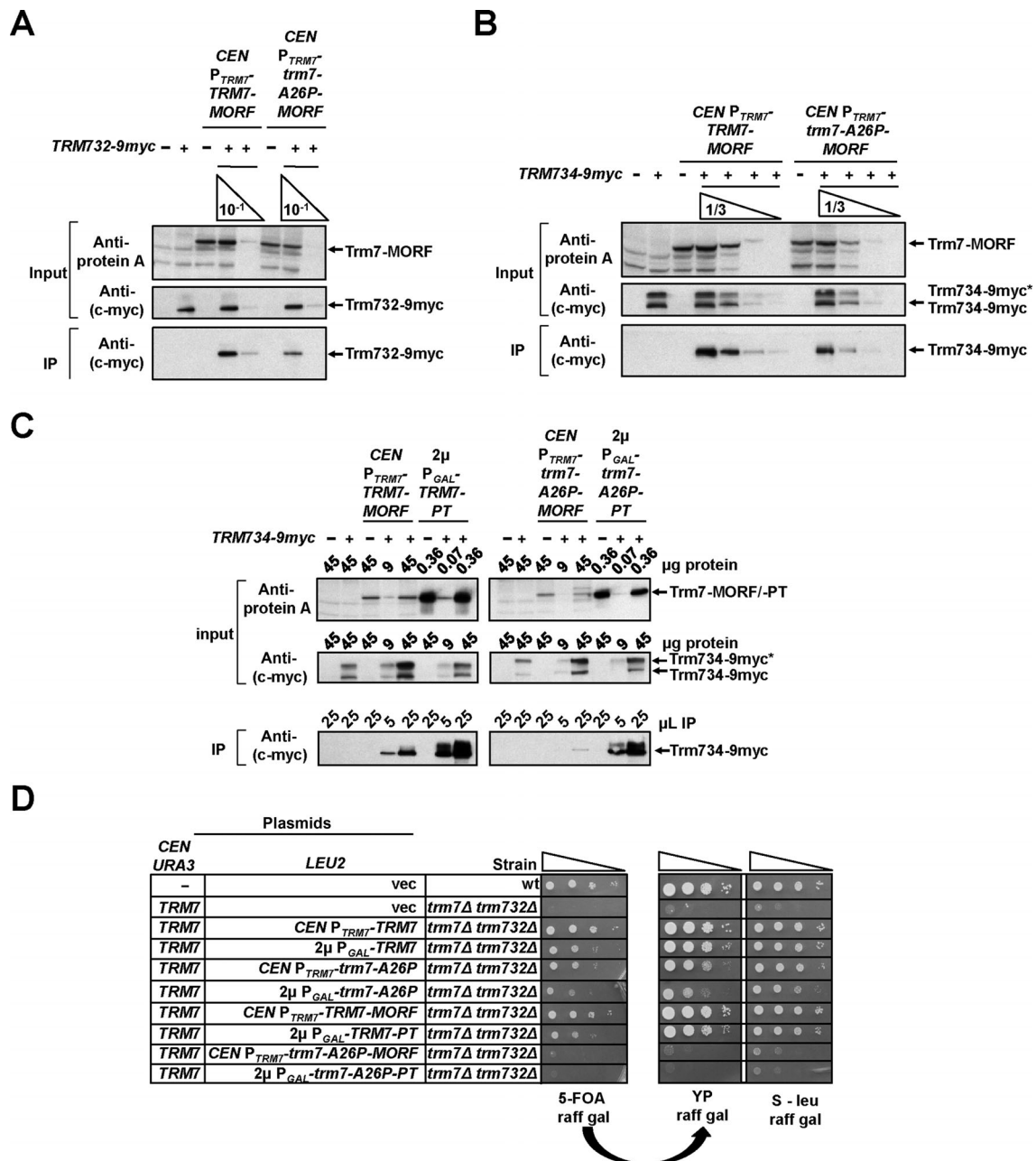


Figure 4. The defect in *S. cerevisiae* Trm7-A26P function is not due to reduced expression or reduced Trm734 binding. **A:** Trm7-A26P-MORF expressed from a *CEN* plasmid efficiently forms a complex with chromosomally expressed Trm732-9myc. Crude extracts were prepared from the indicated strains grown in SD-Leu medium, and proteins were purified with IgG-Sepharose beads (which binds the ZZ domain of the MORF tag), followed by treatment with 3C protease, as described in *Materials and Methods*, and then samples were analyzed by SDS-PAGE and immunoblot analysis as indicated. **B:** Trm7-A26P-MORF expressed from a *CEN* plasmid forms a complex with chromosomally expressed Trm734-9myc. Samples were analyzed as in panel **A**. **C:** Trm7-A26P-PT expressed from a high copy (2 μ) plasmid forms more Trm7-A26P:Trm734-9myc complex than the corresponding Trm7:Trm734-9myc complex in wild-type cells. Indicated samples grown in S-Leu medium containing raffinose and galactose were analyzed as in panel **A**. **D:** Overexpressed *TRM7-A26P* does not fully suppress the slow growth of *S. cerevisiae* *trm7Δ trm732Δ* mutants. Wild-type and *trm7Δ trm732Δ* strains with plasmids as indicated were grown overnight in S-Leu medium containing raffinose and galactose as in Figure 3A, and spotted to the indicated media.

Overexpression of untagged Trm7-A26P also appeared to result in a modest but limited increase in Cm levels on tRNA^{Trp} purified from *trm7Δ* mutants (from 0.13 to 0.31 moles/mole; Supp. Table S7), and modest increases in Cm and ncm⁵Um levels in tRNA^{Leu(UAA)} (Supp. Table S8). Thus, all modification defects associated with normal expression of Trm7-A26P appear to persist even when Trm7-A26P is massively overproduced.

Other Mutations in the Predicted α-Helix Bearing A26 of Trm7 Result in tRNA^{Phe} Specifically Lacking Gm₃₄

We investigated the roles of other Trm7 residues in the region near residue A26 because of its proximity to the catalytic residue K28 and because of the intriguing observation that Trm7-A26P efficiently forms Cm₃₂, but not Gm₃₄, on tRNA^{Phe}. We constructed

[*CEN LEU2 P_{TRM7}-TRM7*] plasmids expressing Trm7 variants bearing other amino acid substitutions at A26, and substitutions in the predicted α -helix bearing A26 (which encompasses residues 23–36, based on *E. coli* RrmJ [Bugl et al., 2000]), and then tested their ability to suppress the growth defects of *trm7* Δ , *trm7* Δ *trm732* Δ , and *trm7* Δ *trm734* Δ mutants.

We found that expression of the *trm7*-A26W or *trm7*-A26G variant resulted in complete complementation of the growth defect in all three strain backgrounds, whereas expression of *trm7*-A26E resulted in a distinct growth defect only in the *trm7* Δ *trm732* Δ strain at 30°C (Fig. 5A), similar to the phenotype resulting from the *trm7*-A26P variant, although there was also a minor growth defect in the *trm7* Δ and *trm7* Δ *trm734* Δ backgrounds that was more obvious at 18°C. Consistent with this result, we found that tRNA^{Phe} from the *trm7* Δ [*CEN LEU2 P_{TRM7}-trm7-A26E*] strain had undetectable Gm levels and about 50% of the normal Cm levels (0.54 vs. 1.00 moles/mole), whereas Cm and Gm of tRNA^{Phe} were at near normal levels in the *trm7* Δ [*CEN LEU2 P_{TRM7}-trm7A26W*] strain (1.03 and 0.54 moles/mole, respectively, compared with 1.0 and 0.81 moles/mole in wild type; Supp. Table S9).

Similar to our observations with the *trm7*-A26P and *trm7*-A26E variants, we found that expression of a *trm7*-L32P variant resulted in a growth defect specifically in the *trm7* Δ *trm732* Δ strain, and not in the *trm7* Δ or *trm7* Δ *trm734* Δ strains (Fig. 5A), and that tRNA^{Phe} from the *trm7* Δ [*CEN LEU2 P_{TRM7}-trm7-L32P*] strain had barely detectable Gm (0.03 moles/mole) but nearly normal levels of Cm (0.90 moles/mole) (Supp. Table S9). By contrast, expression of the *trm7*-L32A variant complemented the growth defect of all three strains and yielded normal tRNA^{Phe} modifications (Fig. 5A; Supp. Table S9), whereas expression of *trm7*-A23P, *trm7*-R24A, or *trm7*-R24P variants resulted in no complementation in any strain background, suggesting lack of function for these variants.

Thus, these results demonstrate that Trm7 variants with other mutations at A26 or elsewhere in the helix specifically lose the ability to form Gm₃₄ on tRNA^{Phe} while retaining Cm₃₂ modification ability.

Discussion

In this report, we have provided strong evidence that human FTSJ1 is required for Cm₃₂ and Gm₃₄ modification of tRNA^{Phe}, since these tRNA^{Phe} modifications were undetectable, or drastically reduced, in each of three LCLs derived from NSXLID patients with two distinct *FTSJ1* loss of function mutations, whereas tRNA^{Phe} from each of two control cell lines derived from healthy individuals with wild-type *FTSJ1* had essentially normal Cm and Gm levels. Since we also observed parallel selective loss of Gm in tRNA^{Phe} from the *FTSJ1*-p.A26P LCLs and from the *S. cerevisiae* *trm7*-A26P mutant (Fig. 5B), and since expression of *FTSJ1* complements the growth defect of an *S. cerevisiae* *trm7* Δ mutant by forming Cm₃₂ on tRNA^{Phe} [Guy and Phizicky, 2015], we conclude that FTSJ1 catalyzes formation of Cm and Gm on tRNA^{Phe} in human cells. Furthermore, since *FTSJ1* mutant alleles segregate with NSXLID in each of the previously reported families from which the LCLs were derived [Freude et al., 2004; Froyen et al., 2007], in the new family with the p.A26P variant reported here, as well as in four other reported families [Freude et al., 2004; Ramser et al., 2004; Takano et al., 2008] and likely in an additional family [Hu et al., 2015], we conclude further that defective 2'-O-methylation of N₃₂ and N₃₄ of the anticodon loop of substrate tRNAs is a major contributing factor in *FTSJ1*-associated NSXLID.

FTSJ1 mutations might contribute to NSXLID through lack of modification of any *FTSJ1* tRNA substrate. Indeed, we provided

evidence that FTSJ1 modifies tRNA^{Trp} in LCLs, and Cm modification of this tRNA was severely reduced in *FTSJ1*-p.A26P LCLs. Moreover, it is possible that FTSJ1 modifies any of the four other documented human tRNA species with Nm₃₂ and/or Nm₃₄ [Machnicka et al., 2013], or any of the numerous uncharacterized tRNAs, and hypomodification of any of these tRNAs could contribute to ID. However, we speculate that defective 2'-O-methylation of tRNA^{Phe} may play a major role in ID, for two reasons. First, reduced function of tRNA^{Phe} is the major cause of the slow growth defect of *S. cerevisiae* and *S. pombe* *trm7* Δ mutants [Guy et al., 2012; Guy and Phizicky, 2015]. Second, the tRNA^{Phe} anticodon loop 2'-O-methylations are very highly conserved, since each of the 17 eukaryotic tRNA^{Phe} species that have been characterized has Cm₃₂, and 16 of the 17 also have Gm₃₄ [Machnicka et al., 2013]. If lack of tRNA^{Phe} modification by FTSJ1 is a major contributor to NSXLID, then it would follow that the defective tRNA^{Phe} would primarily be due to lack of Gm₃₄, because levels of both Cm₃₂ and o₂yW₃₇ are normal in the *FTSJ1*-p.A26P LCL. This importance of Gm₃₄ of tRNA^{Phe} would be consistent with the finding in *S. pombe* that Gm₃₄ of tRNA^{Phe} is much more important for function than Cm₃₂, as measured by growth phenotypes of mutants [Guy and Phizicky, 2015]. We note that NSXLID patients with the *FTSJ1*-p.A26P mutation appear to have ID comparable to that of other *FTSJ1*-associated NSXLID patients (Supp. Table S1), although there have not been any direct, objective comparisons of the extent of ID for each of the NSXLID patients.

These results also underscore the conservation of the intricate circuitry for anticodon loop modification of tRNA^{Phe}, as tRNA^{Phe} from LCLs of patients with splice site and null alleles of *FTSJ1* had significantly reduced levels of o₂yW₃₇, compared with control LCLs, accompanied by increased levels of the o₂yW precursor m¹G. This result suggests that the o₂yW₃₇ modification may be dependent on Cm₃₂ and Gm₃₄ modification, as is the yW₃₇ modification in *S. cerevisiae* and *S. pombe* [Guy et al., 2012; Guy and Phizicky, 2015]. However, unlike in these yeast species, lack of *FTSJ1* in human LCLs did not completely abrogate o₂yW modification of tRNA^{Phe}, lack of Gm₃₄ did not appear to affect o₂yW₃₇ levels, and control LCLs had variable levels of o₂yW, accompanied by correspondingly variable m¹G levels. The apparent dependence of o₂yW₃₇ on Cm₃₂ and Gm₃₄ is consistent with previous observations that tRNA^{Phe} from neuroblastoma cells and a fraction of tRNA^{Phe} from Ehrlich ascites cell lines lack Cm, Gm, and o₂yW [Kuchino et al., 1982], although it is not clear why tRNA^{Phe} was affected in these cell lines.

It is intriguing that both human *FTSJ1*-p.A26P and yeast *Trm7*-A26P are specifically defective in Gm₃₄ modification of tRNA^{Phe}, while retaining normal levels of Cm₃₂ modification. Since our data indicate that overproduction of Trm7 by more than 125-fold can rescue the minor defect in Trm734 binding but not the Gm modification defect, this result suggests that the Trm7-A26P defect is due to some specific aspect of substrate recognition or catalysis for Gm₃₄ modification. This interpretation is consistent with the location of residue 26 near the catalytic residue K28 [Pintard et al., 2002; Feder et al., 2003]. Alternatively, G₃₄ of tRNA^{Phe} may simply be more difficult to modify than C₃₂, and the p.A26P mutation might cause a general loss in methylation activity. This interpretation is consistent with the observation that tRNA^{Phe} from *FTSJ1*-ss LCLs have distinct but low levels of Cm and no detectable Gm, perhaps suggesting a low level of correct mRNA splicing [Freude et al., 2004]. Moreover, Cm₃₂ of tRNA^{Phe} appears to be the most efficient Trm7 modification target in yeast since overproduction of the Trm7-A26P variant only results in partial modification of C₃₂ and N₃₄ of tRNA^{Trp} and tRNA^{Leu(UAA)}, whereas C₃₂ of tRNA^{Phe} is normally modified even when Trm7-A26P is expressed at normal levels. The observation

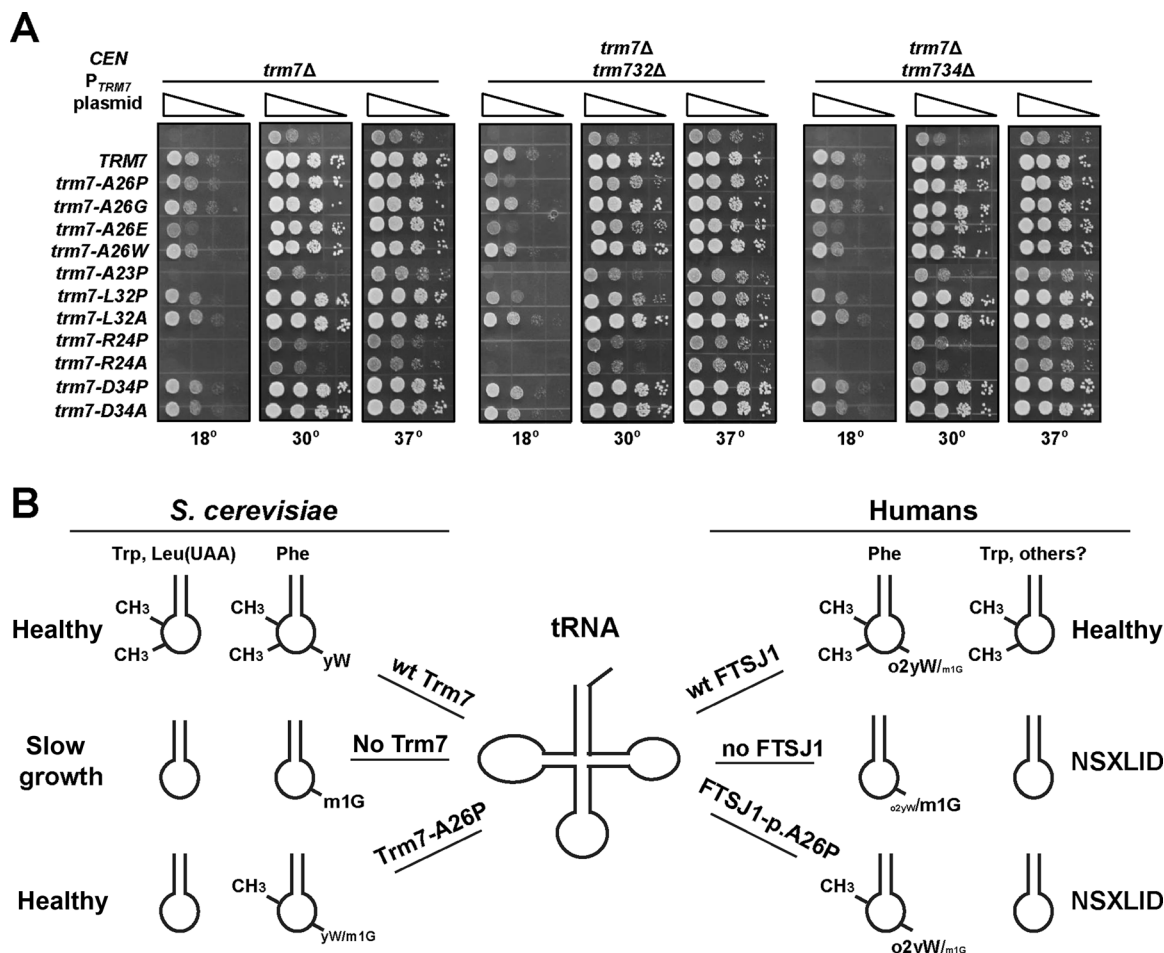


Figure 5. Genetic analysis of Trm7 variant function in *S. cerevisiae*. **A:** Growth assay for Trm7 variant function. *trm7Δ*, *trm7Δ trm732Δ*, and *trm7Δ trm734Δ* strains expressing CEN *trm7* variants as indicated were grown overnight in SD-Leu, diluted, spotted on YPD medium, and incubated at the indicated temperatures. **B:** Schematic of phenotypes associated with loss of FTSJ1/Trm7 activity in humans and yeast. Relative size of yW/o2yW/m¹G corresponds to levels of these modifications on residue 37 of tRNA^{Phe}.

that yeast *trm7Δ* mutants expressing Trm7-A26E or Trm7-L32P at normal levels also selectively modify C₃₂, but not G₃₄, of tRNA^{Phe} emphasizes the sensitivity of the crucial Gm₃₄ modification to perturbations in this helix, but leaves unresolved the mechanism by which this occurs.

Since *FTSJ1*-associated NSXLID patients have no consistent dysmorphic, metabolic, or neuromuscular manifestations of the condition other than ID, it would follow that the functional levels of substrate tRNAs are insufficient for brain development and/or cognitive function, but are sufficient in other tissues and other aspects of development. Although little is known about the differential expression of the 450 tRNA genes in the human genome, comprising ~270 isodecoders (which share an anticodon but have a different tRNA body) [Goodenbour and Pan, 2006; Chan and Lowe, 2009], there is evidence that an isodecoder can be specifically expressed in the central nervous system [Ishimura et al., 2014]. A role for *FTSJ1* in neurological development is consistent with the observation that expression of *FTSJ1* mRNA in the fetus is highest in the brain compared with other tissues tested [Freude et al., 2004].

The linkage between tRNA modifications and NSXLID reported here is part of an emerging theme linking tRNA modifications and neurological function. Previous studies have directly linked defective m⁵C modification to a Dubowitz-like syndrome [Martinez et al.,

2012; Blanco et al., 2014], and defective cm⁵U modification to FD [Anderson et al., 2001; Karlsborn et al., 2014], and lesions in four other predicted or likely tRNA modification genes have been linked to neurological function. Thus, lesions in the *TRMT10A* gene, a homolog of yeast TRM10 [Jackman et al., 2003], are linked to short stature, microcephaly, and defects in glucose homeostasis and diabetes, and the purified protein corresponding to a missense allele lacks tRNA m¹G₉ methyltransferase activity [Igoillo-Esteve et al., 2013; Gillis et al., 2014]. Additionally, a missense allele of human *ADAT3*, the likely homolog of yeast *TAD3*, required for deamination of A₃₄ to I₃₄ [Gerber and Keller, 1999], is linked to strabismus and ARID [Alazami et al., 2013]; a frameshift mutation in human *TRMT1*, the homolog of yeast *TRM1*, required for m^{2,2}G₂₆ activity [Liu and Straby, 2000], is linked to ARID [Najmabadi et al., 2011]; and two distinct missense alleles of human *ELP2*, a member of the ELP complex [Hawkes et al., 2002; Huang et al., 2005], are linked to ARID [Najmabadi et al., 2011]. In addition, allelic variants in the human *ELP3* gene have been linked by genome-wide association studies to amyotrophic lateral sclerosis, and *Elp3* mutants were identified in an accompanying screen for defective neuronal function in *Drosophila* [Simpson et al., 2009]. We note also that tRNA mutations leading to defects in taurine modifications are implicated in MELAS (mitochondrial encephalomyopathy, lactic acidosis, and

stroke-like episodes) and MERFF (myoclonic epilepsy with ragged-red fibers) [Yasukawa et al., 2000a; Yasukawa et al., 2000b; Suzuki et al., 2002], whereas a *TRIT1* mutation resulting in reduced i⁶A modification is linked to myoclonic epilepsy associated with encephalopathy [Yarham et al., 2014].

Since lack of modifications is often overcome by increased dosage of one or more of the unmodified tRNAs [Esberg et al., 2006; Phizicky and Alfonzo, 2010; Guy et al., 2012; Fernandez-Vazquez et al., 2013; Han et al., 2015], the numerous links between tRNA modifications and neurological defects suggest that the available pool of functional tRNAs may somehow be limited during development and function of the central nervous system, presumably leading to defects in translation or its regulation [Begley et al., 2007; Chan et al., 2012]. The specific mechanisms by which defects in tRNA biology impact neurological function remain to be determined.

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References

- Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, Wiczorek D, Motazacker MM, Esmaeli-Nieh S, Cremer K. 2012. Mutations in NSUN2 cause autosomal-recessive intellectual disability. *Am J Hum Genet* 90:847–855.
- Agris PF, Vendeix FA, Graham WD. 2007. tRNA's wobble decoding of the genome: 40 years of modification. *J Mol Biol* 366:1–13.
- Alazami AM, Hijazi H, Al-Dosari MS, Shaheen R, Hashem A, Aldahmesh MA, Mohamed JY, Kentab A, Salih MA, Awaji A. 2013. Mutation in ADAT3, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. *J Med Genet* 50:425–430.
- Alexandrov A, Chernyakov I, Gu W, Hiley SL, Hughes TR, Grayhack EJ, Phizicky EM. 2006. Rapid tRNA decay can result from lack of nonessential modifications. *Mol Cell* 21:87–96.
- Anderson SL, Coli R, Daly IW, Kichula EA, Rork MJ, Volpi SA, Ekstein J, Rubin BY. 2001. Familial dysautonomia is caused by mutations of the IKAP gene. *Am J Hum Genet* 68:753–758.
- Begley U, Dyavaiah M, Patil A, Rooney JP, DiRenzo D, Young CM, Conklin DS, Zitomer RS, Begley TJ. 2007. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Mol Cell* 28:860–870.
- Bekaert M, Rousset JP. 2005. An extended signal involved in eukaryotic -1 frameshifting operates through modification of the E site tRNA. *Mol Cell* 17:61–68.
- Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M. 2014. Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO J* 33:2020–2039.
- Blanco S, Kurowski A, Nichols J, Watt FM, Benitah SA, Frye M. 2011. The RNA-methyltransferase Misu (NSun2) poises epidermal stem cells to differentiate. *PLoS Genet* 7:e1002403.
- Brzezicha B, Schmidt M, Makalowska I, Jarmolowski A, Pienkowska J, Szwejkowska-Kulinska Z. 2006. Identification of human tRNA:m⁵C methyltransferase catalysing intron-dependent m⁵C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). *Nucleic Acids Res* 34:6034–6043.
- Bugl H, Fauman EB, Staker BL, Zheng F, Kushner SR, Saper MA, Bardwell JC, Jakob U. 2000. RNA methylation under heat shock control. *Mol Cell* 6:349–360.
- Chan CT, Pang YL, Deng W, Babu IR, Dyavaiah M, Begley TJ, Dedon PC. 2012. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* 3:937.
- Chan PP, Lowe TM. 2009. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res* 37:D93–D97.
- Chernyakov I, Whipple JM, Kotlawa L, Grayhack EJ, Phizicky EM. 2008. Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. *Genes Dev* 22:1369–1380.
- Cuajungco MP, Leyne M, Mull J, Gill SP, Lu W, Zagzag D, Axelrod FB, Maayan C, Gusella JF, Slaugenhaupt SA. 2003. Tissue-specific reduction in splicing efficiency of IKBKAP due to the major mutation associated with familial dysautonomia. *Am J Hum Genet* 72:749–758.
- de Crecy-Lagard V, Marck C, Grosjean H. 2012. Decoding in *Candidatus Riesia pediculus*, close to a minimal tRNA modification set? *Trends Cell Mol Biol* 7:11–34.
- Dong J, Edelmann L, Bajwa AM, Kornreich R, Desnick RJ. 2002. Familial dysautonomia: detection of the IKBKAP IVS20(+6T → C) and R696P mutations and frequencies among Ashkenazi Jews. *Am J Med Genet* 110:253–257.
- Esberg A, Huang B, Johansson MJ, Bystrom AS. 2006. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell* 24:139–148.
- Fahiminiya S, Almuriekh M, Nawaz Z, Staffa A, Lepage P, Ali R, Hashim L, Schwartzentruber J, Abu Khadija K, Zaineddin S. 2014. Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. *Clin Genet* 86:134–141.
- Feder M, Pas J, Wyrwicz LS, Bujnicki JM. 2003. Molecular phylogenetics of the RrmJ/fibrillarin superfamily of ribose 2'-O-methyltransferases. *Gene* 302:129–138.
- Fernandez-Vazquez J, Vargas-Perez I, Sanso M, Buhne K, Carmona M, Paulo E, Hermand D, Rodriguez-Gabriel M, Ayte J, Leidel S. 2013. Modification of tRNA(Lys) UUU by elongator is essential for efficient translation of stress mRNAs. *PLoS Genet* 9:e1003647.
- Fournier M, Labouesse J, Dirheimer G, Fix C, Keith G. 1978. Primary structure of bovine liver tRNA^{Trp}. *Biochim Biophys Acta* 521:198–208.
- Freude K, Hoffmann K, Jensen LR, Delatycki MB, des Portes V, Moser B, Hamel B, vanBokhoven H, Moraine C, Fryns JP. 2004. Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. *Am J Hum Genet* 75:305–309.
- Froyen G, Bauters M, Boyle J, Van Esch H, Govaerts K, van Bokhoven H, Ropers HH, Moraine C, Chelly J, Fryns JP. 2007. Loss of SLC38A5 and FTSJ1 at Xp11.23 in three brothers with non-syndromic mental retardation due to a microdeletion in an unstable genomic region. *Hum Genet* 121:539–547.
- Gerber AP, Keller W. 1999. An adenosine deaminase that generates inosine at the wobble position of tRNAs. *Science* 286:1146–1149.
- Gillis D, Krishnamohan A, Yaacov B, Shaag A, Jackman JE, Elpeleg O. 2014. TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. *J Med Genet* 51:581–586.
- Goodenbour JM, Pan T. 2006. Diversity of tRNA genes in eukaryotes. *Nucleic Acids Res* 34:6137–6146.
- Guy MP, Phizicky EM. 2015. Conservation of an intricate circuit for crucial modifications of the tRNA^{Phe} anticodon loop in eukaryotes. *RNA* 21:61–74.
- Guy MP, Podyma BM, Preston MA, Shaheen HH, Krivos KL, Limbach PA, Hopper AK, Phizicky EM. 2012. Yeast Trm7 interacts with distinct proteins for critical modifications of the tRNA^{Phe} anticodon loop. *RNA* 18:1921–1933.
- Hall KB, Sampson JR, Uhlenbeck OC, Redfield AG. 1989. Structure of an unmodified tRNA molecule. *Biochemistry* 28:5794–5801.
- Han L, Kon Y, Phizicky EM. 2015. Functional importance of Psi38 and Psi39 in distinct tRNAs, amplified for tRNA^{Gln}(UUG) by unexpected temperature sensitivity of the s2U modification in yeast. *RNA* 21:188–201.
- Hawkes NA, Otero G, Winkler GS, Marshall N, Dahmus ME, Krappmann D, Scheidert C, Thomas CL, Schiavo G, Erdjument-Bromage H. 2002. Purification and characterization of the human elongator complex. *J Biol Chem* 277:3047–3052.
- Helm M, Giege R, Florentz C. 1999. A Watson-Crick base-pair-disrupting methyl group (m1A9) is sufficient for cloverleaf folding of human mitochondrial tRNA^{Lys}. *Biochemistry* 38:13338–13346.
- Hopper AK. 2013. Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast *Saccharomyces cerevisiae*. *Genetics* 194:43–67.
- Hu H, Haas SA, Chelly J, Van Esch H, Raynaud M, de Brouwer AP, Weinert S, Froyen G, Frints SG, Laumonnier F, Zemojtel T, Love MI, et al. 2015. X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. *Mol Psychiatry*. [Epub ahead of print]
- Huang B, Johansson MJ, Bystrom AS. 2005. An early step in wobble uridine tRNA modification requires the Elongator complex. *RNA* 11:424–436.
- Igoillo-Esteve M, Genin A, Lambert N, Desir J, Pirson I, Abdulkarim B, Simonis N, Drielsma A, Marselli L, Marchetti P. 2013. tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. *PLoS Genet* 9:e1003888.
- Ishimura R, Nagy G, Dotu I, Zhou H, Yang XL, Schimmel P, Senju S, Nishimura Y, Chuang JH, Ackerman SL. 2014. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science* 345:455–459.
- Jackman JE, Montange RK, Malik HS, Phizicky EM. 2003. Identification of the yeast gene encoding the tRNA m1G methyltransferase responsible for modification at position 9. *RNA* 9:574–585.
- Kadaba S, Krueger A, Trice T, Krecic AM, Hinnebusch AG, Anderson J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNA^{Met} in *S. cerevisiae*. *Genes Dev* 18:1227–1240.

- Kadaba S, Wang X, Anderson JT. 2006. Nuclear RNA surveillance in *Saccharomyces cerevisiae*: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. *RNA* 12:508–521.
- Karlsborn T, Tukenmez H, Chen C, Bystrom AS. 2014. Familial dysautonomia (FD) patients have reduced levels of the modified wobble nucleoside mcm5s2U in tRNA. *Biochem Biophys Res Commun* 454:441–445.
- Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, Vincent AK, Malli R, Ali G, Khan FS. 2012. Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. *Am J Hum Genet* 90:856–863.
- Kuchino Y, Borek E, Grunberger D, Mushinski JF, Nishimura S. 1982. Changes of post-transcriptional modification of wye base in tumor-specific tRNAPhe. *Nucleic Acids Res* 10:6421–6432.
- LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D. 2005. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* 121:713–724.
- Liu J, Straby KB. 2000. The human tRNA(m(2)(2)G(26))dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. *Nucleic Acids Res* 28:3445–3451.
- Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM. 2013. MOD-OMICS: a database of RNA modification pathways–2013 update. *Nucleic Acids Res* 41:D262–D267.
- Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, Frye M, Al-Gazali L, Gleeson JG. 2012. Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. *J Med Genet* 49:380–385.
- Motorin Y, Grosjean H. 1999. Multisite-specific tRNA:m5C-methyltransferase (Trm4) in yeast *Saccharomyces cerevisiae*: identification of the gene and substrate specificity of the enzyme. *RNA* 5:1105–1118.
- Muramatsu T, Nishikawa K, Nemoto F, Kuchino Y, Nishimura S, Miyazawa T, Yokoyama S. 1988. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. *Nature* 336:179–181.
- Murphy FVt, Ramakrishnan V, Malkiewicz A, Agris PF. 2004. The role of modifications in codon discrimination by tRNA(Lys)UUU. *Nat Struct Mol Biol* 11:1186–1191.
- Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P. 2011. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 478:57–63.
- Noma A, Kirino Y, Ikeuchi Y, Suzuki T. 2006. Biosynthesis of wybutosine, a hyper-modified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J* 25:2142–2154.
- Phizicky EM, Alfonzo JD. 2010. Do all modifications benefit all tRNAs? *FEBS Lett* 584:265–271.
- Pintard L, Lecointe F, Bujnicki JM, Bonnerot C, Grosjean H, Lapeyre B. 2002. Trm7p catalyses the formation of two 2'-O-methylribose in yeast tRNA anticodon loop. *EMBO J* 21:1811–1820.
- Putz J, Florentz C, Benseler F, Giege R. 1994. A single methyl group prevents the mischarging of a tRNA. *Nat Struct Biol* 1:580–582.
- Quartley E, Alexandrov A, Mikucki M, Buckner FS, Hol WG, DeTitta GT, Phizicky EM, Grayhack EJ. 2009. Heterologous expression of L. major proteins in *S. cerevisiae*: a test of solubility, purity, and gene recoding. *J Struct Funct Genomics* 10:233–247.
- Ramser J, Winnepeninckx B, Lenski C, Errijgers V, Platzer M, Schwartz CE, Meindl A, Kooy RF. 2004. A splice site mutation in the methyltransferase gene FTSJ1 in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). *J Med Genet* 41:679–683.
- Schneider C, Anderson JT, Tollervey D. 2007. The exosome subunit Rps44 plays a direct role in RNA substrate recognition. *Mol Cell* 27:324–331.
- Simpson CL, Lemmens R, Miskiewicz K, Broom WJ, Hansen VK, van Vught PW, Landers JE, Sapp P, Van Den Bosch L, Knight J. 2009. Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration. *Hum Mol Genet* 18:472–481.
- Slaugenhaupt SA, Blumenfeld A, Gill SP, Leyne M, Mull J, Cuajungco MP, Liebert CB, Chadwick B, Idelson M, Reznik L. 2001. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. *Am J Hum Genet* 68:598–605.
- Suzuki T, Wada T, Saigo K, Watanabe K. 2002. Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. *EMBO J* 21:6581–6589.
- Takano K, Nakagawa E, Inoue K, Kamada F, Kure S, Goto Y. 2008. A loss-of-function mutation in the FTSJ1 gene causes nonsyndromic X-linked mental retardation in a Japanese family. *Am J Med Genet B Neuropsychiatr Genet* 147B:479–484.
- Tuorto F, Liebers R, Musch T, Schaefer M, Hofmann S, Kellner S, Frye M, Helm M, Stoecklin G, Lyko F. 2012. RNA cytosine methylation by Dnmt2 and NSun2 promotes tRNA stability and protein synthesis. *Nat Struct Mol Biol* 19:900–905.
- Urbonavicius J, Qian Q, Durand JM, Hagervall TG, Bjork GR. 2001. Improvement of reading frame maintenance is a common function for several tRNA modifications. *EMBO J* 20:4863–4873.
- Vanacova S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W. 2005. A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol* 3:e189.
- Waas WF, Druzina Z, Hanan M, Schimmel P. 2007. Role of a tRNA base modification and its precursors in frameshifting in eukaryotes. *J Biol Chem* 282:26026–26034.
- Whipple JM, Lane EA, Chernyakov I, D'Silva S, Phizicky EM. 2011. The yeast rapid tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA. *Genes Dev* 25:1173–1184.
- Yarham JW, Lamichhane TN, Pyle A, Mattijssen S, Baruffini E, Bruni F, Donnini C, Vassilev A, He L, Blakely EL. 2014. Defective i6A37 modification of mitochondrial and cytosolic tRNAs results from pathogenic mutations in TRIT1 and its substrate tRNA. *PLoS Genet* 10:e1004424.
- Yasukawa T, Suzuki T, Ishii N, Ueda T, Ohta S, Watanabe K. 2000a. Defect in modification at the anticodon wobble nucleotide of mitochondrial tRNA(Lys) with the MERRF encephalomyopathy pathogenic mutation. *FEBS Lett* 467:175–178.
- Yasukawa T, Suzuki T, Ueda T, Ohta S, Watanabe K. 2000b. Modification defect at anticodon wobble nucleotide of mitochondrial tRNAs(Leu)(UUR) with pathogenic mutations of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *J Biol Chem* 275:4251–4257.
- Yue D, Kintanar A, Horowitz J. 1994. Nucleoside modifications stabilize Mg²⁺ binding in *Escherichia coli* tRNA(Val): an imino proton NMR investigation. *Biochemistry* 33:8905–8911.
- Zeharia A, Shaag A, Pappo O, Mager-Heckel AM, Saada A, Beinat M, Karicheva O, Mandel H, Ofek N, Segel R. 2009. Acute infantile liver failure due to mutations in the TRMU gene. *Am J Hum Genet* 85:401–407.