HHS Public Access

Author manuscript

Hum Mutat. Author manuscript; available in PMC 2021 March 21.

Published in final edited form as:

Hum Mutat. 2020 March ; 41(3): 600–607. doi:10.1002/humu.23976.

An intellectual disability-associated missense variant in TRMT1 impairs tRNA modification and reconstitution of enzymatic activity

Kejia Zhang[1], **Jenna M Lentini**[1], **Christopher T Prevost**[1], **Mais O Hashem**[2,3], **Fowzan S Alkuraya**[2,3], **Dragony Fu**[1,*]

[1]Department of Biology, Center for RNA Biology, University of Rochester, Rochester, NY 14627

[2]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

[3]Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

Abstract

The human *TRMT1* gene encodes an RNA methyltransferase enzyme responsible for catalyzing dimethylguanosine (m2,2G) formation in tRNAs. Frameshift mutations in *TRMT1* have been shown to cause autosomal-recessive intellectual disability (ID) in the human population but additional TRMT1 variants remain to be characterized. Here, we describe a homozygous *TRMT1* missense variant in a patient displaying developmental delay, ID, and epilepsy. The missense variant changes an arginine residue to a cysteine (R323C) within the methyltransferase domain and is expected to perturb protein folding. Patient cells expressing TRMT1-R323C exhibit a deficiency in m2,2G modifications within tRNAs, indicating that the mutation causes loss-of-function. Notably, the TRMT1 R323C mutant retains tRNA binding but is unable to rescue m2,2G formation in TRMT1-deficient human cells. Our results identify a pathogenic point mutation in TRMT1 that perturbs tRNA modification activity, and demonstrate that m2,2G modifications are disrupted in the cells of patients with TRMT1-associated ID disorders.

Keywords

tRNA modification; TRMT1; dimethylguanosine; intellectual disability

The post-transcriptional modification of tRNA has emerged as a critical modulator of biological processes ranging from gene expression to development (Frye, Harada, Behm, & He, 2018; Ranjan & Leidel, 2019). There are over 100 types of tRNA modifications that range from simple methylation to complex modifications involving multiple chemical groups (El Yacoubi, Bailly, & de Crecy-Lagard, 2012; Ontiveros, Stoute, & Liu, 2019). Notably, defects in tRNA modification have emerged as the cause of diverse neurological

*corresponding author: Dragony Fu, dragonyfu@rochester.edu.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

and neurodevelopmental disorders, thereby highlighting the critical role of tRNA modification in human health and physiology (Angelova et al., 2018; Ramos & Fu, 2018). In particular, the brain appears to be sensitive to any perturbation in translation efficiency and fidelity brought about by defects in tRNA modifications, as evidenced from the numerous cognitive disorders linked to tRNA modification enzymes such as: the Elongator complex (Hawer et al., 2018; Kojic & Wainwright, 2016); ADAT3 (Alazami et al., 2013; El-Hattab et al., 2016; Ramos et al., 2019); NSUN2 (Abbasi-Moheb et al., 2012; Khan et al., 2012; Martinez et al., 2012); FTSJ1 (Dai et al., 2008; Freude et al., 2004; Froyen et al., 2007; Gong et al., 2008; Guy et al., 2015; Ramser et al., 2004; Takano et al., 2008); WDR4 (Chen et al., 2018; Shaheen et al., 2015; Trimouille et al., 2018); KEOPS complex (Braun et al., 2017); PUS3 (Abdelrahman, Al-Shamsi, Ali, & Al-Gazali, 2018; Shaheen, Han, et al., 2016); CTU2 (Shaheen, Al-Salam, El-Hattab, & Alkuraya, 2016; Shaheen, Mark, et al., 2019); TRMT10A (Gillis et al., 2014; Igoillo-Esteve et al., 2013; Narayanan et al., 2015; Yew, McCreight, Colclough, Ellard, & Pearson, 2016; Zung et al., 2015); PUS7 (de Brouwer et al., 2018; Shaheen, Tasak, et al., 2019) and ALKBH8 (Monies, Vagbo, Al-Owain, Alhomaidi, & Alkuraya, 2019).

One of the very first tRNA modification enzymes to be discovered is tRNA methyltransferase 1 (Trm1p) from yeast *Saccharomyces cerevisiae* (Hopper, Furukawa, Pham, & Martin, 1982). *S. cerevisiae* Trm1p is imported into the nucleus and mitochondria, where it catalyzes the methylation of a specific guanosine residue at position 26 in numerous tRNAs to yield the N2,N2-dimethylguanosine (m2,2G) modification (Ellis, Hopper, & Martin, 1987, 1989; Ellis, Morales, Li, Hopper, & Martin, 1986). Two human homologs of yeast Trm1p have been identified by sequence homology that are encoded by the *TRMT1* and *TRMT1L* genes (Buckland, Maule, & Sealey, 1996; Liu & Straby, 2000; Vauti et al., 2007). While the substrates of TRMT1L remain to be discovered, TRMT1 has been demonstrated to be responsible for the majority of m2,2G modifications in the tRNA of human cells (Dewe, Fuller, Lentini, Kellner, & Fu, 2017).

Notably, exome sequencing studies have implicated frameshift mutations in *TRMT1* as the cause for certain forms of autosomal-recessive intellectual disability (ID) disorders (Blaesius et al., 2018; Davarniya et al., 2015; Monies et al., 2017; Najmabadi et al., 2011). The deletion mutations are predicted to cause frameshifts that result in nonsense mediated decay of the mRNA transcript and/or truncated proteins lacking the C-terminal RNA binding domain. The ID-associated TRMT1 mutants have been shown to be defective in tRNA binding and enzymatic activity (Dewe et al., 2017). While frameshift mutations in *TRMT1* have been identified in the human population, *TRMT1* missense alleles that help elucidate the functional consequences of tRNA modification deficiency remain to be found. Moreover, the extent to which m2,2G modifications are impacted in patient cells by the TRMT1 mutations is unknown. Here, we perform a functional characterization of a TRMT1 missense variant in an individual presenting with ID disorder and epilepsy.

We have previously described a “genomics first” approach to patients with ID (Anazi et al., 2017). When combined with the high prevalence of consanguinity in this group, this approach led to a high yield where a likely causal variant was identified in the majority of the >330 patients in that cohort. One of the reported variants in this cohort is a novel

missense variant in the *TRMT1* gene (ClinVar Accession VCV000191099.1, NM_001136035.3 (*TRMT1*):c.967C>T, p.Arg323Cys) (Figure 1A). The variant is absent in >4,000 Saudi exomes and is present at a very low frequency in gnomAD (4 heterozygotes, MAF 0.0000159). While samples from other members of the family could not be obtained, subsequent Sanger sequencing confirmed the homozygous nature of the mutation in the ID-affected index individual (Figure 1B, 13DG1615).

At last evaluation, the female patient was 8 years old with ID and generalized epilepsy (Figure 1C). The patient was conceived via artificial insemination and pregnancy was uneventful. She was delivered at term vaginally but meconium aspiration resulted in respiratory distress and a short admission to neonatal intensive care for 5 days. Her motor development was normal but cognitive development was slow. She scored 73 at the age of 6.5 years on the Vineland scale for adaptive behavior, and she is currently attending special schooling with poor performance. Her medical history is notable for epilepsy that is partially controlled with medications and bilateral sensorineural hearing loss. Physical examination revealed normal head size (52 cm; 62nd percentile), height (127.6 cm, 50th percentile) and weight (26.4 kg; 55th percentile). Brain MRI was also normal with no evidence of pontocerebellar hypoplasia or white matter abnormalities. However, she displayed strabismus and subtle dysmorphism in the form of deep set eyes, epicanthus, smooth philtrum and pointed chin.

The ID-associated variant in the *TRMT1* gene mutates amino acid residue 323 in the SAM-methyltransferase domain of TRMT1 from a positively charged arginine to a non-polar cysteine residue (R323C) (Figure 1D). Based upon protein sequence alignment, the R323 residue of human TRMT1 is absolutely conserved in Trm1 homologs from the Archaea to mammals (Figure 1E). Moreover, the variant has a consistently deleterious prediction by *in silico* tools DANN, LRT, MutationAssessor, MutationTaster, PROVEAN, FATHMM-MKL and SIFT (Choi, Sims, Murphy, Miller, & Chan, 2012; Chun & Fay, 2009; Quang, Chen, & Xie, 2015; Reva, Antipin, & Sander, 2011; Schwarz, Cooper, Schuelke, & Seelow, 2014; Shihab et al., 2015; Sim et al., 2012). Using the crystal structure of Trm1 from the Archaean *Pyrococcus furiosus* (Ihsanawati et al., 2008), we found that the homologous arginine residue is located within the methyltransferase domain near the putative tRNA binding pocket (Supp. Figure S1A). Interestingly, the R323 residue is buried into the interior of Archaeal Trm1 rather than on the surface, unlike most charged side chains which are on the surface of proteins.

To gain insight into the potential effects of the R323C mutation on human TRMT1, we generated a predicted tertiary structure of human TRMT1 using an *in silico* template-based algorithm (Kallberg et al., 2012). Based upon this hypothetical structure, TRMT1 is predicted to fold into two distinct domains coinciding with the SAM-methyltransferase domain and the C-terminal CCCH-type Zinc finger motif (Supp. Figure S1B, TRMT1 methyltransferase domain). Notably, modeling of the R323C mutation using the most favored rotamer conformation would predict a steric clash with a conserved tyrosine side chain present at position 321 (Supp. Figure S1C). Thus, the R323C mutation is likely to be deleterious by perturbing the core packing of the methyltransferase domain of TRMT1 that is responsible for SAM binding, tRNA interaction and catalysis.

To examine the molecular effects of the R323C mutation, we generated a lymphoblastoid cell line (LCL) from the affected human patient harboring the homozygous missense mutation in the *TRMT1* gene (referred to as R323C-LCL). The R323C-LCL was compared to control lymphoblasts generated from ethnically matched, healthy, unrelated individuals (WT-LCLs). We directly measured and compared the levels of more than 20 different tRNA modifications in the patient R323C-LCL versus WT-LCLs through quantitative mass spectrometry of modified ribonucleosides derived from cellular RNA (Cai et al., 2015; Dewe et al., 2017) (Supporting Information, Materials and Methods). Strikingly, the m2,2G modification exhibited a 32-fold decrease in the R323C-LCL compared to WT-LCL (Figure 1F). No other modification displayed a significant change between the WT versus R323C-LCLs.

To validate the perturbation of m2,2G modification in cellular tRNAs, we used a primer extension assay that detects RNA modification status at nucleotide resolution. In this assay, the presence of m2,2G leads to a block of reverse transcriptase (RT) at position 26 of tRNA while a decrease in m2,2G allows for read-through and an extended product up to a subsequent RT-blocking modification (Figure 1G). We selected three nuclear-encoded tRNAs that we have previously shown to contain m2,2G (Dewe et al., 2017), along with mitochondrial tRNA-Ile-GAU, which is the only known mammalian mitochondrial-encoded tRNA to contain m2,2G (Clark, Evans, Dominissini, Zheng, & Pan, 2016; Dewe et al., 2017; Suzuki & Suzuki, 2014). In the absence of RT, only background bands were detected in reactions containing the probe and total cellular RNA from the wildtype LCL (Figure 1H, lane 1 for all tRNAs, background bands denoted by *). Addition of RT led to the appearance of an extension product up to the m2,2G modification at the expected position in both nuclear- and mitochondrial-encoded tRNAs in both WT-LCLs (Figure 1H, lanes 2 and 3 for tRNA-Ala-AGC and Ile-UAU or lanes 2 and 4 for tRNA-Met-CAU and mito-tRNA-Ile-GAU). In contrast, the RT block at position 26 was absent in the nuclear- and mitochondrial-encoded tRNA of the R323C-LCLs (Figure 1H, lane 4 for tRNA-Ala-AGC and Ile-UAU or lane 3 for tRNA-Met-CAU and mito-tRNA-Ile-GAU). Loss of m2,2G modification in the tRNAs allowed for read-through and extension to the next RT-blocking modification. Thus, LCLs from the ID-affected patient with the TRMT1 R323C mutation exhibit a severe deficit in m2,2G formation in cellular tRNAs.

To elucidate the molecular defects associated with the TRMT1-R323C mutant, we investigated the interaction between TRMT1 and tRNAs. As previously shown, human TRMT1 displays a stable interaction with substrate tRNAs that are targets for m2,2G modification (Dewe et al., 2017). Using this system, we expressed a FLAG-tagged version of TRMT1 variants in 293T human embryonic cells followed by affinity purification and analysis of copurifying RNAs. The expressed proteins represent either: 1) wildtype (WT) TRMT1, 2) the R323C mutant, and 3) Y445fs, a previously described, ID-associated TRMT1 mutant that lacks RNA binding due to the truncation of the RNA recognition motif (Figure 2A). Immunoblotting confirmed the expression and purification of each TRMT1 variant on anti-FLAG resin (Fig. 2B). In the control purification from vector-transfected cells, we detected only background contaminating 5.8S and 5S rRNAs (Figure 2C, lane 5). In contrast, the purification of WT-TRMT1 resulted in the considerable enrichment of tRNAs along with rRNAs as we have previously shown (Figure 2C, lane 6) (Dewe et al.,

2017). Interestingly, we found that similar levels of tRNA were enriched with either TRMT1-WT or TRMT1-R323C mutant (Figure 2C, compare lanes 6 and 7). As expected, the Y445fs mutant exhibited only background RNA signal indicative of defective tRNA binding (Figure 2C, lane 8). Thus, the TRMT1-R323C mutant differs from other ID-associated TRMT1 variants by retaining the ability to bind tRNA.

We next used a previously-described TRMT1-knock out (KO) cell line derived from 293T human cells to dissect the effects of the R323C mutation on TRMT1 function (Dewe et al., 2017). This human 293T cell line lacks TRMT1 expression resulting in the near complete loss of m2,2G modifications in tRNA and the absence of m2,2G modifications in all tested tRNAs. Using transient transfection of mammalian constructs, we expressed either WT-TRMT1 or the R323 variant in the WT or TRMT1-KO 293T cell lines (Supp. Figure S2). We then assessed for rescue of m2,2G formation in tRNA-Ala-AGC using the primer extension assay described above. As expected, non-transfected or vector-transfected WT 293T cells exhibited an RT block at position 26 of tRNA-Ala-AGC indicative of the m2,2G modification (Figure 2D and E, lanes 1 and 2). No read-through product was detected for either tRNA in WT 293T cells suggesting that nearly all endogenous tRNA-Ala-AGC is modified with m2,2G. Consistent with this observation, increased expression of TRMT1 in WT 239T cells had no noticeable effect on m2,2G modification in tRNA-Ala-AGC (Figure 2D, lane 3). Intriguingly though, over-expression of the TRMT1-R323C mutant in WT 293T cells led to increased read-through product indicative of decreased m2,2G modification (Figure 2D, lane 4, quantified in 2E). The increase in read-through product suggests that TRMT1-R323C could have a dominant-negative effect on m2,2G modification when over-expressed in the presence of WT-TRMT1.

We next tested the TRMT1-R323C mutant for the ability to rescue m2,2G formation in the TRMT1-KO cell line. As expected, the m2,2G modification was absent in tRNA-Ala-AGC isolated from the non-transfected or vector-transfected TRMT1-KO cell line leading to read-through to the next RT block (Figure 2D, lanes 5 and 6, quantified in 2E). Re-expression of TRMT1-WT in the TRMT1-KO cell line was able to restore m2,2G formation (Figure 2D, lane 7). Due to variable TRMT1 expression caused by incomplete transfection efficiency, the level of m2,2G modification was increased but not completely rescued to the level of the WT cell line. Notably, the TRMT1-R323 mutant displayed greatly reduced ability to reconstitute m2,2G formation in the TRMT1-KO cell line (Figure 2D, lane 8, quantified in 2E). These results indicate that even though TRMT1-R323C can retain binding to tRNAs, it is compromised in its capacity to generate m2,2G in cellular tRNA. Thus, the TRMT1-R323C alteration appears to be a loss-of-function mutation, consistent with the severe deficiency in m2,2G modification in the tRNAs of the affected patient with the R323C mutation.

Previously characterized TRMT1-ID mutations result in translation frameshifting that lead to truncation of the carboxyl-terminal zinc finger motif. These mutants have been shown to be defective in RNA binding and reconstitution of methyltransferase activity *in vivo* (Dewe et al., 2017). Unlike the frameshift mutants, the R323C mutant is still able to efficiently interact with tRNA substrates. The retention of tRNA binding by the R323C mutant is consistent with the location of the R323 residue on the interior of TRMT1 within the methyltransferase motif and outside of the putative zinc finger motif that interacts with

tRNA. While the R323C mutant can still bind RNA, it exhibits a severe defect in the reconstitution of m2,2G formation in TRMT1-KO human cells. Due to the location of the R323 residue in the core of the TRMT1 methyltransferase domain, the R323C mutation could severely distort the tertiary structure of TRMT1 leading to defects in substrate orientation, SAM binding and/or catalysis.

The m2,2G modification has been predicted to prevent the folding of certain nuclear-encoded tRNAs into alternative conformers found in mitochondrial tRNAs (Steinberg & Cedergren, 1995). Moreover, previous studies in *S. cerevisiae* have found that certain tRNA isoacceptors exhibit decreased accumulation in the absence of Trm1, suggesting that the m2,2G modification plays a role in the proper folding of certain tRNAs (Dewe, Whipple, Chernyakov, Jaramillo, & Phizicky, 2012; Vakiloroayaei, Shah, Oeffinger, & Bayfield, 2017). Interestingly, we have found that TRMT1-deficient human cells exhibit similar steady-state levels of all tested nuclear- and mitochondrial-encoded tRNAs (Dewe et al., 2017). However, it is possible that the m2,2G modification is more important for certain human tRNA isodecoders in terms of their stability and accumulation, akin to the situation in *S. cerevisiae*.

The m2,2G modification could also play a role in proper tRNA interaction with the ribosome to ensure efficient translation. Indeed, studies in yeast S. cerevisiae have found that *Trm1* deletion mutants display alterations in ribosome profiles indicative of translation aberrations (Chou, Donnard, Gustafsson, Garber, & Rando, 2017). Moreover, studies in human cells have found that global translation is reduced upon ablation of TRMT1 (Dewe et al., 2017). The alterations in translation are correlated with perturbations in redox homeostasis and heightened sensitivity to oxidative stress. Future studies using ribosome profiling in patient cells could provide insight into the biological pathways that are perturbed upon loss of the m2,2G modification that contribute to the spectrum of neurodevelopmental phenotypes exhibited by individuals with TRMT1 mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the study family for agreeing to participate and members of the Fu Lab for helpful discussion on the manuscript. This work was supported by the Saudi Human Genome Program, and King Salman Center for Disability Research (F.S.A.), and a University of Rochester Furth Fund Award and National Science Foundation CAREER Award 1552126 to D.F..

References

Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, . . . Kuss A. (2012). Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet, 90(5), 847–855. doi:10.1016/j.ajhg.2012.03.021 [PubMed: 22541559]

Abdelrahman HA, Al-Shamsi AM, Ali BR, & Al-Gazali L. (2018). A null variant in PUS3 confirms its involvement in intellectual disability and further delineates the associated neurodevelopmental disease. Clin Genet. doi:10.1111/cge.13443

Alazami AM, Hijazi H, Al-Dosari MS, Shaheen R, Hashem A, Aldahmesh MA, . . . Alkuraya FS (2013). Mutation in ADAT3, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. J Med Genet, 50(7), 425–430. doi:10.1136/jmedgenet-2012-101378 [PubMed: 23620220]

Anazi S, Maddirevula S, Faqeih E, Alsedairy H, Alzahrani F, Shamseldin HE, . . . Alkuraya FS (2017). Clinical genomics expands the morbid genome of intellectual disability and offers a high diagnostic yield. Mol Psychiatry, 22(4), 615–624. doi:10.1038/mp.2016.113 [PubMed: 27431290]

Angelova MT, Dimitrova DG, Dinges N, Lence T, Worpenberg L, Carre C, & Roignant JY (2018). The Emerging Field of Epitranscriptomics in Neurodevelopmental and Neuronal Disorders. Front Bioeng Biotechnol, 6, 46. doi:10.3389/fbioe.2018.00046 [PubMed: 29707539]

Blaesius K, Abbasi AA, Tahir TH, Tietze A, Picker-Minh S, Ali G, . . . Kaindl A. (2018). Mutations in the tRNA methyltransferase 1 gene TRMT1 cause congenital microcephaly, isolated inferior vermian hypoplasia and cystic leukomalacia in addition to intellectual disability. Am J Med Genet A, 176(11), 2517–2521. doi:10.1002/ajmg.a.38631 [PubMed: 30289604]

Braun DA, Rao J, Mollet G, Schapiro D, Daugeron MC, Tan W, . . . Hildebrandt F. (2017). Mutations in KEOPS-complex genes cause nephrotic syndrome with primary microcephaly. Nat Genet, 49(10), 1529–1538. doi:10.1038/ng.3933 [PubMed: 28805828]

Buckland RA, Maule JC, & Sealey PG (1996). A cluster of transfer RNA genes (TRM1, TRR3, and TRAN) on the short arm of human chromosome 6. Genomics, 35(1), 164–171. doi:10.1006/geno.1996.0335 [PubMed: 8661117]

Cai WM, Chionh YH, Hia F, Gu C, Kellner S, McBee ME, . . . Dedon PC (2015). A Platform for Discovery and Quantification of Modified Ribonucleosides in RNA: Application to Stress-Induced Reprogramming of tRNA Modifications. Methods Enzymol, 560, 29–71. doi:10.1016/bs.mie.2015.03.004 [PubMed: 26253965]

Chen X, Gao Y, Yang L, Wu B, Dong X, Liu B, . . . Wang H. (2018). Speech and language delay in a patient with WDR4 mutations. Eur J Med Genet. doi:10.1016/j.ejmg.2018.03.007

Choi Y, Sims GE, Murphy S, Miller JR, & Chan AP (2012). Predicting the functional effect of amino acid substitutions and indels. PLoS One, 7(10), e46688. doi:10.1371/journal.pone.0046688 [PubMed: 23056405]

Chou HJ, Donnard E, Gustafsson HT, Garber M, & Rando OJ (2017). Transcriptome-wide Analysis of Roles for tRNA Modifications in Translational Regulation. Mol Cell, 68(5), 978–992 e974. doi:10.1016/j.molcel.2017.11.002 [PubMed: 29198561]

Chun S, & Fay JC (2009). Identification of deleterious mutations within three human genomes. Genome Res, 19(9), 1553–1561. doi:10.1101/gr.092619.109 [PubMed: 19602639]

Clark WC, Evans ME, Dominissini D, Zheng G, & Pan T. (2016). tRNA base methylation identification and quantification via high-throughput sequencing. RNA. doi:10.1261/rna.056531.116

Dai L, Xing L, Gong P, Zhang K, Gao X, Zheng Z, . . . Zhang F. (2008). Positive association of the FTSJ1 gene polymorphisms with nonsyndromic X-linked mental retardation in young Chinese male subjects. J Hum Genet, 53(7), 592–597. doi:10.1007/s10038-008-0287-x [PubMed: 18401546]

Davarniya B, Hu H, Kahrizi K, Musante L, Fattahi Z, Hosseini M, . . . Najmabadi H. (2015). The Role of a Novel TRMT1 Gene Mutation and Rare GRM1 Gene Defect in Intellectual Disability in Two Azeri Families. PLoS One, 10(8), e0129631. doi:10.1371/journal.pone.0129631

de Brouwer APM, Abou Jamra R, Kortel N, Soyris C, Polla DL, Safra M, . . . Schwartz S. (2018). Variants in PUS7 Cause Intellectual Disability with Speech Delay, Microcephaly, Short Stature, and Aggressive Behavior. Am J Hum Genet, 103(6), 1045–1052. doi:10.1016/j.ajhg.2018.10.026 [PubMed: 30526862]

Dewe JM, Fuller BL, Lentini JM, Kellner SM, & Fu D. (2017). TRMT1-Catalyzed tRNA Modifications Are Required for Redox Homeostasis To Ensure Proper Cellular Proliferation and Oxidative Stress Survival. Mol Cell Biol, 37(21). doi:10.1128/MCB.00214-17

Dewe JM, Whipple JM, Chernyakov I, Jaramillo LN, & Phizicky EM (2012). The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs

lacking one or more of several modifications. RNA, 18(10), 1886–1896. doi:10.1261/rna.033654.112 [PubMed: 22895820]

El Yacoubi B, Bailly M, & de Crecy-Lagard V. (2012). Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Annu Rev Genet, 46, 69–95. doi:10.1146/annurev-genet-110711-155641 [PubMed: 22905870]

El-Hattab AW, Saleh MA, Hashem A, Al-Owain M, Asmari AA, Rabei H, . . . Alkuraya FS (2016). ADAT3-related intellectual disability: Further delineation of the phenotype. Am J Med Genet A, 170A(5), 1142–1147. doi:10.1002/ajmg.a.37578 [PubMed: 26842963]

Ellis SR, Hopper AK, & Martin NC (1987). Amino-terminal extension generated from an upstream AUG codon is not required for mitochondrial import of yeast N2,N2-dimethylguanosine-specific tRNA methyltransferase. Proc Natl Acad Sci U S A, 84(15), 5172–5176. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3299379 [PubMed: 3299379]

Ellis SR, Hopper AK, & Martin NC (1989). Amino-terminal extension generated from an upstream AUG codon increases the efficiency of mitochondrial import of yeast N2,N2-dimethylguanosine-specific tRNA methyltransferases. Mol Cell Biol, 9(4), 1611–1620. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2657400 [PubMed: 2657400]

Ellis SR, Morales MJ, Li JM, Hopper AK, & Martin NC (1986). Isolation and characterization of the TRM1 locus, a gene essential for the N2,N2-dimethylguanosine modification of both mitochondrial and cytoplasmic tRNA in Saccharomyces cerevisiae. J Biol Chem, 261(21), 9703–9709. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2426253 [PubMed: 2426253]

Freude K, Hoffmann K, Jensen LR, Delatycki MB, des Portes V, Moser B, . . . Ropers HH (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(2), 305–309. doi:10.1086/422507 [PubMed: 15162322]

Froyen G, Bauters M, Boyle J, Van Esch H, Govaerts K, van Bokhoven H, . . . Turner G. (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(5), 539–547. doi:10.1007/s00439-007-0343-1 [PubMed: 17333282]

Frye M, Harada BT, Behm M, & He C. (2018). RNA modifications modulate gene expression during development. Science, 361(6409), 1346–1349. doi:10.1126/science.aau1646 [PubMed: 30262497]

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(9), 581–586. doi:10.1136/jmedgenet-2014-102282 [PubMed: 25053765]

Gong P, Li J, Dai L, Zhang K, Zheng Z, Gao X, & Zhang F. (2008). Genetic variations in FTSJ1 influence cognitive ability in young males in the Chinese Han population. J Neurogenet, 22(4), 277–287. doi:10.1080/01677060802337299 [PubMed: 19012053]

Guy MP, Shaw M, Weiner CL, Hobson L, Stark Z, Rose K, . . . Phizicky EM (2015). Defects in tRNA Anticodon Loop 2'-O-Methylation Are Implicated in Nonsyndromic X-Linked Intellectual Disability due to Mutations in FTSJ1. Hum Mutat, 36(12), 1176–1187. doi:10.1002/humu.22897 [PubMed: 26310293]

Hawer H, Hammermeister A, Ravichandran KE, Glatt S, Schaffrath R, & Klassen R. (2018). Roles of Elongator Dependent tRNA Modification Pathways in Neurodegeneration and Cancer. Genes (Basel), 10(1). doi:10.3390/genes10010019

Hopper AK, Furukawa AH, Pham HD, & Martin NC (1982). Defects in modification of cytoplasmic and mitochondrial transfer RNAs are caused by single nuclear mutations. Cell, 28(3), 543–550. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7074684 [PubMed: 7074684]

Igoillo-Esteve M, Genin A, Lambert N, Desir J, Pirson I, Abdulkarim B, . . . Cnop M. (2013). tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. PLoS Genet, 9(10), e1003888. doi:10.1371/journal.pgen.1003888

Ihsanawati, Nishimoto M, Higashijima K, Shirouzu M, Grosjean H, Bessho Y, & Yokoyama S. (2008). Crystal structure of tRNA N2,N2-guanosine dimethyltransferase Trm1 from Pyrococcus horikoshii. J Mol Biol, 383(4), 871–884. doi:10.1016/j.jmb.2008.08.068 [PubMed: 18789948]

Kallberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, & Xu J. (2012). Template-based protein structure modeling using the RaptorX web server. Nat Protoc, 7(8), 1511–1522. doi:10.1038/nprot.2012.085 [PubMed: 22814390]

Khan M, Rafiq M, Noor A, Hussain S, Flores J, Rupp V, . . . Vincent J. (2012). Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet, 90(5), 856–863. doi:10.1016/j.ajhg.2012.03.023 [PubMed: 22541562]

Kojic M, & Wainwright B. (2016). The Many Faces of Elongator in Neurodevelopment and Disease. Front Mol Neurosci, 9, 115. doi:10.3389/fnmol.2016.00115 [PubMed: 27847465]

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(18), 3445–3451. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10982862 [PubMed: 10982862]

Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, . . . Gleeson JG (2012). Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet, 49(6), 380–385. doi:10.1136/jmedgenet-2011-100686 [PubMed: 22577224]

Monies D, Abouelhoda M, AlSayed M, Alhassnan Z, Alotaibi M, Kayyali H, . . . Alkuraya FS (2017). The landscape of genetic diseases in Saudi Arabia based on the first 1000 diagnostic panels and exomes. Hum Genet, 136(8), 921–939. doi:10.1007/s00439-017-1821-8 [PubMed: 28600779]

Monies D, Vagbo CB, Al-Owain M, Alhomaidi S, & Alkuraya FS (2019). Recessive Truncating Mutations in ALKBH8 Cause Intellectual Disability and Severe Impairment of Wobble Uridine Modification. Am J Hum Genet, 104(6), 1202–1209. doi:10.1016/j.ajhg.2019.03.026 [PubMed: 31079898]

Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini S, Chen W, . . . Ropers H. (2011). Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature, 478(7367), 57–63. doi:10.1038/nature10423 [PubMed: 21937992]

Narayanan M, Ramsey K, Grebe T, Schrauwen I, Szelinger S, Huentelman M, . . . Group, C. R. R. (2015). Case Report: Compound heterozygous nonsense mutations in TRMT10A are associated with microcephaly, delayed development, and periventricular white matter hyperintensities. F1000Res, 4, 912. doi:10.12688/f1000research.7106.1 [PubMed: 26535115]

Ontiveros RJ, Stoute J, & Liu KF (2019). The chemical diversity of RNA modifications. Biochem J, 476(8), 1227–1245. doi:10.1042/BCJ20180445 [PubMed: 31028151]

Quang D, Chen Y, & Xie X. (2015). DANN: a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics, 31(5), 761–763. doi:10.1093/bioinformatics/btu703 [PubMed: 25338716]

Ramos J, & Fu D. (2018). The emerging impact of tRNA modifications in the brain and nervous system. Biochim Biophys Acta Gene Regul Mech. doi:10.1016/j.bbagrm.2018.11.007

Ramos J, Han L, Li Y, Hagelskamp F, Kellner SM, Alkuraya FS, . . . Fu D. (2019). Formation of tRNA Wobble Inosine in Humans Is Disrupted by a Millennia-Old Mutation Causing Intellectual Disability. Mol Cell Biol, 39(19). doi:10.1128/MCB.00203-19

Ramser J, Winnepenninckx B, Lenski C, Errijgers V, Platzer M, Schwartz CE, . . . 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(9), 679–683. doi:10.1136/jmg.2004.019000 [PubMed: 15342698]

Ranjan N, & Leidel SA (2019). The epitranscriptome in translation regulation: mRNA and tRNA modifications as the two sides of the same coin? FEBS Lett, 593(13), 1483–1493. doi:10.1002/1873-3468.13491 [PubMed: 31206634]

Reva B, Antipin Y, & Sander C. (2011). Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res, 39(17), e118. doi:10.1093/nar/gkr407 [PubMed: 21727090]

Schwarz JM, Cooper DN, Schuelke M, & Seelow D. (2014). MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods, 11(4), 361–362. doi:10.1038/nmeth.2890 [PubMed: 24681721]

Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, . . . Alkuraya FS (2015). Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of

microcephalic primordial dwarfism. Genome Biol, 16, 210. doi:10.1186/s13059-015-0779-x [PubMed: 26416026]

Shaheen R, Al-Salam Z, El-Hattab AW, & Alkuraya FS (2016). The syndrome dysmorphic facies, renal agenesis, ambiguous genitalia, microcephaly, polydactyly and lissencephaly (DREAM-PL): Report of two additional patients. Am J Med Genet A, 170(12), 3222–3226. doi:10.1002/ajmg.a.37877 [PubMed: 27480277]

Shaheen R, Han L, Faqeih E, Ewida N, Alobeid E, Phizicky EM, & Alkuraya FS (2016). A homozygous truncating mutation in PUS3 expands the role of tRNA modification in normal cognition. Hum Genet, 135(7), 707–713. doi:10.1007/s00439-016-1665-7 [PubMed: 27055666]

Shaheen R, Mark P, Prevost CT, AlKindi A, Alhag A, Estwani F, . . . Alkuraya FS (2019). Biallelic variants in CTU2 cause DREAM-PL syndrome and impair thiolation of tRNA wobble U34. Hum Mutat. doi:10.1002/humu.23870

Shaheen R, Tasak M, Maddirevula S, Abdel-Salam GMH, Sayed ISM, Alazami AM, . . . Alkuraya FS (2019). PUS7 mutations impair pseudouridylation in humans and cause intellectual disability and microcephaly. Hum Genet, 138(3), 231–239. doi:10.1007/s00439-019-01980-3 [PubMed: 30778726]

Shihab HA, Rogers MF, Gough J, Mort M, Cooper DN, Day IN, . . . Campbell C. (2015). An integrative approach to predicting the functional effects of non-coding and coding sequence variation. Bioinformatics, 31(10), 1536–1543. doi:10.1093/bioinformatics/btv009 [PubMed: 25583119]

Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, & Ng PC (2012). SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res, 40(Web Server issue), W452–457. doi:10.1093/nar/gks539 [PubMed: 22689647]

Steinberg S, & Cedergren R. (1995). A correlation between N2-dimethylguanosine presence and alternate tRNA conformers. RNA, 1(9), 886–891. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8548653 [PubMed: 8548653]

Suzuki T, & Suzuki T. (2014). A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. Nucleic Acids Res, 42(11), 7346–7357. doi:10.1093/nar/gku390 [PubMed: 24831542]

Takano K, Nakagawa E, Inoue K, Kamada F, Kure S, Goto Y. i., & Japanese Mental Retardation C. (2008). A loss-of-function mutation in the FTSJ1 gene causes nonsyndromic X-linked mental retardation in a Japanese family. American journal of medical genetics. Part B, Neuropsychiatric genetics, 147B(4), 479–484. doi:10.1002/ajmg.b.30638

Trimouille A, Lasseaux E, Barat P, Deiller C, Drunat S, Rooryck C, . . . Lacombe D. (2018). Further delineation of the phenotype caused by biallelic variants in the WDR4 gene. Clin Genet, 93(2), 374–377. doi:10.1111/cge.13074 [PubMed: 28617965]

Vakiloroayaei A, Shah NS, Oeffinger M, & Bayfield MA (2017). The RNA chaperone La promotes pre-tRNA maturation via indiscriminate binding of both native and misfolded targets. Nucleic Acids Res, 45(19), 11341–11355. doi:10.1093/nar/gkx764 [PubMed: 28977649]

Vauti F, Goller T, Beine R, Becker L, Klopstock T, Hölter S, . . . Arnold H-H (2007). The mouse Trm1-like gene is expressed in neural tissues and plays a role in motor coordination and exploratory behaviour. Gene, 389(2), 174–185. doi:10.1016/j.gene.2006.11.004 [PubMed: 17198746]

Yew TW, McCreight L, Colclough K, Ellard S, & Pearson ER (2016). tRNA methyltransferase homologue gene TRMT10A mutation in young adult-onset diabetes with intellectual disability, microcephaly and epilepsy. Diabet Med, 33(9), e21–25. doi:10.1111/dme.13024 [PubMed: 26526202]

Zung A, Kori M, Burundukov E, Ben-Yosef T, Tatoor Y, & Granot E. (2015). Homozygous deletion of TRMT10A as part of a contiguous gene deletion in a syndrome of failure to thrive, delayed puberty, intellectual disability and diabetes mellitus. Am J Med Genet A, 167A(12), 3167–3173. doi:10.1002/ajmg.a.37341 [PubMed: 26297882]

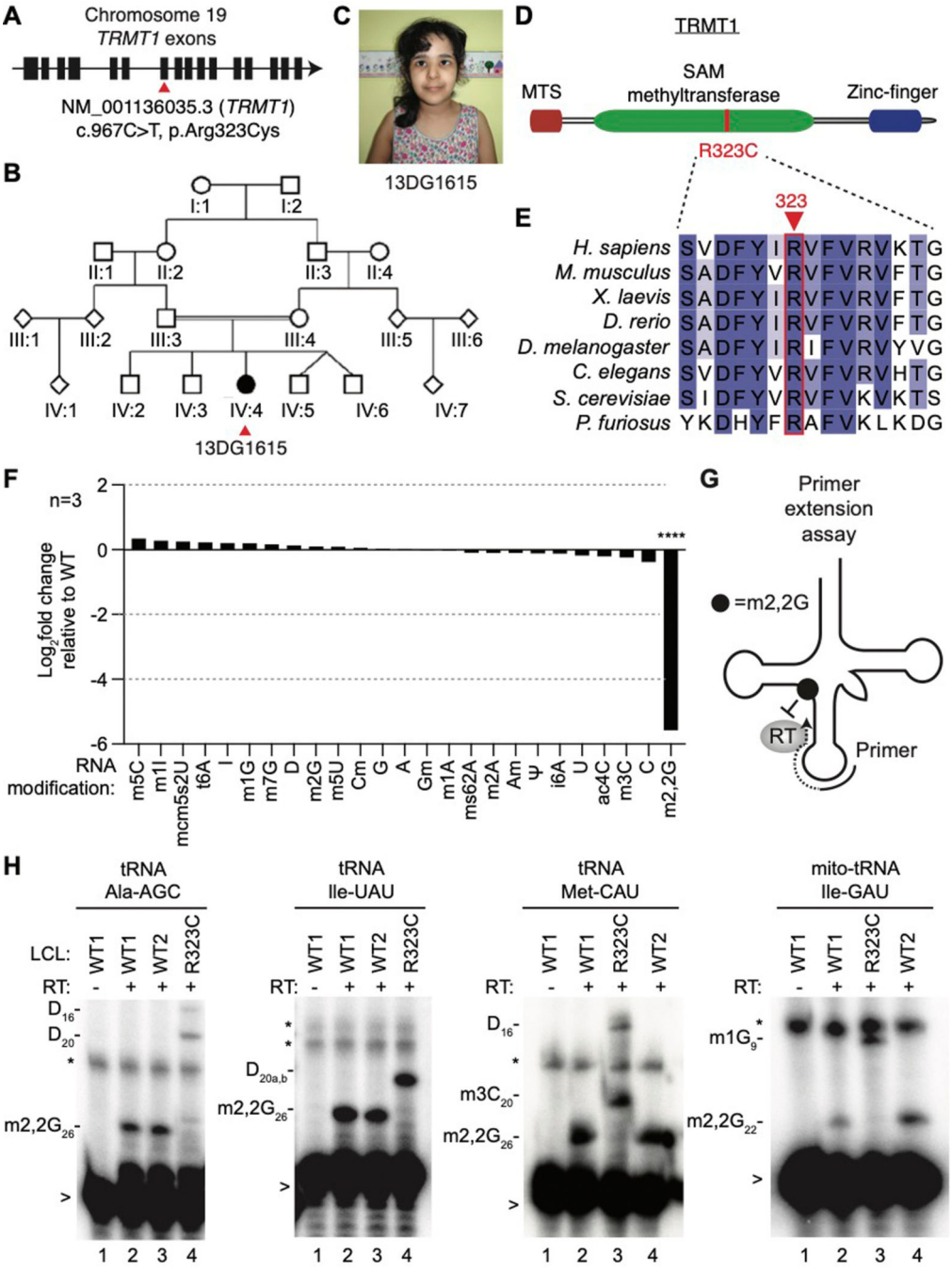

Figure 1.

Characterization of a missense mutation in TRMT1 linked to ID. (A) Exon organization of the *TRMT1* locus with the location of the single C>T point mutation highlighted in red. (B) Pedigree of the family harboring the missense mutation in the *TRMT1* gene and the patient that is homozygous for the mutation. (C) Patient 13DG1615 who is homozygous for the TRMT1 missense mutation. (D) Schematic of human TRMT1 with protein domains denoted; MTS (mitochondrial targeting signal), SAM-methyltransferase, and Zinc-finger motif. The location of the R323C mutation is denoted in red. (E) Protein sequence

alignments of TRMT1 from human to Archaea. The R323 residue is boxed in red. (F) Comparison of tRNA modification levels between R323C versus WT-LCLs. Nucleosides from digested tRNA samples were analyzed by LC-MS. Y-axis represents the log2-fold change in the levels of the indicated tRNA modification between the R323C patient and WT individual. Samples were measured in triplicate. ****, $P < 0.0001$. (G) Schematic of primer extension assay to monitor m2,2G in tRNAs. RT, reverse transcriptase. (H) Representative gels of primer extension assays to monitor the presence of m2,2G in tRNA from the indicated LCLs. >, labeled oligonucleotide used for primer extension; D, dihydrouridine; m3C, 3-methylcytosine; m1G, 1-methylguanosine; *, background signal.

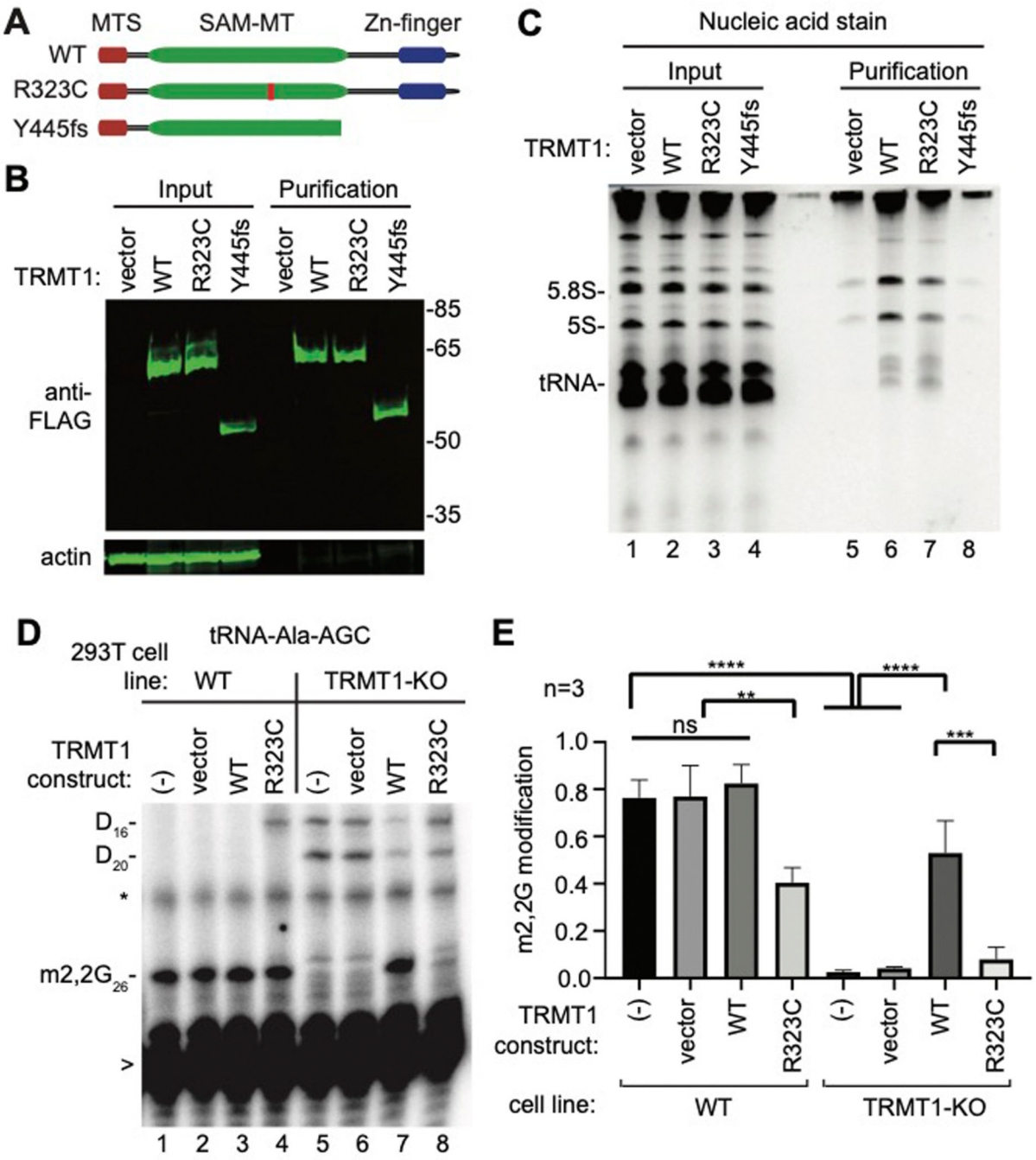

Figure 2.

The TRMT1-R323C mutant retains RNA binding but is impaired in reconstitution of tRNA modification activity. (A) Schematic of TRMT1 domains and variants. WT, wildtype; R323C, ID-associated point mutant; Y445fs, TRMT1 variant encoded by an ID-causing frameshift mutation. (B) Immunoblot of whole cell extracts prepared from each human cell line transfected with the indicated constructs. Molecular weight in kiloDalton is denoted on the right. (C) Nucleic acid stain of RNAs extracted from the indicated input or purified samples after denaturing PAGE. The migration pattern of tRNAs, 5.8S and 5S rRNA is

denoted. (D) Representative primer extension assay to monitor the presence of m2,2G in tRNA-Ala-AGC from cell lines transfected with the indicated constructs. >, labeled oligonucleotide used for primer extension; m2,2G, dimethylguanosine; D, dihydrouridine; * background signal. (E) Quantification of m2,2G modification levels in tRNA-Ala-AGC. Primer extensions were performed three times and error bars represent the standard error of the mean. Comparison were performed using one-way ANOVA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.