Franz *et al. J Transl Genet Genom* 2020;4:50-70
DOI: 10.20517/jtgg.2020.13

Journal of Translational Genetics and Genomics

Review

Open Access

Role of transfer RNA modification and aminoacylation in the etiology of congenital intellectual disability

Martin Franz#, Lisa Hagenau#, Lars R. Jensen, Andreas W. Kuss

Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald 17475, Germany.
#Authors cotributed equally.

Correspondence to: Prof. Andreas W. Kuss; Dr. Lars R. Jensen, Department of Functional Genomics, University Medicine Greifswald, C_FunGene, Felix-Hausdorff-Str. 8, Greifswald 17475, Germany.
E-mail: kussa@uni-greifswald.de; jensenl@uni-greifswald.de

How to cite this article: Franz M, Hagenau L, Jensen LR, Kuss AW. Role of transfer RNA modification and aminoacylation in the etiology of congenital intellectual disability. *J Transl Genet Genom* 2020;4:50-70.
http://dx.doi.org/10.20517/jtgg.2020.13

Received: 14 Feb 2020 **First Decision**: 17 Mar 2020 **Revised**: 30 Mar 2020 **Accepted**: 23 Apr 2020 **Available online**: 16 May 2020

Science Editor: Tjitske Kleefstra **Copy Editor**: Jing-Wen Zhang **Production Editor**: Tian Zhang

Abstract

Transfer RNA (tRNA) modification and aminoacylation are post-transcriptional processes that play a crucial role in the function of tRNA and thus represent critical steps in gene expression. Knowledge of the exact processes and effects of the defects in various tRNAs remains incomplete, but a rapidly increasing number of publications over the last decade has shown a growing amount of evidence as to the importance of tRNAs for normal human development, including brain formation and the development and maintenance of higher cognitive functions as well. In this review, we present a synopsis of the literature focusing on tRNA-modifying enzymes and aminoacyl-tRNA synthetases (ARSs) that have been found to be involved in the etiology of hereditary forms of intellectual disability. Our overview shows several parallels but also differences in the symptomatic spectrum observed in individuals affected by intellectual disability caused by mutations in tRNA modifier and/or ARS genes. This observation suggests that tRNAs seem to assume diverse roles in a variety of cellular processes possibly even beyond translation and that not only the abundance but also the modification and aminoacylation levels of tRNAs contribute to cell functions in ways that still remain to be understood.

Keywords: transfer RNA modification, aminoacylation, intellectual disability, aminoacyl-tRNA synthetases, ARS, human cognition, cognitive impairment, brain development

INTRODUCTION

Cognitive impairment features among the most important problems in healthcare, one prominent example being intellectual disability (ID) with a prevalence between 1% and 3%. The majority of severe forms of ID have specific yet very heterogeneous genetic causes, including numerous X-chromosomal as well as autosomal gene defects and disease-causing copy-number variants[1-5]. Thus, with the exception of a few more prominent syndromes (for pertinent reviews see e.g., Salcedo-Arellano *et al.*[6], 2020, Glasson *et al.*[7], 2020, Antonarakis *et al.*[8], 2020), individual genes only account for an often extremely low proportion of cases.

Accumulating evidence, however, indicates that while there are no major players on a genetic level, there are functional contexts or pathways that play a prominent role in the etiology of hereditary forms of ID and are thus of major importance for the development and maintenance of higher cognitive functions. One such feature is the molecular and functional integrity of transfer RNA (tRNA), and we and others have recently put forward the notion that a full as well as a fully functional complement of tRNAs is vital for human cognition[9,10]. This is corroborated by the results of a survey of the recent literature, which shows a steep increase in the number of articles featuring tRNA-related issues in the context of impaired human cognition over the last few years [Figure 1]. In support of the hypothesis that tRNAs play a major role in the basis of human cognitive features, our review aims to provide a synopsis of the presently available literature on tRNA modifiers and aminoacyl-tRNA synthetases (ARSs) that were found to play a role in the etiology of cognitive dysfunction.

tRNA STRUCTURE AND FUNCTION

tRNAs are important mediator molecules that facilitate the reading and translation process of the triplet genetic code from messenger RNA (mRNA) to corresponding polypeptides during protein biosynthesis[11]. The human genome contains more than 500 tRNA genes[12]; however, tRNA expression is cell- and tissue-specific and approximately half of the genes are not or poorly expressed[13].

The typical tRNA secondary structure, consisting of hydrogen-bonded stems and associated loops, is shown in Figure 2. This results in a complex three-dimensional folding of the molecule, so that in their tertiary structure all tRNAs assume an L-shape. The 3' end of this structure serves as the amino acid attachment site. The anticodon loop, which is exposed at the tip of the L-shape, is used for mRNA codon recognition. Base pairing with the first and third residue of the anticodon can be flexible so that some tRNAs can recognize various codons.

The translation of proteins from their coding mRNAs, where tRNAs play a central role, is an absolutely essential process. It begins with the formation of the pre-initiation complex, which is formed from the 40S subunit of a ribosome, the initiator $tRNA^{Met}$, GTP and various initiation factors. mRNA binds to this complex at its 5' end and translation is initiated when a start codon (AUG) is recognized. Elongation starts with the binding of the initiator tRNA to the peptidyl site of the ribosome, the second binding site of the ribosome, and the aminoacyl site is then occupied by the next tRNA. A peptide bond is formed between the methionine of the initiator tRNA and the amino acid of the following tRNA. The ribosome then moves one position further on the mRNA and binds another aminoacylated tRNA. This elongation continues until a stop codon is reached, after which the polypeptide leaves the ribosome[14]. This happens at a rate of approximately ten tRNAs per second.

To ensure that protein synthesis runs smoothly, tRNA molecules are chemically modified[15-18]. These alterations include methylation (guanosine → 7-methylguanosine), deamination (adenine → inosine), Sulfur substitution (uridine → 4-thiouridine), intramolecular rearrangements (uridine → pseudouridine) and the saturation of existing double bonds (uridine → dihydrouridine). Some of the non-standard ribonucleosides

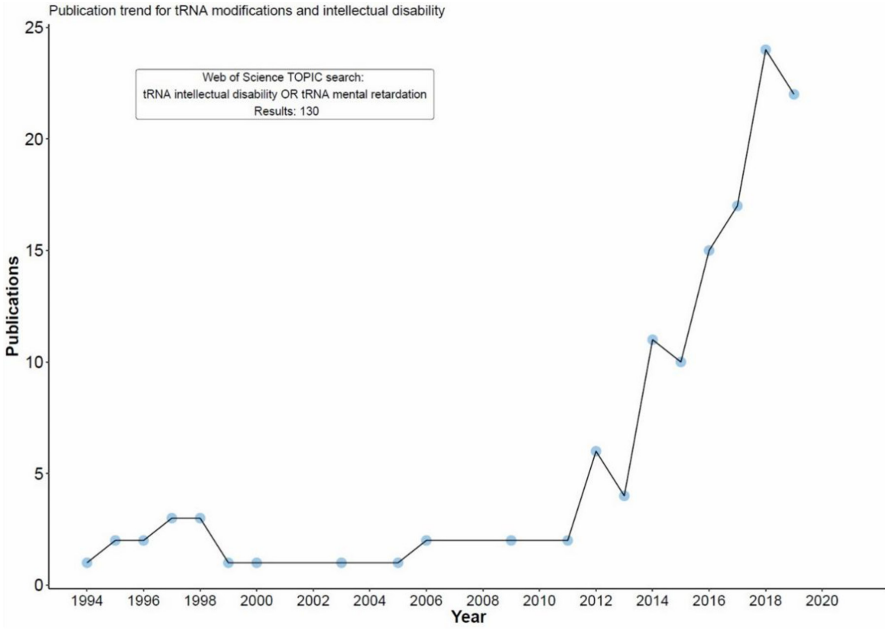

Figure 1. Number of articles featuring tRNA-related issues in the context of impaired human cognition between 1994 and the present

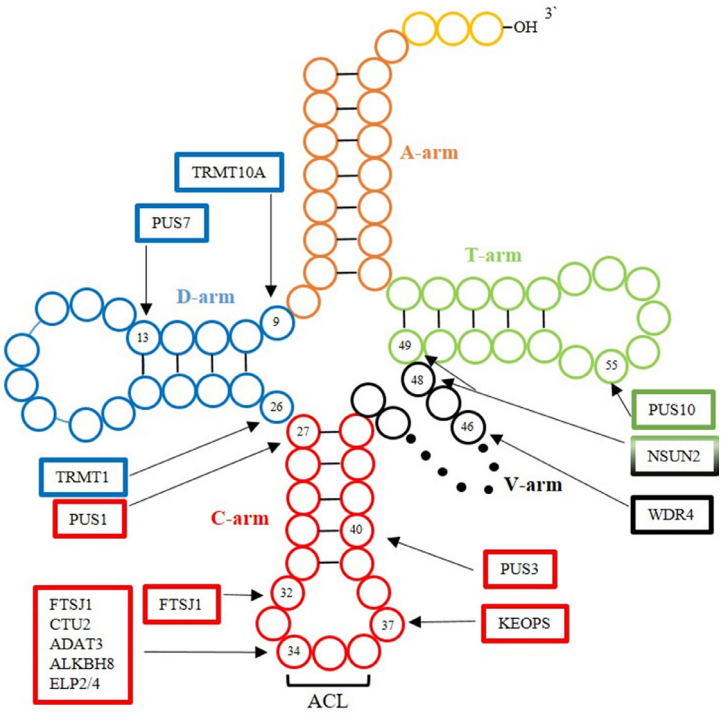

Figure 2. Overview of the main target nucleotides of the indicated tRNA modifiers involved in the etiology of ID. A-arm: acceptor stem; D-arm: dihydrouracil arm; C-arm: anticodon arm; ACL: anticodon loop; V-arm: variable arm; T-arm: ribothymidine arm; ID: intellectual disability

are believed to be important for tRNA stability and folding, or to improve codon-anticodon recognition[19-21]. The wobble-uridine modification 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U), for example, was found to be associated with improving codon-anticodon recognition[22-25], and mcm^5s^2U plays a role in improving tRNA binding to the ribosomal aminoacyl-tRNA binding site[26]. Without the modification, reduced binding at the aminoacyl-site leads to downstream effects, including slowing of the ribosomes and

associated protein folding defects[23,27-29]. Defects in tRNA modifications, which sometimes only represent a single atom, can trigger serious neurodegenerative diseases[30]. For example, if tRNA molecules lack only a single chemical group, protein biosynthesis can stop at innumerable sites in the mRNA (reviewed in Torres *et al.*[31] 2014). The result is an increase in protein aggregates that the cells can no longer remove. Nerve cells in particular are very sensitive to such aggregates, as is well known from Alzheimer's and Parkinson's diseases[27]. Moreover, ribosome profiling experiments have shown that ribosomes in cells with defects in tRNAs take longer to read certain sections of the mRNA[27]. The fact that protein biosynthesis does not occur at a constant rate plays a major role in this context, because changes in protein synthesis rate can influence protein conformation, as proteins take on their active form at the same time as they are produced[32].

Another important function in protein biosynthesis is performed by the ARSs. These enzymes are essential for translation, since they catalyze the binding of the proteinogenic amino acids to their respective associated tRNAs to form aminoacylated tRNAs.

There are 37 ARSs known - 17 occur only in the cytoplasm, 17 are mitochondria-specific, and three encode bifunctional proteins that charge tRNAs in both compartments[33]. It is known that mutations in genes coding for ARSs play an important role in many human inherited diseases, both with recessive and dominant inheritance patterns. In homozygous carriers, recessive mutations in ARSs often cause early-onset disorders with a severe course, not only affecting nerve cells but also impairing the function of many other tissues. A total of 31 of the 37 human ARSs have been linked to a genetic phenotype. These range from later-onset peripheral neuropathy to severe multi-system development syndromes[34-36] with ID.

In the following sections, we will first give an overview of tRNA modifiers that have been found to play a role in the etiology of hereditary forms of cognitive impairment, focusing on the major tRNA sites targeted by these enzymes. Subsequently, we will introduce the ID-associated ARSs known to date, based on their cytosolic or mitochondrial occurrence.

TRNA MODIFICATION AND ID

A list of currently known tRNA modifiers, which have been associated with ID, is given in Table 1 (see Part A).

The tRNA schematic in Figure 2 gives an overview over the main target nucleotides of tRNA modifiers involved in the etiology of ID, showing that there are 4 main sites that are of particular importance for human cognition: the C-arm (anticodon arm), V-arm (variable arm), D-arm (dihydrouridine-arm) and T-arm (ribothymidine arm).

Anticodon arm

The anticodon arm of a tRNA molecule contains the anticodon site and is the most heavily modified part of the tRNA molecule.

Currently, six different ID proteins catalyzing tRNA modifications in this tRNA region have been identified. These include the following enzymes.

FTSJ1

FTSJ1 (filamentous temperature-sensitive J, *E. coli* homolog 1) is an X-linked tRNA 2'-*O*-methyltransferase that catalyzes ribose methylation at tRNA positions 32 and 34. The homologous gene was originally isolated from an *E. coli* in 1991[178], and the crystal structure with the methyl donor *S*-adenosyl-methionine was later solved[179].

Yeast has been the model of choice for investigations concerning FTSJ1 as human FTSJ1 is able to complement yeast Trm7 growth defects[180]. In yeast, two different interaction partners have been identified

Table 1. List of currently known tRNA modifiers (Part A: modification) and aminoacyl-tRNA synthetases (Part B: aminoacylation) that have been associated with ID

Gene	Mutation	Modification	Chromosome location	Localization	Recessive/ dominant	ID	Microcephaly	Hypotonia	Ataxia	Seizures	Myopathy	Hypomyelination	Neuropathy	Others	Ref.
Part A: modification															
FTSJ1	c.[192-2A>G] (G65Cfs*18); c.[G121+1delG] (G41fs*V*19); c.[196C>T] (Q66*); c.[655G>A] (D219N)	Cm, Gm, ncm^5Um	Xp11.23	Cytopl	Recessive	x									[37-54]
NSUN2	c.[679C-T] (Q227X); c.[1114C-T] (Q372X); c.[2035G-A] (G679R)	m^5C	5p15.31	Nucleus	Recessive	x	x							x	[46-55]
WDR4	c.[509G-T] (R170L); c.[509G-A] (R170Q); c.[491A-C] (D164A); c.[940dupC] (L314Pfs*16); c.[911_927dup] (Q310Gfs*30)	m^7G	21q22.3	Cytopl/ nucleus	Recessive	x	x								[56-63]
TRMT1	c.[1506+1G-T]; c.[1332_1333delGT] (Y445Lfs*28); c.[657_688del32] (Q219Hfs*22)	m^2_2G	19p13.13	Mito/nucleus	Recessive	x	x							x	[64-70]
ADAT3	c.[382G-A] (V128M); c.[99_106dupGAGCCCGG] (E36Gfs*44)	I	19p13.3	Cytopl	Recessive	x	x							x	[71-77]
ALKBH8	c.[1660C-T] (R554X); c.[1794delC] (W599Gfs*19)	mcm^5U, mcm^5s^2U	11q22.3	Cytopl/ nucleus	Recessive	x									[78-81]
KEOPS	c.[407T-C] (L136P); c.[446A-G] (Y149C)	t^6A	2p13.1	Cytopl/ nucleus	Recessive	x	x							x	[82-85]
TRMT10A	c.[379G-A] (R127X); c.616G-A (G206R)	m^1G	4q23	Nucleus	Recessive	x	x							x	[86-92]
ELP2	[chr18.31,993,951A-C] (T555P); [chr18.31,990,536G-T] (R462L); c.[1579C-T] (R527W); c.[812A-G] (H271R)	mcm^5U, mcm^5s^2U	18q12.2	Cytopl/ nucleus	Recessive	x								x	[67,93-98]
ELP4	chr [11: 31,685,945] (G>T)	mcm^5s^2U	11p13	Cytopl/ nucleus	Dominant	x								x	[97-101]
CTU2	c.[873G-A] (T247T)	mcm^5s^2U	16q24.3	Cytopl	Recessive	x	x							x	[102-107]
PUS3	c.[1303C-T] (R435X); c.[1181_1182delCT] (S394Cfs*18)	Ψ	11q24.2	Cytopl/ nucleus	Recessive	x	x							x	[56,108-111]
PUS1	C.[656C-T] (R116W); c.[658G-T] (E220X); c.[883C-T] (R295W)	Ψ	12q24.33	Cytopl	Recessive	x								x	[112-117]

PUS7	c.[89_90del] (T30Kfs*20); c[.329_332delCTGA] (T110Rfs*4); c.[1348C-T] (R450X); c.[1507G-T] (D503Y)	Ψ	7q22.3	Nucleus	Recessive	x							[111,118,119]
PUS10		Ψ	2p16.1-p15	Cytopl/ nucleus	Recessive	x						x	[56,107,110,120,121]
Part B: aminoacylation													
AARS	c.[242A-C] (K81T); c.2251A-G (R751G)		16q22.1	Cytopl	Recessive	x	x	x		x			[122,123]
RARS	c.[5A-G] (D2G); c.[45+1G-T]; c.[1535G-A] (R512Q); c.[1367C-T] (S456L); c.[1846_1847delTA] (Y61Lfs*6); c.[96_97del] (C32Wfs*39)		5q34	Cytopl	Recessive	x	x		x		x		[124,125]
DARS	c.[1459C-T] (R487C); c.[1099G-T] (D367Y); c.[821C-T] (A274V); c.[766A-C] (M256L); c.[1379G-A] (R460H); c.[1480C-T] (R494G)		2q21.3	Cytopl	Recessive	x	x	x	x	x	x		[126]
LARS	c.[1118A-G] (Y373C); c.[245 A-G] (K82R)		5q32	Cytopl	Recessive	x	x	x		x			[126-129]
MARS	c.[1700C-T] (S567L); c.[1177G-A](A393T)		12q13.3	Cytopl	Recessive	x	x	x					[130-132]
YARS	c.[586G-A] (E196K); c.[241_242GA-AT] (D81I)		1p35.1	Cytopl	Recessive	x	x	x					[133]
IARS	c.[3521T-A] (I1174N); c.[1310C-T] (P437L); c.[2974A-G] (N992D); c.[2215C>T];[1667 T>C] (R739C); c.[1252C>T];[3521 T>A] (R418X); c.[760C>T];[1310C>T] (R254X); c.[1109 T>G];[2974A>G] (V370G)		9p22.31	Cytopl	Recessive	x		x				x	[134-138]
SARS	c.[514G-A] (D172N)		1p13.3	Cytopl	Recessive	x	x		x	x		x	[9,129,139]
VARS	c.[2653C-T] (L885F); c.[3173G-A] (R1058Q); c.[3192G-A] (M1064I); c.[2840G-A] (R947H); c.[3355C-T] (R1119C); c.[1300C-G] (L434V); c.[2464G-A] (G822S); c.[2825G-A] (R942Q); c.[1210C-T] (R404W); c.[219_232dup] (L78Rfs*35)		6p21.33	Cytopl	Recessive	x	x			x			[129,140-145]

QARS	c.[1426G>A] (V476I); c.[1207C-T] (R403W); c.[134G-T] (G45V); c.[1543C-T] (R515W)	3p21.31	Cytopl/mito	Recessive	x	x			x		x		x	[146-151]
KARS	c.[398T-A] (L133H); c.[517T-C] (Y173H); c.[1129G-A] (D377N)	16q23.1	Cytopl/mito	Recessive	x							x	x	[129,152-158]
NARS2	c.[822G-C] (Q274H); c.[641C-T] (P214L); c.[969T-A] (Y323X); c.[1142A-G] (N381S); c.[637G-T] (V213F); c.[167A-G] (Q56R); c.[631T-A] (F211I); c.[707T-G] (F236C); c.[1184T-G] (L395R); c.[151C-T] (R51C); c.[500A-G] (H167R)	11p14.1	Mito	Recessive	x				x	x				[159-164]
PARS2	c.[836C-T] (S279L); c.[1091C-G] (P364R); c.[239T-C] (I80T); c.[283G-A] (V95I); c.[607G-A] (E203K); c.[604G-C] (R202G); c.[1130dupC] (K378fs*1)	1p32.3	Mito	Recessive	x				x	x				[160,163,165-168]
RARS2	c.[1024A-G] (M342V); c.[35A-G] (Q12R)	6q15	Mito	Recessive	x								x	[166,169-173]
WARS2	c.[37T-G] (W13G); c.[938A-T] (K313M); c.[134G-T] (G45V); c.532G-C (V178L) c.[298_300delCTT] (delK100); c.[797delC] (Pro266Rfs*10); c.[325delA] (S109Afs*15)	1p12	Mito	Recessive	x	x		x	x				x	[9,159,174-176]

cytopl: localization in cytoplasm; mito: localization in mitochondria; others: prenatal growth retardation, speech and hearing impairment, aggressive behavior, encephalocardiomyopathy, pontocerebellar hypoplasia, multiple respiratory chain complex defects, infantile onset developmental delay; abbreviations for tRNA modifications are according to the MODOMICS database[177]

that are necessary for methylation at positions 32 and 34 in the anticodon loop, respectively. Trm7 interacts with Trm732 to methylate tRNAs encoding Trp (CCA), Phe (GAA) and Leu (UAA) at position 32 and with Trm734 to methylate at position 34 of $tRNA^{Trp}$ (CCA), $tRNA^{Phe}$ (GAA) and $tRNA^{Lys}$ ($cmnm^5UmUU$)[181]. The $tRNA^{Lys}$ residue $cmnm^5Um$ is a 5-carboxymethylaminomethyl 2'-*O*-methyluridine. The human homologs of Trm732 and 734 are *THADA* and *WDR6*, respectively. So far, *WDR6* has not been found mutated in a Mendelian disorder, whereas translocations disrupting *THADA* have been found in certain thyroid adenoma cells[182]. However, the tRNA modification status in these cells has not been investigated.

Yeast is a good model for molecular investigations into tRNA modifications, but far from identical to the human situation. For example, yeast Trm7 methylates tRNAs encoding phenylalanine, leucine and tryptophan whereas human FTSJ1 methylates tRNAs encoding phenylalanine, asparagine, glutamine, alanine and methionine[181].

In ID patients, protein-truncating mutations have been reported in 5 families[37,41,183], and a missense change has also been found in patients with non-syndromic ID[180]. In addition, duplication or microdeletions involving *FTSJ1* and other ID genes were also found in ID families[184-187]. Recently, a mouse model for *FTSJ1* deficiency was reported. In combination with a mild ID phenotype, these mice presented with additional phenotypic features, some of which were also found in affected humans upon reexamination of patients who were previously considered to have a non-syndromic phenotype[40].

ADAT3

ADAT3 (adenosine deaminase TRNA specific 3) is part of an enzyme complex involved in inosine formation through hydrolytic deamination of adenosine at the tRNA wobble position. This protein was also first characterized in yeast and is specific for modification of the tRNA wobble position. In yeast, Adat3 complexes with Adat2 to function as a deaminase, and the coding genes for both are essential for yeast viability[73]. In human cells, ADAT2 and 3 form a complex, localized in the nucleus that is required for inosine formation at the tRNA precursor level[188].

ID caused by *ADAT3* mutations is inherited in an autosomal recessive manner and several consanguineous families have been analyzed mainly in the Middle East. *ADAT3* mutations were first identified in 24 individuals from eight consanguineous Arab ID families that all presented with ID and strabismus[71]. The missense change c.382G>A, V128M, is located in an ancient haplotype that is approximately 1600 years old and considered to be the most common cause for autosomal recessive ID in Arabia[71]. Other clinical symptoms in ID patients with the *V128M* mutation apart from ID and strabismus were reported to include growth failure, microcephaly and tone abnormality[72]. The authors concluded that despite a distinct facial profile, this syndrome should be considered also for ID patients from apparently non-consanguineous ID families originating from Arabia[72]. Recently, an 8-bp duplication in *ADAT3* was found in a patient with mild ID, microcephaly and hyperactivity but without strabismus[75].

In a patient cell line, it was recently shown that the *ADAT3* mutation *V128M* indeed reduces adenosine deaminase activity and inosine formation at the tRNA wobble position[74].

ALKBH8

ALKBH8 (alkylated DNA repair protein AlkB homolog 8) was originally investigated because it is expressed in human cancers, including bladder cancer[80]. Knockdown of ALKBH8 in cell lines has shown several effects including reduced H_2O_2 generation, induction of JNK- and p38-mediated apoptosis, phosphorylation of the histone 2 variant H2AX and reduced gall bladder cancer growth[80]. In a mouse model for ALKBH8 deficiency, Alkbh8 was identified as a methyltransferase necessary for 5-methoxycarbonylmethyluridine (mcm^5u) formation of wobble uridine residues[189]. Generation of mcm^5u is required for ALKBH8 hydroxylation of wobble uridine to 5-methoxycarbonylhydroxymethyluridine in certain tRNAs[78,190]. Although Alkbh8-deficient mice seemed normal, the authors observed aberrant modification of selenocysteine-specific $tRNA^{Sec}$[189].

Recently, truncating *ALKBH8* mutations were found in ID patients from two consanguineous families with different mutations (c.1660C>T, p. Arg554Ter and c.1794delC, Trp599GlyfsTer19). tRNA from the investigated patients showed complete loss of wobble uridine modifications. All seven investigated patients had ID and showed global developmental delay. Out of the seven patients, only one affected sister did not present with epilepsy[79].

CTU2

CTU2 (cytosolic thiouridylase 2) is also a highly conserved gene that was first identified in yeast and found necessary for tRNA thiolation in yeast, *C. elegans* and even plants[102,104,191]. In these organisms, CTU1 and CTU2 homologs form a complex that catalyzes tRNA thiolation of wobble uridine. Inactivation of the complex leads to loss of thiolation at the tRNA wobble uridine and abnormal phenotypes[102,104,191]. Interestingly, proteins involved in thiolation of the uridine wobble base are also important for the altered protein synthesis driven by the $BRAF^{V600E}$ oncogene transformation in melanomas, and melanomas depend on these tRNA-modifying proteins for survival[103].

The first human *CTU2* mutations were reported in three families from Saudi Arabia and two families from the United Arab Emirates, and they were all homozygous for the same haplotype and splice site mutation (c.873G>A, Thr247AlafsTer21). The affected individuals presented with dysmorphic faces, renal agenesis, ambiguous genitalia, polydactyly and lissencephaly, and the authors suggested the acronym DREAM-PL for this syndromic form of ID[105,107].

Five more patients with the DREAM-PL phenotype were recently reported, all showing a reduced ratio of thiolated wobble uridine to unmodified wobble uridine[106].

KEOPS

KEOPS (kinase, endopeptidase and other proteins of small size) and the KEOPS complex were originally identified in yeast as a complex involved in telomere capping and elongation[192]. In 2010, yeast KEOPS was found to be necessary for N^6-threonyl-carbamoyl-adenosine modification of yeast tRNA adenosine (t^6A), which is present at position 37 in all tRNAs that pair with ANN codons[193]. Although telomere regulation seems to be independent of t^6A modifications[66], yeast cells lacking t^6A modifications show severe growth defects.

The human and yeast KEOPS complex each consist of four homologous subunits (OSGEP, TP53RK, TPRKB, LAGE3 and kae1, Bud32, Cgi121, Pcc1, respectively), and mutations were found in genes encoding any of the four subunits in Galloway-Mowat syndrome (GAMOS, MIM#251300) patients[83]. These patients were all affected by early-onset nephrotic syndrome, primary microcephaly, developmental delay and propensity for seizures of which most patients died in early childhood[83]. None of the patients carried truncating mutations on both alleles[83].

Due to the multiple functions involving the KEOPS complex it is difficult to determine the effect of a missing t^6A modification on the patient phenotype. However, as overlapping phenotypes are observed in patients with *WDR4* mutations, missing t6A modifications are likely to contribute to the observed GAMOS phenotype[82].

ELP2 and ELP4

The Elongator protein complex (ELP) is composed of six highly conserved subunits (ELP1-6) and, as the name suggests, was initially thought to promote elongation of transcription. Recently, it was discovered that its primary role is to modify the uridine at position 34 of tRNAs (mcm^5s^2U)[194,195]. Mutations in two of the Elongator subunits have been linked to ID. Missense mutations in the *ELP2* gene have been identified in three families with ID[67,93]. Microdeletions in the *ELP4* gene have been linked to ID and speech delay, although deletion of part of the regulatory regions of *PAX6* may contribute to the phenotype[99,100]. Previously, mutations in the *ELP4* gene have been implicated in Rolandic epilepsy[101]. It is now accepted that the diverse disease phenotypes caused by defects in Elongator are likely due to hypomodified tRNAs, but it remains to be seen whether rescue experiments with elevated tRNA levels prevent the phenotypes in multicellular organisms[97,98].

PUS1, PUS3 and PUS7

Pseudouridine is a common tRNA modification, and to date, three ID proteins that play a role in pseudouridinylation have been identified. These are PUS1, PUS3 and PUS7 (pseudouridine synthases), which are involved in the conversion of uridine to pseudouridine at different specific tRNA positions.

Yeast Pus1 was the first eukaryotic tRNA pseudouridine synthase to be characterized and shown to be involved in the conversion of tRNA uridines at multiple positions of introns containing $tRNA^{Ile}$[196]. Pus1 targets both cytoplasmic and mitochondrial tRNA[112] and was later shown also to target U2 snRNA in yeast[115].

The first reported human *PUS1* mutation was a homozygous missense change (*R116W*) found in all affected individuals in two Italian families who suffered from mitochondrial myopathy and sideroblastic anemia (MLASA; MIM 600462) but without ID[113]. tRNA pseudouridinylation was later shown to be greatly reduced in patient cell lines[116]. *PUS1*-dependent ID was first reported in a patient with the same (*R116W*) missense change by Zeharia *et al.*[117]. In two brothers with MLASA and a truncating *PUS1* mutation (*E220X*), one had ID whereas the other had an elevated intelligence quotient above normal levels[114].

PUS3 is a pseudouridine synthase, originally isolated from yeast, that catalyzes pseudouridine formation at positions 38 and 39 in the anticodon stem of certain tRNAs. Yeast Pus3 deletion strains are viable but grow slowly, especially at elevated temperatures[197]. The protein was found to be evolutionarily conserved, and like mouse Pus3, it can convert uridine at position 38 or 39 to pseudouridine in yeast and human tRNA *in vitro*, albeit with different efficiency[109].

ID caused by PUS3 deficiency is inherited as an autosomal recessive disorder. The first report of *PUS3* mutations described 3 affected sisters that were homozygous for the nonsense mutation c.1303C>T, R435X, and the phenotype in these patients was largely brain specific[110]. A second report presented a single child from consanguineous parents, carrying a frameshift mutation (c.1181_1182delCT, Ser394CysfsTer18) and no detectable PUS3 transcript. The child suffered from ID, microcephaly, hypotonia, seizures, and vision and hearing loss[56]. Furthermore, two compound heterozygous mutations were reported in a Brazilian and a Chinese family[198,199]. Although all reported patients presented with additional features, ID was the only consistent characteristic.

PUS7 is a multi-substrate pseudouridine synthase that in yeast targets several tRNA uridines at position 13, the pre-$tRNA^{Tyr}$ at position 35[200], small nucleolar RNA U2 (U2 snRNA) at position 35[201] and also 5S and 5.8S rRNA[202] and mRNA[203]. Interestingly, uridine conversion of snRNA U2 at positions 56 and 93 can be induced in yeast by nutrient deprivation or heat shock[204]. In human stem cells, PUS7 pseudouridinylation was found to activate small tRNA-derived fragments that inhibit protein synthesis by targeting the initiation complex. PUS7 inactivation leads to defective germ layer specification[205].

Homozygous truncating *PUS7* mutations were recently reported to cause ID with speech delay, short stature, microcephaly, and aggressive behavior in patients from three different families[118]. Two ID families with homozygous *PUS7* mutations, a missense change or a deletion leading to a frameshift, were also reported. The patients also suffered from microcephaly, whereas short stature was not seen in all patients[111]. Recently, another ID family of Afghan origin was reported, carrying a Gly128Arg missense change. The phenotype of the patient was milder without microcephaly or short stature, but still with speech delay and aggressive behavior[119]. In this last study, pseudouridine levels were not investigated[119], whereas markedly reduced pseudouridine levels at tRNA position 13 were found in all investigated ID patients[111,118].

Variable arm

The variable arm of tRNAs is located between the anticodon (or C) and the T arms. The length of the variable arm depends on the tRNA and can be between 3 and 21 nucleotides long. Generally speaking, class

I tRNAs have shorter variable arms (between 4-5 nucleotides) than class II tRNAs (> 10 nucleotides)[206,207]. The variable arm functions as a stabilizer of the tertiary structure as well as in the specific recognition of the ARS. So far, two modifications of nucleotides in the variable arm by two different genes have been linked to ID.

NSUN2

NSUN2 (Nop2/Sun RNA methyltransferase family member 2) is one of three cytosine-5 tRNA methyltransferases and is responsible for methylating tRNAs that carry a cytosine at positon 48 or 49. There have been several reports linking mutations in the *NSUN2* gene to ID[46,49,51,208]. Two reports observed a Dubowitz-like syndrome in patients[46,51]. Other common symptoms described include microcephaly, facial dysmorphism and growth retardation.

The likely molecular mechanism in NSUN2-deficient cells is increased angiogenin-induced fragmentation of tRNA which inhibits protein translation[55]. Methylation of cytosine at the variable loop in healthy cells protects tRNAs from binding to angiogenin.

WDR4

WDR4 (WD repeat domain 4) encodes the noncatalytic subunit of the tRNA (guanine-N7-)-methyltransferase which is necessary for the 7-methylguanosine modification (m^7G) at position 46[62]. It has been described to cause primordial dwarfism, a phenotypically diverse syndrome with several subtypes, characterized by ID as well as pre- and postnatal growth deficiency[58,62,63]. More recently, WDR4 deficiency has also been linked to the Galloway-Mowat syndrome[57]. WDR4 knockouts result in a complete loss of m^7G modification in tRNAs and consequently to disturbed codon recognition and ribosome stalling. It has also been shown that depletion of WDR4 in mice impairs the neural lineage differentiation capacity in mESCs[60].

D-arm

The D-arm of tRNAs is located between the anticodon and acceptor arms. It is of variable length, but the modification of the D-loop nucleotides is highly conserved in all kingdoms. Its function is mainly the stabilization of tRNA structure through tertiary interaction with the T-arm, but it is also involved in aminoacyl tRNA synthase recognition. Defects in two tRNA methyltransferases that modify different positions in the D-arm have been shown to cause ID.

TRMT1

The *TRMT1* (TRNA methyltransferase 1) gene encodes for a tRNA methyltransferase that dimethylates G at position 26 in the D-arm of most tRNAs. It was first connected to non-syndromic ID in a deep sequencing-based screen for novel genes for cognitive disorders in 2011[67]. More recent reports confirm this finding and describe facial dysmorphism, general developmental delay and in some cases muscle weakness and spasticity as TRMT1-specific symptoms in patients[64,65,70]. Apart from decreased protein translation and cell proliferation, TRMT1-deficient cells show disturbed redox homeostasis and hypersensitivity to oxidative reagents, which might explain some of the neurological defects observed[69]. The causative mechanism at the tRNA level is still unclear; however, loss of m^2_2G could affect tRNA structure or stability[209,210] or modulate translation activity[211].

TRMT10A

TRMT10A (TRNA methyltransferase 10A) is a tRNA methyltransferase that is responsible for methylating the G at position 9 of tRNAs (m1G9). A missing, shortened or otherwise non-functioning *TRMT10A* gene causes ID, microcephaly and general developmental delay[86]. Interestingly, some reports describe early-onset diabetes or hypoglycemia in patients with mutations in the gene[86,88,91,92].

A lack of m^1G_9 modification in yeast has been shown to play a role in tRNA stability and translation terminating efficiency[212,213]. In human $tRNA^{Lys}$, which has an adenine at position 9, a lack of methylation prevented the tRNA to be folded into the cloverleaf form[214]. However, how exactly the lack of methylation is connected to the variety of symptoms is still not fully understood and continues to be the subject of ongoing research.

T-arm

PUS10

So far, no modifications on the T-arm of tRNAs have been shown to cause ID specifically. While microduplications or -deletions of the 2p16.1p15 locus, which contains the pseudouridine synthase 10 gene (*PUS10*) among several other genes, have been linked to ID and developmental and speech delay[215-217], there is growing evidence that in these cases, BCL11A is the cause for ID[218,219]. Still, it cannot be ruled out that PUS10, which pseudouridinylates tRNAs at positions 54 and 55, contributes to the phenotype, but clinical cases with PUS10-specific mutations linked to ID have not yet been described so far.

ARSS AND ID

Cytoplasmic ARSs

The main task of ARSs is to transfer and bind amino acids to the appropriate tRNA molecules. The charged tRNAs are then used by the ribosomes to carry out protein synthesis. Their availability therefore plays an essential role in the regulatory processes of cell functions[220]. All ARSs are ubiquitously expressed and highly conserved. There is one ARS enzyme for each amino acid to facilitate binding with the appropriate tRNA. Of the 37 known *ARS* genes, 17 encode purely cytoplasmic enzymes[33]. Like mitochondrial ARSs (mt-ARSs), all cytosolic ARSs (ct-ARSs) are encoded by nuclear genes. They are complemented by three ARSs that function in both the cytoplasm and mitochondria to match the full complement of amino acids. It has already been mentioned that biallelic mutations in 31 ARS genes lead to serious recessive, early onset diseases, ranging from later-onset peripheral neuropathy to severe multi-system development syndromes. Here, however, we will focus only on ARSs, which have been found to play a role in the etiology of diseases associated with ID [Table 1, see Part B].

In VARS, for example, Friedman *et al.*[140] found different biallelic mutations in several families, leading to a very heterogeneous symptomatic picture including, developmental delay, epileptic encephalopathy and primary or progressive microcephaly. Another interesting case is the glutaminyl-tRNA synthetase gene (*QARS*). This gene encodes both the cytosolic as well as the mitochondrial *QARS* and shows a strong level of expression in the brain of the developing fetus. A very often found missense mutation (*V476I*) in *QARS* was shown to cause a reduction in its aminoacylation activity[148]. Mutations in *QARS* have severe consequences in affected individuals including not only ID but also progressive microcephaly, cerebral cerebellar atrophy and seizures that are difficult to treat. Altogether 11 patients have so far been described with *QARS* mutations[147,149,151,221], all of whom consistently show a severe so-called global development delay but none reaching any significant milestone. An initially normal occipito-frontal circumference (OFC) quickly and clearly changed to postnatal microcephaly. Various degrees of severity of ID from mild to severe were described in several case studies [Table 1B]. In addition to other serious symptoms, the condition is ultimately fatal for a large proportion of patients[148]. These examples shows the breadth and variability of the phenotypic spectrum associated with *ARS* mutations.

There are, however, recurrent motives among the features accompanying ARS-dependent ID, such as microcephaly, which is observed in carriers of mutations in *AARS, RARS, DARS, LARS, MARS, YARS, QARS, SARS, VARS* and *WARS2* [Table 1B]. An association with the occurrence of seizures (*AARS, DARS, LARS, SARS, VARS, QARS, NARS2, PARS2 and WARS2*) and hypotonia (*AARS, DARS, LARS, MARS, YARS and IARS*) is also frequently observed. Less common features among affected individuals range from ataxia,

cerebral atrophy, neonatal choleastasis, muscular hypotension, infantile hepatopathy and hypomyelination to speech disorders and aggressive behavior. Finally, it should be mentioned that the non-canonical functions of ARSs could also be responsible for the wide phenotypic spectra that can be observed in the diseases related to their mal- or dysfunction.

Mitochondrial ARSs

Human mitochondrial ARSs (mt-ARSs) are essential for the synthesis of 17 mt-DNA-encoded proteins, which are all subunits of the respiratory chain complexes. Therefore, they are involved in the generation of the major source of cellular energy, i.e., ATP. Like cytosolic ARSs, all mt-ARSs are encoded by nuclear genes, which are, however, different from those coding for the cytosolic ARSs. Three *ARS* genes encode enzymes that are active in both mitochondria and cytosol: glycyl-tRNA synthetase (GARS), lysyl-tRNA synthetase (KARS), and glutaminyl-tRNA synthetase (QARS). Only QARS, however, has so far been found to be associated with an ID phenotype [Table 1B]. The first correlation between an mt-ARS mutation and a human disorder was published in 2007 by Scheper *et al.*[222], who found autosomal recessive mutations in the *DARS2* gene in individuals suffering from leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL). Since then, numerous other pathogenic mutations in mt-ARSs have been described, so that to date, at least 17 out of the 19 *mt-ARSs* genes have been implicated in human genetic disorders involving damage to the central nervous system[35].

It is noteworthy at this point that in 2017, Moulinier *et al.*[223] introduced MiSynPat, an integrated knowledge base that links clinical, genetic, and structural data for disease-causing mutations in human *mt-ARSs*. According to the authors, this tool provides a "comprehensive knowledge base together with an ergonomic Web server designed to organize and access all pertinent information (sequences, multiple sequence alignments, structures, disease descriptions, mutation characteristics, original literature) (http://misynpat.org/misynpat/AboutMisynpat.rvt last accessed 2020-01-09).

Mutations in at least six *mt-ARS* genes (Table 1B - aminoacylation, including *QARS*) are involved in the etiology of ID. All of these lead to a syndromic phenotype. Mutations in *NARS2* and *PARS2*, for example, cause Alpers syndrome, and homozygous *RARS2* defects lead to pontocerebellar hypoplasia, which is characterized by not only overall delayed development, impaired brain development, movement problems and ID but also progressive atrophy, particularly of the pons and cerebellum. *WARS2* mutation carriers show a phenotype that is very similar to patients with mutations in cytosolic *SARS* (Table 1B - aminoacylation). Other than that seen for ct-ARSs, there are no clearly prominent recurrent motives in homozygous or compound heterozygous carriers of mt-ARS mutations (Table 1B - aminoacylation) with the possible exception of seizures that are observed with a notably increased frequency (*NARS2*, *PARS2* and *QARS*).

CONCLUSION

The literature compilation we present here makes a compelling case for an important if not pivotal role of a fully functional tRNA complement for the development and maintenance of higher cognitive functions. Interestingly, disease-causing *ARSs* mutations often only result in a reduction of enzyme activity without causing complete inhibition[158,224,225]. This points to the sensitivity of cognitive features towards even slight disturbances in this basic cellular process.

In addition, there is much evidence that tRNA molecules assume possibly unknown biological functions in eukaryotes, which have not yet been fully elucidated[17] but could be influenced by disruption of tRNA function. This opens up a myriad of further possibilities for tRNA involvement in the formation of cognitive features and underlines the importance of further research in this field.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the article, performed literature research and interpretation and were involved in the writing and editing of the manuscript as well: Franz M, Hagenau L, Jensen LR, Kuss AW

Franz M and Hagenau Lcontributed equally to the article.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Musante L, Ropers HH, Genetics of recessive cognitive disorders. Trends Genet 2014;30:32-9.
2. Chiurazzi P, Pirozzi F, Advances in understanding - genetic basis of intellectual disability. F1000Res 2016;5:F1000 Faculty Rev-599.
3. Khan MA, Khan S, Windpassinger C, Badar M, Nawaz Z, et al. The molecular genetics of autosomal recessive nonsyndromic intellectual disability: a mutational continuum and future recommendations. Ann Hum Genet 2016;80:342-68.
4. Jamra R, Genetics of autosomal recessive intellectual disability. Med Genet 2018;30:323-7.
5. Hu H, Kahrizi K, Musante L, Fattahi Z, Herwig R, et al. Genetics of intellectual disability in consanguineous families. Mol Psychiatry 2019;24:1027-39.
6. Salcedo-Arellano MJ, Dufour B, McLennan Y, Martinez-Cerdeno V, Hagerman R. Fragile X syndrome and associated disorders: clinical aspects and pathology. Neurobiol Dis 2020;136:104740.
7. Glasson EJ, Buckley N, Chen W, Leonard H, Epstein A, et al. Systematic review and meta-analysis: mental health in children with neurogenetic disorders associated with intellectual disability. J Am Acad Child Adolesc Psychiatry 2020:S0890-8567(20)30008-3.
8. Antonarakis SE, Skotko BG, Rafii MS, Strydom A, Pape SE, et al. Down syndrome. Nat Rev Dis Primers 2020;6:9.
9. Musante L, Puttmann L, Kahrizi K, Garshasbi M, Hu H, et al. Mutations of the aminoacyl-tRNA-synthetases SARS and WARS2 are implicated in the etiology of autosomal recessive intellectual disability. Hum Mutat 2017;38:621-36.
10. Abedini SS, Kahrizi K, de Pouplana LR, Najmabadi H. tRNA methyltransferase defects and intellectual disability. Arch Iran Med 2018;21:478-85.
11. Pan T. Modifications and functional genomics of human transfer RNA. Cell Res 2018;28:395-404.
12. Chan PP, Lowe TM. GtRNAdb 2.0: an expanded database of transfer RNA genes identified in complete and draft genomes. Nucleic Acids Res 2015;44:D184-9.
13. Torres AG. Enjoy the silence: nearly half of human trna genes are silent. Bioinform Biol Insights 2019;13:1177932219868454.
14. Matthaei JH, Jones OW, Martin RG, Nirenberg MW. Characteristics and composition of rna coding units. Proc Natl Acad Sci U S A 1962;48:666-77.
15. Nishikura K, De Robertis EM. RNA processing in microinjected Xenopus oocytes. Sequential addition of base modifications in the spliced transfer RNA. J Mol Biol 1981;145:405-20.
16. Jiang HQ, Motorin Y, Jin YX, Grosjean H. Pleiotropic effects of intron removal on base modification pattern of yeast tRNAPhe: an in vitro study. Nucleic Acids Res 1997;25:2694-701.
17. Phizicky EM, Hopper AK. tRNA biology charges to the front. Genes Dev 2010;24:1832-60.
18. Ohira T, Suzuki T. Retrograde nuclear import of tRNA precursors is required for modified base biogenesis in yeast. Proc Natl Acad Sci U

S A 2011;108:10502-7.
19. Agris PF, Eruysal ER, Narendran A, Vare VYP, Vangaveti S, et al. Celebrating wobble decoding: half a century and still much is new. RNA Biol 2018;15:537-53.
20. Vare VY, Eruysal ER, Narendran A, Sarachan KL, Agris PF. Chemical and conformational diversity of modified nucleosides affects trna structure and function. Biomolecules 2017;7:29.
21. Grosjean H, Westhof E. An integrated, structure- and energy-based view of the genetic code. Nucleic Acids Res 2016;44:8020-40.
22. Johansson MJ, Esberg A, Huang B, Bjork GR, Bystrom AS. Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. Mol Cell Biol 2008;28:3301-12.
23. Rezgui VA, Tyagi K, Ranjan N, Konevega AL, Mittelstaet J, et al. tRNA tKUUU, tQUUG, and tEUUC wobble position modifications fine-tune protein translation by promoting ribosome A-site binding. Proc Natl Acad Sci U S A 2013;110:12289-94.
24. Vendeix FA, Murphy FVt, Cantara WA, Leszczynska G, Gustilo EM, et al. Human tRNA(Lys3)(UUU) is pre-structured by natural modifications for cognate and wobble codon binding through keto-enol tautomerism. J Mol Biol 2012;416:467-85.
25. Ranjan N, Rodnina MV. Thio-modification of tRNA at the wobble position as regulator of the kinetics of decoding and translocation on the ribosome. J Am Chem Soc 2017;139:5857-64.
26. Roovers M, Oudjama Y, Kaminska KH, Purta E, Caillet J, et al. Sequence-structure-function analysis of the bifunctional enzyme MnmC that catalyses the last two steps in the biosynthesis of hypermodified nucleoside mnm5s2U in tRNA. Proteins 2008;71:2076-85.
27. Nedialkova DD, Leidel SA. Optimization of codon translation rates via tRNA modifications maintains proteome integrity. Cell 2015;161:1606-18.
28. Tukenmez H, Xu H, Esberg A, Bystrom AS. The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes. Nucleic Acids Res 2015;43:9489-99.
29. Klassen R, Bruch A. Schaffrath R. Independent suppression of ribosomal +1 frameshifts by different tRNA anticodon loop modifications. RNA Biol 2017;14:1252-9.
30. Woese CR, Olsen GJ, Ibba M, Soll D. Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. Microbiol Mol Biol Rev 2000;64:202-36.
31. Torres AG, Batlle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. Trends Mol Med 2014;20:306-14.
32. Pechmann S, Willmund F, Frydman J. The ribosome as a hub for protein quality control. Mol Cell 2013;49:411-21.
33. Antonellis A, Green ED. The role of aminoacyl-tRNA synthetases in genetic diseases. Annu Rev Genomics Hum Genet 2008;9:87-107.
34. Meyer-Schuman R, Antonellis A. Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. Hum Mol Genet 2017;26:R114-27.
35. Sissler M, Gonzalez-Serrano LE, Westhof E. Recent advances in mitochondrial aminoacyl-tRNA synthetases and disease. Trends Mol Med 2017;23:693-708.
36. Fuchs SA, Schene IF, Kok G, Jansen JM, Nikkels PGJ, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. Genet Med 2019;21:319-30.
37. Freude K, Hoffmann K, Jensen LR, Delatycki MB, des Portes V, et al. Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. Am J Hum Genet 2004;75:305-9.
38. Hamel BC, Smits AP, van den Helm B, Smeets DF, Knoers NV, et al. Four families (MRX43, MRX44, MRX45, MRX52) with nonspecific X-linked mental retardation: clinical and psychometric data and results of linkage analysis. Am J Med Genet 1999;85:290-304.
39. Hirata A, Okada K, Yoshii K, Shiraishi H, Saijo S, et al. Structure of tRNA methyltransferase complex of Trm7 and Trm734 reveals a novel binding interface for tRNA recognition. Nucleic Acids Res 2019;47:10942-55.
40. Jensen LR, Garrett L, Holter SM, Rathkolb B, Racz I, et al. A mouse model for intellectual disability caused by mutations in the X-linked 2'Omethyltransferase Ftsj1 gene. Biochim Biophys Acta Mol Basis Dis 2019;1865:2083-93.
41. Ramser J, Winnepenninckx B, Lenski C, Errijgers V, Platzer M, et al. 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 2004;41:679-83.
42. Ropers HH, Hoeltzenbein M, Kalscheuer V, Yntema H, Hamel B, et al. Nonsyndromic X-linked mental retardation: where are the missing mutations? Trends Genet 2003;19:316-20.
43. Wang R, Lei T, Fu F, Li R, Jing X, et al. Application of chromosome microarray analysis in patients with unexplained developmental delay/intellectual disability in South China. Pediatr Neonatol 2019;60:35-42.
44. Willems P, Vits L, Buntinx I, Raeymaekers P, Van Broeckhoven C, et al. Localization of a gene responsible for nonspecific mental retardation (MRX9) to the pericentromeric region of the X chromosome. Genomics 1993;18:290-4.
45. Pintard L, Kressler D, Lapeyre B. Spb1p is a yeast nucleolar protein associated with Nop1p and Nop58p that is able to bind S-adenosyl-L-methionine in vitro. Mol Cell Biol 2000;20:1370-81.
46. Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, et al. Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 2012;90:847-55.
47. Brzezicha B, Schmidt M, Makalowska I, Jarmolowski A, Pienkowska J, et al. 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 2006;34:6034-43.
48. Frye M, Watt FM. The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. Curr Biol 2006;16:971-81.
49. Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, et al. Mutation in NSUN2, which encodes an RNA methyltransferase, causes

autosomal-recessive intellectual disability. Am J Hum Genet 2012;90:856-63.
50. Kuss AW, Garshasbi M, Kahrizi K, Tzschach A, Behjati F, et al. Autosomal recessive mental retardation: homozygosity mapping identifies 27 single linkage intervals, at least 14 novel loci and several mutation hotspots. Hum Genet 2011;129:141-8.
51. Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, et al. Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet 2012;49:380-5.
52. Najmabadi H, Motazacker MM, Garshasbi M, Kahrizi K, Tzschach A, et al. Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci. Hum Genet 2007;121:43-8.
53. Sakita-Suto S, Kanda A, Suzuki F, Sato S, Takata T, et al. Aurora-B regulates RNA methyltransferase NSUN2. Mol Biol Cell 2007;18:1107-17.
54. Tuorto F, Liebers R, Musch T, Schaefer M, Hofmann S, et al. RNA cytosine methylation by Dnmt2 and NSun2 promotes tRNA stability and protein synthesis. Nat Struct Mol Biol 2012;19:900-5.
55. Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, et al. Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. EMBO J 2014;33:2020-39.
56. Abdelrahman HA, Al-Shamsi AM, Ali BR, Al-Gazali L. A null variant in PUS3 confirms its involvement in intellectual disability and further delineates the associated neurodevelopmental disease. Clin Genet 2018;94:586-7.
57. Braun DA, Shril S, Sinha A, Schneider R, Tan W, et al. Mutations in WDR4 as a new cause of Galloway-Mowat syndrome. Am J Med Genet A 2018;176:2460-5.
58. Chen X, Gao Y, Yang L, Wu B, Dong X, et al. Speech and language delay in a patient with WDR4 mutations. Eur J Med Genet 2018;61:468-72.
59. Claudio JO, Liew CC, Ma J, Heng HH, Stewart AK, et al. Cloning and expression analysis of a novel WD repeat gene, WDR3, mapping to 1p12-p13. Genomics 1999;59:85-9.
60. Lin S, Liu Q, Lelyveld VS, Choe J, Szostak JW, et al. Mettl1/Wdr4-mediated m(7)G tRNA methylome is required for normal mRNA translation and embryonic stem cell self-renewal and differentiation. Mol Cell 2018;71:244-55.e5.
61. Michaud J, Kudoh J, Berry A, Bonne-Tamir B, Lalioti MD, et al. Isolation and characterization of a human chromosome 21q22.3 gene (WDR4) and its mouse homologue that code for a WD-repeat protein. Genomics 2000;68:71-9.
62. Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, et al. Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. Genome Biol 2015;16:210.
63. Trimouille A, Lasseaux E, Barat P, Deiller C, Drunat S, et al. Further delineation of the phenotype caused by biallelic variants in the WDR4 gene. Clin Genet 2017;93:374-7.
64. Blaesius K, Abbasi AA, Tahir TH, Tietze A, Picker-Minh S, et al. 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 2018;176:2517-21.
65. Davarniya B, Hu H, Kahrizi K, Musante L, Fattahi Z, et al. The role of a novel TRMT1 gene mutation and rare GRM1 gene defect in intellectual disability in two azeri families. PLoS One 2015;10:e0129631.
66. Liu JM, Straby KB. The human tRNA(m(2)(2)G(26))dimethyltransferase: functional expression and characterization of a cloned hTRM1 gene. Nucleic Acids Res 2000;28:3445-51.
67. Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, et al. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 2011;478:57-63.
68. Xu F, Zhou Y, Bystrom AS, Johansson MJO. Identification of factors that promote biogenesis of tRNA(CGA)(Ser). RNA Biol 2018;15:1286-94.
69. Dewe JM, Fuller BL, Lentini JM, Kellner SM, Fu D. TRMT1-catalyzed tRNA modifications are required for redox homeostasis to ensure proper cellular proliferation and oxidative stress survival. Mol Cell Biol 2017;37:e00214-17.
70. Zhang K, Lentini JM, Prevost CT, Hashem MO, Alkuraya FS, et al. An intellectual disability-associated missense variant in TRMT1 impairs tRNA modification and reconstitution of enzymatic activity. Hum Mutat 2020;41:600-7.
71. Alazami AM, Hijazi H, Al-Dosari MS, Shaheen R, Hashem A, et al. Mutation in ADAT3, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. J Med Genet 2013;50:425-30.
72. El-Hattab AW, Saleh MA, Hashem A, Al-Owain M, Asmari AA, et al. ADAT3-related intellectual disability: further delineation of the phenotype. Am J Med Genet A 2016;170A:1142-7.
73. Gerber AP, Keller W. An adenosine deaminase that generates inosine at the wobble position of tRNAs. Science 1999;286:1146-9.
74. Ramos J, Han L, Li Y, Hagelskamp F, Kellner SM, et al. Formation of tRNA wobble inosine in humans is disrupted by a millennia-old mutation causing intellectual disability. Mol Cell Biol 2019;39:e00203-19.
75. Salehi Chaleshtori AR, Miyake N, Ahmadvand M, Bashti O, Matsumoto N, et al. A novel 8-bp duplication in ADAT3 causes mild intellectual disability. Hum Genome Var 2018;5:7.
76. Sharkia R, Zalan A, Jabareen-Masri A, Zahalka H, Mahajnah M. A new case confirming and expanding the phenotype spectrum of ADAT3-related intellectual disability syndrome. Eur J Med Genet 2019;62:103549.
77. Thomas E, Lewis AM, Yang Y, Chanprasert S, Potocki L, et al. Novel missense variants in ADAT3 as a cause of syndromic intellectual disability. J Pediatr Genet 2019;8:244-51.
78. Fu Y, Dai Q, Zhang W, Ren J, Pan T, et al. The AlkB domain of mammalian ABH8 catalyzes hydroxylation of 5-methoxycarbonylmethyluridine at the wobble position of tRNA. Angew Chem Int Ed Engl 2010;49:8885-8.

79. Monies D, Vagbo CB, Al-Owain M, Alhomaidi S, Alkuraya FS. Recessive truncating mutations in ALKBH8 cause intellectual disability and severe impairment of wobble uridine modification. Am J Hum Genet 2019;104:1202-9.
80. Shimada K, Nakamura M, Anai S, De Velasco M, Tanaka M, et al. A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. Cancer Res 2009;69:3157-64.
81. Tsujikawa K, Koike K, Kitae K, Shinkawa A, Arima H, et al. Expression and sub-cellular localization of human ABH family molecules. J Cell Mol Med 2007;11:1105-16.
82. Arrondel C, Missoury S, Snoek R, Patat J, Menara G, et al. Defects in t(6)A tRNA modification due to GON7 and YRDC mutations lead to Galloway-Mowat syndrome. Nat Commun 2019;10:3967.
83. Braun DA, Rao J, Mollet G, Schapiro D, Daugeron MC, et al. Mutations in KEOPS-complex genes cause nephrotic syndrome with primary microcephaly. Nat Genet 2017;49:1529-38.
84. Edvardson S, Prunetti L, Arraf A, Haas D, Bacusmo JM, et al. tRNA N6-adenosine threonylcarbamoyltransferase defect due to KAE1/TCS3 (OSGEP) mutation manifest by neurodegeneration and renal tubulopathy. Eur J Hum Genet 2017;25:545-51.
85. Miyoshi A, Kito K, Aramoto T, Abe Y, Kobayashi N, et al. Identification of CGI-121, a novel PRPK (p53-related protein kinase)-binding protein. Biochem Biophys Res Commun 2003;303:399-405.
86. Gillis D, Krishnamohan A, Yaacov B, Shaag A, Jackman JE, et al. TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. J Med Genet 2014;51:581-6.
87. Howell NW, Jora M, Jepson BF, Limbach PA, Jackman JE. Distinct substrate specificities of the human tRNA methyltransferases TRMT10A and TRMT10B. RNA 2019;25:1366-76.
88. Igoillo-Esteve M, Genin A, Lambert N, Desir J, Pirson I, et al. tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. PLoS Genet 2013;9:e1003888.
89. Krishnamohan A, Jackman JE. Mechanistic features of the atypical tRNA m1G9 SPOUT methyltransferase, Trm10. Nucleic Acids Res 2017;45:9019-29.
90. Krishnamohan A, Jackman JE. A family divided: distinct structural and mechanistic features of the SpoU-TrmD (SPOUT) methyltransferase superfamily. Biochemistry 2019;58:336-45.
91. Yew TW, McCreight L, Colclough K, Ellard S, Pearson ER. tRNA methyltransferase homologue gene TRMT10A mutation in young adult-onset diabetes with intellectual disability, microcephaly and epilepsy. Diabet Med 2016;33:e21-5.
92. Zung A, Kori M, Burundukov E, Ben-Yosef T, Tatoor Y, et al. 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 2015;167A:3167-73.
93. Cohen JS, Srivastava S, Farwell KD, Lu HM, Zeng W, et al. ELP2 is a novel gene implicated in neurodevelopmental disabilities. Am J Med Genet A 2015;167:1391-5.
94. Dalwadi U, Yip CK. Structural insights into the function of Elongator. Cell Mol Life Sci 2018;75:1613-22.
95. Dauden MI, Kosinski J, Kolaj-Robin O, Desfosses A, Ori A, et al. Architecture of the yeast Elongator complex. EMBO Rep 2017;18:264-79.
96. Hawkes NA, Otero G, Winkler GS, Marshall N, Dahmus ME, et al. Purification and characterization of the human elongator complex. J Biol Chem 2002;277:3047-52.
97. Johansson MJO, Xu F, Byström AS. Elongator—a tRNA modifying complex that promotes efficient translational decoding. Biochim Biophys Acta Gene Regul Mech 2018;1861:401-8.
98. Karlsborn T, Tukenmez H, Mahmud AK, Xu F, Xu H, et al. Elongator, a conserved complex required for wobble uridine modifications in eukaryotes. RNA Biol 2014;11:1519-28.
99. Addis L, Ahn JW, Dobson R, Dixit A, Ogilvie CM, et al. Microdeletions of ELP4 are associated with language impairment, autism spectrum disorder, and mental retardation. Hum Mutat 2015;36:842-50.
100. Hu P, Meng L, Ma D, Qiao F, Wang Y, et al. A novel 11p13 microdeletion encompassing PAX6 in a Chinese Han family with aniridia, ptosis and mental retardation. Mol Cytogenet 2015;8:3.
101. Strug LJ, Clarke T, Chiang T, Chien M, Baskurt Z, et al. Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). Eur J Hum Genet 2009;17:1171-81.
102. Dewez M, Bauer F, Dieu M, Raes M, Vandenhaute J, et al. The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. Proc Natl Acad Sci U S A 2008;105:5459-64.
103. Rapino F, Delaunay S, Rambow F, Zhou Z, Tharun L, et al. Codon-specific translation reprogramming promotes resistance to targeted therapy. Nature 2018;558:605-9.
104. Schlieker CD, Van der Veen AG, Damon JR, Spooner E, Ploegh HL. A functional proteomics approach links the ubiquitin-related modifier Urm1 to a tRNA modification pathway. Proc Natl Acad Sci U S A 2008;105:18255-60.
105. Shaheen R, Al-Salam Z, El-Hattab AW, Alkuraya FS. The syndrome dysmorphic facies, renal agenesis, ambiguous genitalia, microcephaly, polydactyly and lissencephaly (DREAM-PL): report of two additional patients. Am J Med Genet A 2016;170:3222-6.
106. Shaheen R, Mark P, Prevost CT, AlKindi A, Alhag A, et al. Biallelic variants in CTU2 cause DREAM-PL syndrome and impair thiolation of tRNA wobble U34. Hum Mutat 2019;40:2108-20.
107. Shaheen R, Patel N, Shamseldin H, Alzahrani F, Al-Yamany R, et al. Accelerating matchmaking of novel dysmorphology syndromes through clinical and genomic characterization of a large cohort. Genet Med 2016;18:686-95.
108. Alfares A, Alfadhel M, Wani T, Alsahli S, Alluhaydan I, et al. A multicenter clinical exome study in unselected cohorts from a consanguineous population of Saudi Arabia demonstrated a high diagnostic yield. Mol Genet Metab 2017;121:91-5.
109. Chen J, Patton JR. Pseudouridine synthase 3 from mouse modifies the anticodon loop of tRNA. Biochemistry 2000;39:12723-30.

110. Shaheen R, Han L, Faqeih E, Ewida N, Alobeid E, et al. A homozygous truncating mutation in PUS3 expands the role of tRNA modification in normal cognition. Hum Genet 2016;135:707-13.
111. Shaheen R, Tasak M, Maddirevula S, Abdel-Salam GMH, Sayed ISM, et al. PUS7 mutations impair pseudouridylation in humans and cause intellectual disability and microcephaly. Hum Genet 2019;138:231-9.
112. Becker HF, Motorin Y, Planta RJ, Grosjean H. The yeast gene YNL292w encodes a pseudouridine synthase (Pus4) catalyzing the formation of psi55 in both mitochondrial and cytoplasmic tRNAs. Nucleic Acids Res 1997;25:4493-9.
113. Bykhovskaya Y, Casas K, Mengesha E, Inbal A, Fischel-Ghodsian N. Missense mutation in pseudouridine synthase 1 (PUS1) causes mitochondrial myopathy and sideroblastic anemia (MLASA). Am J Hum Genet 2004;74:1303-8.
114. Fernandez-Vizarra E, Berardinelli A, Valente L, Tiranti V, Zeviani M. Nonsense mutation in pseudouridylate synthase 1 (PUS1) in two brothers affected by myopathy, lactic acidosis and sideroblastic anaemia (MLASA). J Med Genet 2007;44:173-80.
115. Massenet S, Motorin Y, Lafontaine DL, Hurt EC, Grosjean H, et al. Pseudouridine mapping in the Saccharomyces cerevisiae spliceosomal U small nuclear RNAs (snRNAs) reveals that pseudouridine synthase pus1p exhibits a dual substrate specificity for U2 snRNA and tRNA. Mol Cell Biol 1999;19:2142-54.
116. Patton JR, Bykhovskaya Y, Mengesha E, Bertolotto C, Fischel-Ghodsian N. Mitochondrial myopathy and sideroblastic anemia (MLASA): missense mutation in the pseudouridine synthase 1 (PUS1) gene is associated with the loss of tRNA pseudouridylation. J Biol Chem 2005;280:19823-8.
117. Zeharia A, Fischel-Ghodsian N, Casas K, Bykhocskaya Y, Tamari H, et al. Mitochondrial myopathy, sideroblastic anemia, and lactic acidosis: an autosomal recessive syndrome in Persian Jews caused by a mutation in the PUS1 gene. J Child Neurol 2005;20:449-52.
118. Brouwer APM, Abou Jamra R, Kortel N, Soyris C, Polla DL, et al. Variants in PUS7 cause intellectual disability with speech delay, microcephaly, short stature, and aggressive behavior. Am J Hum Genet 2018;103:1045-52.
119. Darvish H, Azcona LJ, Alehabib E, Jamali F, Tafakhori A, et al. A novel PUS7 mutation causes intellectual disability with autistic and aggressive behaviors. Neurol Genet 2019;5:e356.
120. Aza-Blanc P, Cooper CL, Wagner K, Batalov S, Deveraux QL, et al. Identification of modulators of TRAIL-induced apoptosis via RNAi-based phenotypic screening. Mol Cell 2003;12:627-37.
121. McCleverty CJ, Hornsby M, Spraggon G, Kreusch A. Crystal structure of human Pus10, a novel pseudouridine synthase. J Mol Biol 2007;373:1243-54.
122. Nakayama T, Wu J, Galvin-Parton P, Weiss J, Andriola MR, et al. Deficient activity of alanyl-tRNA synthetase underlies an autosomal recessive syndrome of progressive microcephaly, hypomyelination, and epileptic encephalopathy. Hum Mutat 2017;38:1348-54.
123. Simons C, Griffin LB, Helman G, Golas G, Pizzino A, et al. Loss-of-function alanyl-tRNA synthetase mutations cause an autosomal-recessive early-onset epileptic encephalopathy with persistent myelination defect. Am J Hum Genet 2015;96:675-81.
124. Nafisinia M, Sobreira N, Riley L, Gold W, Uhlenberg B, et al. Mutations in RARS cause a hypomyelination disorder akin to Pelizaeus-Merzbacher disease. Eur J Hum Genet 2017;25:1134-41.
125. Wolf NI, Salomons GS, Rodenburg RJ, Pouwels PJ, Schieving JH, et al. Mutations in RARS cause hypomyelination. Ann Neurol 2014;76:134-9.
126. Taft RJ, Vanderver A, Leventer RJ, Damiani SA, Simons C, et al. Mutations in DARS cause hypomyelination with brain stem and spinal cord involvement and leg spasticity. Am J Hum Genet 2013;92:774-80.
127. Casey JP, McGettigan P, Lynam-Lennon N, McDermott M, Regan R, et al. Identification of a mutation in LARS as a novel cause of infantile hepatopathy. Mol Genet Metab 2012;106:351-8.
128. Casey JP, Slattery S, Cotter M, Monavari AA, Knerr I, et al. Clinical and genetic characterisation of infantile liver failure syndrome type 1, due to recessive mutations in LARS. J Inherit Metab Dis 2015;38:1085-92.
129. Lo WS, Gardiner E, Xu Z, Lau CF, Wang F, et al. Human tRNA synthetase catalytic nulls with diverse functions. Science 2014;345:328-32.
130. Hadchouel A, Wieland T, Griese M, Baruffini E, Lorenz-Depiereux B, et al. Biallelic mutations of methionyl-tRNA synthetase cause a specific type of pulmonary alveolar proteinosis prevalent on reunion island. Am J Hum Genet 2015;96:826-31.
131. Sun Y, Hu G, Luo J, Fang D, Yu Y, et al. Mutations in methionyl-tRNA synthetase gene in a Chinese family with interstitial lung and liver disease, postnatal growth failure and anemia. J Hum Genet 2017;62:647-51.
132. van Meel E, Wegner DJ, Cliften P, Willing MC, White FV, et al. Rare recessive loss-of-function methionyl-tRNA synthetase mutations presenting as a multi-organ phenotype. BMC Med Genet 2013;14:106.
133. Nowaczyk MJ, Huang L, Tarnopolsky M, Schwartzentruber J, Majewski J, et al. A novel multisystem disease associated with recessive mutations in the tyrosyl-tRNA synthetase (YARS) gene. Am J Med Genet A 2017;173:126-34.
134. Kopajtich R, Murayama K, Janecke AR, Haack TB, Breuer M, et al. Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. Am J Hum Genet 2016;99:414-22.
135. Nichols RC, Blinder J, Pai SI, Ge Q, Targoff IN, et al. Assignment of two human autoantigen genes-isoleucyl-tRNA synthetase locates to 9q21 and lysyl-tRNA synthetase locates to 16q23-q24. Genomics 1996;36:210-3.
136. Nichols RC, Raben N, Boerkoel CF, Plotz PH. Human isoleucyl-tRNA synthetase: sequence of the cDNA, alternative mRNA splicing, and the characteristics of an unusually long C-terminal extension. Gene 1995;155:299-304.
137. Orenstein N, Weiss K, Oprescu SN, Shapira R, Kidron D, et al. Bi-allelic IARS mutations in a child with intra-uterine growth retardation, neonatal cholestasis, and mild developmental delay. Clin Genet 2017;91:913-7.
138. Smigiel R, Biela M, Biernacka A, Stembalska A, Sasiadek M, et al. New evidence for association of recessive IARS gene mutations with hepatopathy, hypotonia, intellectual disability and growth retardation. Clin Genet 2017;92:671-3.

139. Vincent C, Tarbouriech N, Hartlein M. Genomic organization, cDNA sequence, bacterial expression, and purification of human seryl-tRNA synthase. Eur J Biochem 1997;250:77-84.
140. Friedman J, Smith DE, Issa MY, Stanley V, Wang R, et al. Biallelic mutations in valyl-tRNA synthetase gene VARS are associated with a progressive neurodevelopmental epileptic encephalopathy. Nat Commun 2019;10:707.
141. Hsieh SL, Campbell RD. Evidence that gene G7a in the human major histocompatibility complex encodes valyl-tRNA synthetase. Biochem J 1991;278:809-16.
142. Karaca E, Harel T, Pehlivan D, Jhangiani SN, Gambin T, et al. Genes that affect brain structure and function identified by rare variant analyses of mendelian neurologic disease. Neuron 2015;88:499-513.
143. Okur V, Ganapathi M, Wilson A, Chung WK. Biallelic variants in VARS in a family with two siblings with intellectual disability and microcephaly: case report and review of the literature. Cold Spring Harb Mol Case Stud 2018;4:a003301.
144. Siekierska A, Stamberger H, Deconinck T, Oprescu SN, Partoens M, et al. Biallelic VARS variants cause developmental encephalopathy with microcephaly that is recapitulated in vars knockout zebrafish. Nat Commun 2019;10:708.
145. Stephen J, Nampoothiri S, Banerjee A, Tolman NJ, Penninger JM, et al. Loss of function mutations in VARS encoding cytoplasmic valyl-tRNA synthetase cause microcephaly, seizures, and progressive cerebral atrophy. Hum Genet 2018;137:293-303.
146. Datta A, Ferguson A, Simonson C, Zannotto F, Michoulas A, et al. Case report. J Child Neurol 2017;32:403-7.
147. Kodera H, Osaka H, Iai M, Aida N, Yamashita A, et al. Mutations in the glutaminyl-tRNA synthetase gene cause early-onset epileptic encephalopathy. J Hum Genet 2015;60:97-101.
148. Leshinsky-Silver E, Ling J, Wu J, Vinkler C, Yosovich K, et al. Severe growth deficiency, microcephaly, intellectual disability, and characteristic facial features are due to a homozygous QARS mutation. Neurogenetics 2017;18:141-6.
149. Salvarinova R, Ye CX, Rossi A, Biancheri R, Roland EH, et al. Expansion of the QARS deficiency phenotype with report of a family with isolated supratentorial brain abnormalities. Neurogenetics 2015;16:145-9.
150. Vinkler C, Leshinsky-Silver E, Michelson M, Haas D, Lerman-Sagie T, et al. A newly recognized syndrome of severe growth deficiency, microcephaly, intellectual disability, and characteristic facial features. Eur J Med Genet 2014;57:288-92.
151. Zhang X, Ling J, Barcia G, Jing L, Wu J, et al. Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. Am J Hum Genet 2014;94:547-58.
152. Basit S, Lee K, Habib R, Chen L, Umm-e-Kalsoom, et al. DFNB89, a novel autosomal recessive nonsyndromic hearing impairment locus on chromosome 16q21-q23.2. Hum Genet 2011;129:379-85.
153. Dickinson ME, Flenniken AM, Ji X, Teboul L, Wong MD, et al. High-throughput discovery of novel developmental phenotypes. Nature 2016;537:508-14.
154. Kohda M, Tokuzawa Y, Kishita Y, Nyuzuki H, Moriyama Y, et al. A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies. PLoS Genet 2016;12:e1005679.
155. McLaughlin HM, Sakaguchi R, Liu C, Igarashi T, Pehlivan D, et al. Compound heterozygosity for loss-of-function lysyl-tRNA synthetase mutations in a patient with peripheral neuropathy. Am J Hum Genet 2010;87:560-6.
156. McMillan HJ, Humphreys P, Smith A, Schwartzentruber J, Chakraborty P, et al. Congenital Visual impairment and progressive microcephaly due to Lysyl-transfer ribonucleic acid (RNA) synthetase (KARS) mutations: the expanding phenotype of aminoacyl-transfer RNA synthetase mutations in human disease. J Child Neurol 2015;30:1037-43.
157. Murray CR, Abel SN, McClure MB, Foster J, Walke MI, et al. Novel causative variants in DYRK1A, KARS, and KAT6A associated with intellectual disability and additional phenotypic features. J Pediatr Genet 2017;6:77-83.
158. Santos-Cortez RL, Lee K, Azeem Z, Antonellis PJ, Pollock LM, et al. Mutations in KARS, encoding lysyl-tRNA synthetase, cause autosomal-recessive nonsyndromic hearing impairment DFNB89. Am J Hum Genet 2013;93:132-40.
159. Bonnefond L, Fender A, Rudinger-Thirion J, Giege R, Florentz C, et al. Toward the full set of human mitochondrial aminoacyl-tRNA synthetases: characterization of AspRS and TyrRS. Biochemistry 2005;44:4805-16.
160. Mizuguchi T, Nakashima M, Kato M, Yamada K, Okanishi T, et al. PARS2 and NARS2 mutations in infantile-onset neurodegenerative disorder. J Hum Genet 2017;62:525-9.
161. Seaver LH, DeRoos S, Andersen NJ, Betz B, Prokop J, et al. Lethal NARS2-related disorder associated with rapidly progressive intractable epilepsy and global brain atrophy. Pediatr Neurol 2018;89:26-30.
162. Simon M, Richard EM, Wang X, Shahzad M, Huang VH, et al. Mutations of human NARS2, encoding the mitochondrial asparaginyl-tRNA synthetase, cause nonsyndromic deafness and Leigh syndrome. PLoS Genet 2015;11:e1005097.
163. Sofou K, Kollberg G, Holmstrom M, Davila M, Darin N, et al. Whole exome sequencing reveals mutations in NARS2 and PARS2, encoding the mitochondrial asparaginyl-tRNA synthetase and prolyl-tRNA synthetase, in patients with Alpers syndrome. Mol Genet Genomic Med 2015;3:59-68.
164. Vanlander AV, Menten B, Smet J, De Meirleir L, Sante T, et al. Two siblings with homozygous pathogenic splice-site variant in mitochondrial asparaginyl-tRNA synthetase (NARS2). Hum Mutat 2015;36:222-31.
165. Ciara E, Rokicki D, Lazniewski M, Mierzewska H, Jurkiewicz E, et al. Clinical and molecular characteristics of newly reported mitochondrial disease entity caused by biallelic PARS2 mutations. J Hum Genet 2018;63:473-85.
166. Edvardson S, Shaag A, Kolesnikova O, Gomori JM, Tarassov I, et al. Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. Am J Hum Genet 2007;81:857-62.
167. Pronicka E, Piekutowska-Abramczuk D, Ciara E, Trubicka J, Rokicki D, et al. New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. J Transl Med 2016;14:174.
168. Yin X, Tang B, Mao X, Peng J, Zeng S, et al. The genotypic and phenotypic spectrum of PARS2-related infantile-onset encephalopathy. J

Hum Genet 2018;63:971-80.
169. Alkhateeb AM, Aburahma SK, Habbab W, Thompson IR. Novel mutations in WWOX, RARS2, and C10orf2 genes in consanguineous Arab families with intellectual disability. Metab Brain Dis 2016;31:901-7.
170. Cassandrini D, Cilio MR, Bianchi M, Doimo M, Balestri M, et al. Pontocerebellar hypoplasia type 6 caused by mutations in RARS2: definition of the clinical spectrum and molecular findings in five patients. J Inherit Metab Dis 2013;36:43-53.
171. Li Z, Schonberg R, Guidugli L, Johnson AK, Arnovitz S, et al. A novel mutation in the promoter of RARS2 causes pontocerebellar hypoplasia in two siblings. J Hum Genet 2015;60:363-9.
172. Rankin J, Brown R, Dobyns WB, Harington J, Patel J, et al. Pontocerebellar hypoplasia type 6: a British case with PEHO-like features. Am J Med Genet A 2010;152A:2079-84.
173. Tzagoloff A, Shtanko A. Mitochondrial and cytoplasmic isoleucyl-, glutamyl- and arginyl-tRNA synthetases of yeast are encoded by separate genes. Eur J Biochem 1995;230:582-6.
174. Martinez-Dominguez MT, Justesen J, Kruse TA, Hansen LL. Assignment of the human mitochondrial tryptophanyl-tRNA synthetase (WARS2) to 1p13.3-->p13.1 by radiation hybrid mapping. Cytogenet Cell Genet 1998;83:249-50.
175. Theisen BE, Rumyantseva A, Cohen JS, Alcaraz WA, Shinde DN, et al. Deficiency of WARS2, encoding mitochondrial tryptophanyl tRNA synthetase, causes severe infantile onset leukoencephalopathy. Am J Med Genet A 2017;173:2505-10.
176. Wortmann SB, Timal S, Venselaar H, Wintjes LT, Kopajtich R, et al. Biallelic variants in WARS2 encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy. Hum Mutat 2017;38:1786-95.
177. Boccaletto P, Machnicka MA, Purta E, Piątkowski P, Bagiński B, et al. MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res 2018;46:D303-7.
178. Ogura T, Tomoyasu T, Yuki T, Morimura S, Begg KJ, et al. Structure and function of the ftsH gene in Escherichia coli. Res Microbiol 1991;142:279-82.
179. Bugl H, Fauman EB, Staker BL, Zheng F, Kushner SR, et al. RNA methylation under heat shock control. Mol Cell 2000;6:349-60.
180. Guy MP, Shaw M, Weiner CL, Hobson L, Stark Z, et al. Defects in tRNA anticodon loop 2'-O-Methylation are implicated in nonsyndromic X-linked intellectual disability due to mutations in FTSJ1. Hum Mutat 2015;36:1176-87.
181. Marchand V, Pichot F, Thuring K, Ayadi L, Freund I, et al. Next-generation sequencing-based ribomethseq protocol for analysis of tRNA 2'-O-Methylation. Biomolecules 2017;7:13.
182. Panebianco F, Kelly LM, Liu P, Zhong S, Dacic S, et al. THADA fusion is a mechanism of IGF2BP3 activation and IGF1R signaling in thyroid cancer. Proc Natl Acad Sci U S A 2017;114:2307-12.
183. Takano K, Nakagawa E, Inoue K, Kamada F, Kure S, et al. 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 2008;147B:479-84.
184. Honda S, Hayashi S, Imoto I, Toyama J, Okazawa H, et al. Copy-number variations on the X chromosome in Japanese patients with mental retardation detected by array-based comparative genomic hybridization analysis. J Hum Genet 2010;55:590-9.
185. Bonnet C, Gregoire MJ, Brochet K, Raffo E, Leheup B, et al. Pure de-novo 5 Mb duplication at Xp11.22-p11.23 in a male. J Hum Genet 2006;51:815.
186. El-Hattab AW, Bournat J, Eng PA, Wu JBS, Walker BA, et al. Microduplication of Xp11.23p11.3 with effects on cognition, behavior, and craniofacial development. Clin Genet 2011;79:531-8.
187. Froyen G, Bauters M, Boyle J, van Esch H, Govaerts K, et al. 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 2007;121:539-47.
188. Torres AG, Pineyro D, Rodriguez-Escriba M, Camacho N, Reina O, et al. Inosine modifications in human tRNAs are incorporated at the precursor tRNA level. Nucleic Acids Res 2015;43:5145-57.
189. Songe-Moller L, van den Born E, Leihne V, Vagbo CB, Kristoffersen T, et al. Mammalian ALKBH8 possesses tRNA methyltransferase activity required for the biogenesis of multiple wobble uridine modifications implicated in translational decoding. Mol Cell Biol 2010;30:1814-27.
190. van den Born E, Vagbo CB, Songe-Moller L, Leihne V, Lien GF, et al. ALKBH8-mediated formation of a novel diastereomeric pair of wobble nucleosides in mammalian tRNA. Nat Commun 2011;2:172.
191. Philipp M, John F, Ringli C. The cytosolic thiouridylase CTU2 of Arabidopsis thaliana is essential for posttranscriptional thiolation of tRNAs and influences root development. BMC Plant Biol 2014;14:109.
192. Downey M, Houlsworth R, Maringele L, Rollie A, Brehme M, et al. A genome-wide screen identifies the evolutionarily conserved KEOPS complex as a telomere regulator. Cell 2006;124:1155-68.
193. Srinivasan M, Mehta P, Yu Y, Prugar E, Koonin EV, et al. The highly conserved KEOPS/EKC complex is essential for a universal tRNA modification, t6A. EMBO J 2011;30:873-81.
194. Huang B, Johansson MJ, Bystrom AS. An early step in wobble uridine tRNA modification requires the Elongator complex. RNA 2005;11:424-36.
195. Esberg A, Huang B, Johansson MJ, Bystrom AS. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. Mol Cell 2006;24:139-48.
196. Simos G, Tekotte H, Grosjean H, Segref A, Sharma K, et al. Nuclear pore proteins are involved in the biogenesis of functional tRNA. EMBO J 1996;15:2270-84.
197. Lecointe F, Simos G, Sauer A, Hurt EC, Motorin Y, et al. Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of psi 38 and psi 39 in tRNA anticodon loop. J Biol Chem 1998;273:1316-23.
198. Paiva ARB, Lynch DS, Melo US, Lucato LT, Freua F, et al. PUS3 mutations are associated with intellectual disability,

leukoencephalopathy, and nephropathy. Neurol Genet 2019;5:e306.
199. Fang H, Zhang L, Xiao B, Long H, Yang L. Compound heterozygous mutations in PUS3 gene identified in a Chinese infant with severe epileptic encephalopathy and multiple malformations. Neurol Sci 2020;41:465-7.
200. Behm-Ansmant I, Urban A, Ma X, Yu YT, Motorin Y, et al. The Saccharomyces cerevisiae U2 snRNA:pseudouridine-synthase Pus7p is a novel multisite-multisubstrate RNA:Psi-synthase also acting on tRNAs. RNA 2003;9:1371-82.
201. Ma X, Zhao X, Yu YT. Pseudouridylation (Psi) of U2 snRNA in S. cerevisiae is catalyzed by an RNA-independent mechanism. EMBO J 2003;22:1889-97.
202. Decatur WA, Schnare MN. Different mechanisms for pseudouridine formation in yeast 5S and 5.8S rRNAs. Mol Cell Biol 2008;28:3089-100.
203. Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell 2014;159:148-62.
204. Wu G, Xiao M, Yang C, Yu YT. U2 snRNA is inducibly pseudouridylated at novel sites by Pus7p and snR81 RNP. EMBO J 2011;30:79-89.
205. Guzzi N, Ciesla M, Ngoc PCT, Lang S, Arora S, et al. Pseudouridylation of tRNA-derived fragments steers translational control in stem cells. Cell 2018;173:1204-16.e26.
206. Brennan T, Sundaralingam M. Structure of transfer RNA molecules containing the long variable loop. Nucleic Acids Res 1976;3:3235-50.
207. Sun FJ, Caetano-Anollés G. The evolutionary significance of the long variable arm in transfer RNA. Complexity 2009;14:26-39.
208. Komara M, Al-Shamsi AM, Ben-Salem S, Ali BR, Al-Gazali L. A novel single-nucleotide deletion (c.1020delA) in NSUN2 causes intellectual disability in an emirati child. J Mol Neurosci 2015;57:393-9.
209. Steinberg S, Cedergren R. A correlation between N2-dimethylguanosine presence and alternate tRNA conformers. RNA 1995;1:886-91.
210. Vakiloroayaei A, Shah NS, Oeffinger M, Bayfield MA. The RNA chaperone La promotes pre-tRNA maturation via indiscriminate binding of both native and misfolded targets. Nucleic Acids Res 2017;45:11341-55.
211. Chou HJ, Donnard E, Gustafsson HT, Garber M, Rando OJ. Transcriptome-wide analysis of roles for tRNA modifications in translational regulation. Mol Cell 2017;68:978-92.e4.
212. Gustavsson M, Ronne H. Evidence that tRNA modifying enzymes are important in vivo targets for 5-fluorouracil in yeast. RNA 2008;14:666-74.
213. Torabi N, Kruglyak L. Variants in SUP45 and TRM10 underlie natural variation in translation termination efficiency in Saccharomyces cerevisiae. PLoS Genet 2011;7:e1002211.
214. Helm M, Brule H, Degoul F, Cepanec C, Leroux JP, et al. The presence of modified nucleotides is required for cloverleaf folding of a human mitochondrial tRNA. Nucleic Acids Res 1998;26:1636-43.
215. Chen CP, Chern SR, Wu PS, Chen SW, Lai ST, et al. Prenatal diagnosis of a 3.2-Mb 2p16.1-p15 duplication associated with familial intellectual disability. Taiwan J Obstet Gynecol 2018;57:578-82.
216. Piccione M, Piro E, Serraino F, Cavani S, Ciccone R, et al. Interstitial deletion of chromosome 2p15-16.1: report of two patients and critical review of current genotype-phenotype correlation. Eur J Med Genet 2012;55:238-44.
217. Lovrecic L, Gnan C, Baldan F, Franzoni A, Bertok S, et al. Microduplication in the 2p16.1p15 chromosomal region linked to developmental delay and intellectual disability. Mol Cytogenet 2018;11:39.
218. Peter B, Matsushita M, Oda K, Raskind W. De novo microdeletion of BCL11A is associated with severe speech sound disorder. Am J Med Genet A. 2014;164A:2091-6.
219. Balci TB, Sawyer SL, Davila J, Humphreys P, Dyment DA. Brain malformations in a patient with deletion 2p16.1: a refinement of the phenotype to BCL11A. Eur J Med Genet 2015;58:351-4.
220. Schimmel P. The emerging complexity of the tRNA world: mammalian tRNAs beyond protein synthesis. Nat Rev Mol Cell Biol 2018;19:45-58.
221. Waltl S. Progressive microcephaly is caused by compound-heterozygous mutations in QARS. Clin Genet 2014;86:508-9.
222. Scheper GC, van der Klok T, van Andel RJ, van Berkel CG, Sissler M, et al. Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. Nat Genet 2007;39:534-9.
223. Moulinier L, Ripp R, Castillo G, Poch O, Sissler M. MiSynPat: an integrated knowledge base linking clinical, genetic, and structural data for disease-causing mutations in human mitochondrial aminoacyl-tRNA synthetases. Hum Mutat 2017;38:1316-24.
224. Puffenberger EG, Jinks RN, Sougnez C, Cibulskis K, Willert RA, et al. Genetic mapping and exome sequencing identify variants associated with five novel diseases. PLoS One 2012;7:e28936.
225. Pierce SB, Chisholm KM, Lynch ED, Lee MK, Walsh T, et al. Mutations in mitochondrial histidyl tRNA synthetase HARS2 cause ovarian dysgenesis and sensorineural hearing loss of Perrault syndrome. Proc Natl Acad Sci U S A 2011;108:6543-8.