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Role of tRNA modifications in human diseases

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Transfer RNAs (tRNAs) are key for efficient and accurate protein translation. To be fully active, tRNAs need to be heavily modified post-transcriptionally. Growing evidence indicates that tRNA modifications and the enzymes catalyzing such modifications may play important roles in complex human pathologies. Here, we have compiled current knowledge that directly link tRNA modifications to human diseases such as cancer, type 2 diabetes (T2D), neurological disorders, and mitochondrial-linked disorders. The molecular mechanisms behind these connections remain, for the most part, unknown. As we progress towards the understanding of the roles played by hypomodified tRNAs in human disease, novel areas of therapeutic intervention may be discovered.

Structure of tRNAs and molecular role of tRNA modifications

tRNAs (see Glossary) are key adaptor molecules in the protein translation machinery. In their mature form, they are approximately 70–100 nucleotides long and fold into a 'clover leaf' secondary structure (Figure 1) and an L-shaped tertiary structure. After maturation, tRNAs are charged with their cognate amino acid at the 3′-end and, through their anticodon loop, pair specifically with codons in messenger RNAs (mRNAs). The nucleobases in the tRNA anticodon that interact with the mRNA triplets are those located at positions 34, 35 and 36 of the tRNA. Position 34 can wobble and pair with different nucleotides at the third position of the mRNA codon via non-Watson–Crick interactions. This flexibility allows the genetic code to be degenerate at the third position of codons (i.e., the third position of the codon does not alter the amino acid decoding); therefore, wobbling at position 34 of tRNAs is important because it allows some tRNAs to decode different sets of codons coding for the same amino acid, and some codons to be recognized by more than one anticodon sequence [1].

tRNAs are heavily modified post-transcriptionally during their maturation process. In Eukarya there are more than 50 different chemical modifications described affecting different positions on the tRNA (The tRNA Modification Database: http://mods.rna.albany.edu/home). Most of these modifications and the enzymes responsible for catalyzing them are well described in the yeast *Saccharomyces cerevisiae* [2]; however, in recent years the human homologs for many of those enzymes and the biological role of the modifications they catalyze have started to be documented [3]. With this novel information, a link between tRNA modifications and human diseases is becoming increasingly clear (Table 1).

Chemical modifications are crucial for tRNA structure, function, and stability. In general, hypomodified tRNAs are targeted for degradation, thus a primary role of tRNA modifications is to prevent tRNAs from entering specific degradation pathways [2]. From a functional point of view, specific modifications in the anticodon loop can directly affect the behavior of tRNAs during gene translation. For example, several modifications at position 37 (adjacent to the anticodon) help to stabilize codon:anticodon interactions by providing base-stacking interactions at this position. Such modifications were shown to prevent translational frameshifting [4]. Modifications at position 34 are generally associated with decoding, because base modifications at this position are usually necessary for codon:anticodon wobbling to occur; however, such modifications can also prevent translational frameshifting. Typical examples of wobble modifications include uridine (U) 34 modifications such as incorporation of hydroxyl, methyl, and thiol groups, and adenosine (A) 34 modifications such as adenosine-to-inosine (A-to-I) editing [2,5].

Modifications in the main body of the tRNA usually have structural and stabilizing roles in tRNAs. Those modifications that drive the sugar conformation of the nucleobase into the C3′-endo increase binding affinity and rigidify the tRNA structure (e.g., pseudouridines); whereas other modifications, such as dihydrouridines, help to maintain the flexibility of the tRNA structure [4]. In some cases, certain modifications serve as identity elements for tRNAs (e.g., aminoacyl tRNA synthetase recognition). Post-transcriptional addition of a guanosine (G) at the 5′-end of $tRNA^{His}$ is critical for charging the tRNA with histidine by histidinyl-tRNA synthetase [6]. Another example is the 2′O-ribosyl phosphate modification at position 64 of $tRNA^{Met}$ from *S. cerevisiae*, which was shown to serve for discrimination between the initiator $tRNA^{Met}$ and the elongator $tRNA^{Met}$ [7]. Chemical structures of the tRNA modifications discussed are depicted in Figure 2.

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Glossary

Anticodon: a sequence formed by residues 34, 35, and 36 on tRNAs that recognize specific codons in mRNAs.
Base stacking: noncovalent interactions between the aromatic rings of the nucleotide bases.
Codon: a sequence of three nucleotides present in mRNAs that encode for a specific amino acid (or stop signal) during protein synthesis.
Dubowitz syndrome (DS): rare genetic disorder characterized by microcephaly, growth and mental retardation, eczema and peculiar facial features (small and round face, broad nose, wide set eyes with drooping eyelids).
Exon skipping: the omission of an exon by the splicing machinery during mRNA maturation. In some circumstances, therapies can induce the splicing machinery to leave out a mutated or misaligned exon, resulting in a transcript encoding for a truncated but functional protein.
Familial dysautonomia (FD): a hereditary sensory and autonomic neuropathy characterized by complex clinical traits such as decreased sensitivity to pain and temperature; cardiovascular, respiratory, and gastrointestinal dysfunction; lack of overflow tears; excessive sweating; and hypertension.
Gene therapy: the use of DNA as a therapeutic agent to treat a disease (e.g., supplement the patient with a DNA encoding for the functional version of a gene to replace the endogenous mutated one).
Homolog: protein or DNA sequences from different organisms that are similar due to a shared phylogenetic ancestry.
Insulin: a peptide hormone produced in the pancreas to regulate the carbohydrate and fat metabolism. It induces cells in skeletal muscles and liver and fat tissue to absorb glucose from the blood.
Intellectual disability: refers to patients with limitations in cognitive function and deficits in two or more adaptive behaviors such as reduced communication or social skills.
Isoacceptors: different tRNA species that code for the same amino acid. They carry a different anticodon sequence and hence recognize a different mRNA codon for a particular amino acid.
Messenger RNA (mRNA): a family of RNA molecules that are transcribed from the genomic DNA sequence and carry the information for protein translation. The coding region of mRNAs is defined by codons which are recognized by tRNAs to translate its nucleotide sequence into a peptidic sequence.
Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a syndrome linked to mutations in the mitochondrial genome. As such, it is inherited from the female parent. It primarily affects the nervous system and muscles.
Myoclonus epilepsy associated with ragged-red fibers (MERRF): a syndrome linked to mutations in the mitochondrial genome. As such, it is inherited from the female parent. It is characterized by progressive myoclonus epilepsy, short stature, hearing loss, lactic acidosis, and exercise intolerance.
Noonan syndrome: relatively common congenital disorder characterized by heart problems (pulmonary valve stenosis, atrial septal defects, hypertrophic cardiomyopathy, etc.) and multiple malformations (widely set eyes, low set ears, webbed neck, chest deformity, etc.). Patients also develop mental retardation in approximately 25% of cases.
Non-syndromic intellectual disability: a form of mental retardation that is milder than the syndromic intellectual disability with no other phenotypic abnormalities. It can be linked to the X chromosome in which case males are affected whereas females are carriers.
Oncogene: a gene that has the potential to produce cancer by inducing cell survival and proliferation.
Ortholog: a homolog derived from a speciation event.
Post-transcriptional modification: modifications that occur on RNA molecules after being transcribed from DNA and are therefore not encoded in the genome.
Protein translation accuracy: refers to the accurate incorporation of the correct amino acid into the growing polypeptide chain.
Protein translation efficiency: refers to the speed at which a protein is being translated and directly reflects on the protein abundance.
Rolandic epilepsy: a benign form of childhood epilepsy. It can start at age 3 years but usually stops at around age 14–18 years.
Strabismus: a condition where the eyes are not properly aligned with each other (also known as heterotropia).
Squamous cell carcinoma: a type of non-melanoma skin cancer.
Transfer RNA (tRNA): a family of non-coding RNA of approximately 70–100 nucleotides in length that serve as an adaptor molecule between mRNAs and the growing polypeptide chain during protein translation. tRNAs carry a specific amino acid at its 3′-end and specifically recognizes codons in the mRNA through the tRNA anticodon.
Translational frameshift: refers to a shift in the ribosome reading frame where the second or the third position of the mRNA codon is read as the first position of the codon.
Type 2 diabetes (T2D): metabolic disorder resulting in elevated levels of glucose in the blood either due to resistance to insulin or due to a relative deficiency in insulin production.
Urothelial carcinoma: a type of cancer that occurs in the urinary system (kidneys, urinary bladder, and accessory organs).
Watson–Crick pairing: nucleotide pairing between a purine and a pyrimidine (guanine:cytosine and adenine:thymine/adenine:uracil).
Wobble pairing: non-Watson–Crick pairing between residue 34 of tRNAs and the third residue of the mRNA codon.
Xenograft: a tissue graft or organ transplant from a donor of a different species than the recipient.

Because tRNA modifications can affect translation accuracy and efficiency as well as general tRNA stability, it would be expected that lack of such modifications could have profound and generalized effects on protein synthesis. By contrast, certain tRNA modifications might affect the translation of only a defined subset of transcripts enriched in certain types of codons, which could all be linked to a common cellular pathway [8]. In either case, we can hypothesize that the regulation of the tRNA modification levels could be used as a method to modulate protein expression and regulate complex cellular processes. In agreement with this hypothesis, many reports point at a clear link between defects in tRNA modifications and human diseases such as cancer, T2D, neurological disorders, and mitochondrial-linked disorders (Table 1). We therefore propose that tRNA modifications play crucial roles in human diseases, and that novel therapeutics based on modulation of such modifications could lead the way for tackling complex human pathologies for which, to date, there are no effective treatments.

tRNA modifications and neurological disorders

Intellectual disability is a major health problem worldwide with an estimated prevalence of up to 3% in the total population. In approximately 25% of cases, the disease is due to chromosomal rearrangements that are either cytogenetically visible (e.g., Down's syndrome trisomy 21) or submicroscopic (smaller deletions or duplications in the DNA). However, the remaining 75% of cases are believed to be caused by different single gene mutations that may or may not be linked to the X chromosome [9]. Several reports associate human intellectual disability and mutations in genes that encode for tRNA modification enzymes [10–17].

The FtsJ RNA methyltransferase homolog 1 (*FTSJ1*) gene has been described as the closest human homolog to the yeast tRNA methyltransferase 7 (*TRM7*) gene, which encodes for a methyltransferase acting at positions 32 and 34 on $tRNA^{Leu}$, $tRNA^{Phe}$, and $tRNA^{Trp}$ [3,18] (Figure 1). In humans, *FTSJ1* maps to the X chromosome, and genetic analyses revealed mutations in this gene to be associated with non-syndromic X-linked mental retardation [11–14]. Additionally, a strong correlation between the genetic variations in *FTSJ1* and cognitive functions in young males of the Chinese Han population has also been observed [19]. Mutant *FTSJ1* transcripts were reported to be very unstable and, in at least one case, the aberrant transcript was shown to be degraded via nonsense-mediated mRNA decay [11,14]. Northern blot analysis on a panel of normal human tissues revealed that wild type *FTSJ1* was expressed in several fetal tissues, most prevalently in the fetal brain [11]. In a different study, the expression of the gene in a panel of adult tissues was found to be highest in the heart and liver, and very low in the brain [12]. Thus, current data point towards a critical role

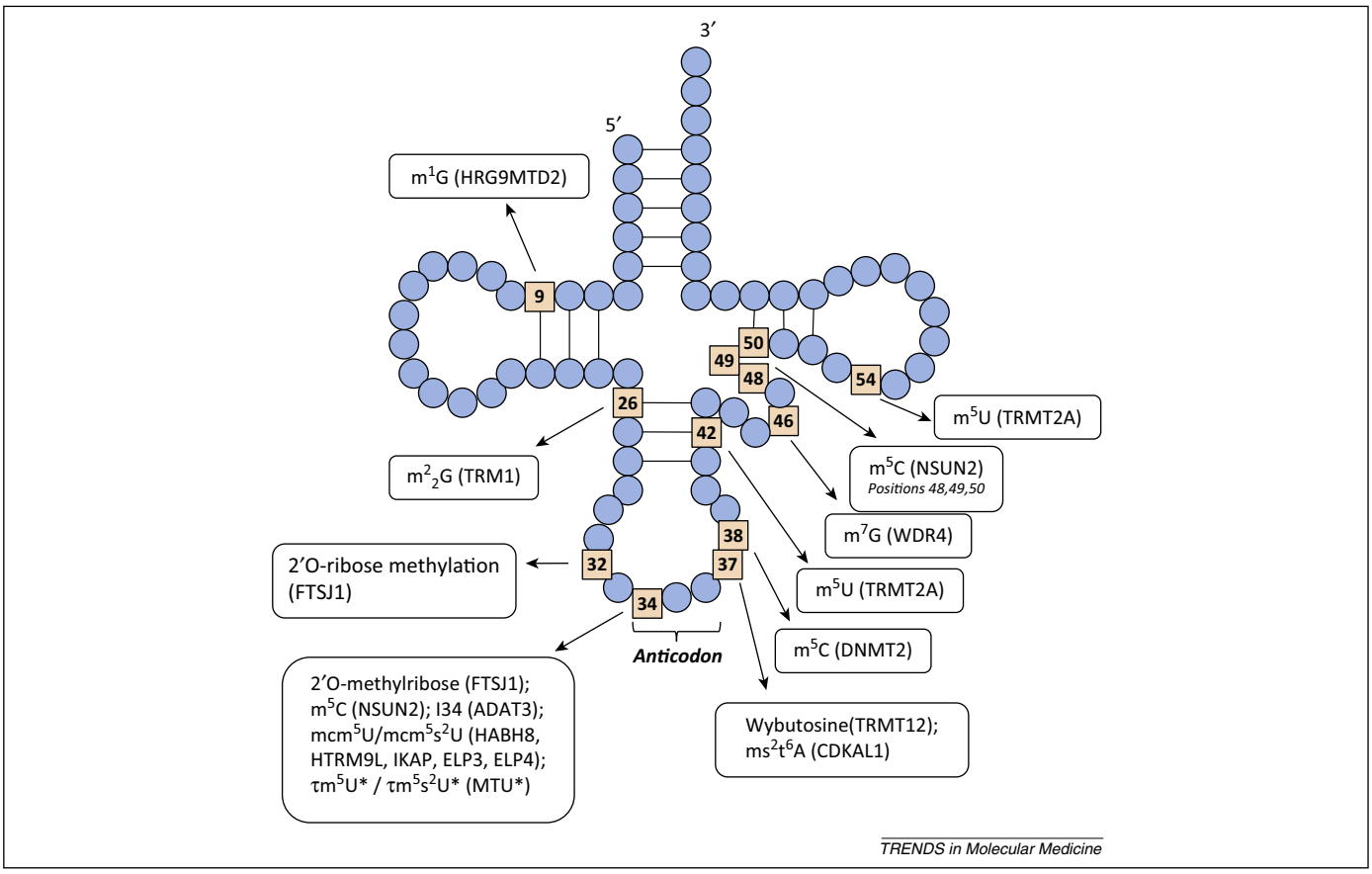

Figure 1. tRNA structure and modifications discussed in this opinion article. Schematic representation of the 'clover leaf' tRNA secondary structure. Blue dots represent RNA bases. Connecting lines between RNA residues indicate base pairing. Positions where aberrant residue modifications have been linked to human pathologies (squared numbers) and the proteins responsible for such modifications (in parentheses) are also shown. Asterisks denote modification only present in mitochondrial tRNA. Abbreviations: tRNA, transfer RNA; m^1G, 1-methylguanosine; $m^2{}_2G$: N2,N2-dimethyl guanosine; m^5C: 5-methylcytosine; I34: inosine at position 34; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; τm^5U, 5-taurinomethyluridine; τm^5s^2U, 5-taurinomethyl-2-thiouridine; ms^2t^6A, 2-methylthio-N6-threonyl carbamoyladenosine; m^5U, 5-methyl uridine; m^7G, 7-methylguanosine; HRG9MTD2, human RNA (guanine-9-)methyltransferase domain containing 2; TRM or TRMT, tRNA methyltransferase; FTSJ1, FtsJ RNA methyltransferase homolog 1; NSUN2, NOP2/Sun RNA methyltransferase family member 2; ADAT3, adenosine deaminase acting on tRNA 3; HABH8, human AlkB homolog 8; IKAP, IκB kinase complex-associated protein; ELP, Elongator protein homolog; MTU, mitochondrial tRNA-specific-2-thiouridylase 1; CDKAL1, CDK5 regulatory subunit associated protein 1-like 1; DNMT2, DNA methyltransferase 2; WDR4, WD repeat domain 4.

for *FTSJ1*, mainly in the developing brain; whereas its potential role in adulthood remains to be explored.

Other mutations in tRNA modification enzymes mapping outside the X chromosome have also been linked to intellectual disability. Human tRNA methyltransferase 1 (*TRM1*) encodes a methyltransferase in charge of dimethylating guanosines ($m^2{}_2G$) at position 26 of tRNAs [20] (Figure 1). A homozygous frameshift mutation that inactivates this gene has been reported as a novel marker for recessive cognitive disorders [15]. A second human homolog of yeast *TRM1*, for which the methyltransferase activity has not been confirmed, has also been described, named '*hTrm1-like*' [3]. Interestingly, in mice this gene was found to be expressed in neural tissues and was reported to play a role in motor coordination and exploratory behavior [21].

NOP2/Sun RNA methyltransferase family member 2 (*NSUN2*) encodes for a methyltransferase that catalyzes the formation of 5-methylcytosine (m^5C) at position 34 of $tRNA^{Leu}$(CCA) [22] and also positions 48, 49, and 50 on several tRNAs [23,24] (Figure 1). Two independent reports showed that mutations in this gene are associated with autosomal-recessive intellectual disability, according to genetic analyses in affected individuals from different populations [16,25]. Moreover, Khan *et al.* [25] showed that wild type NSUN2 localized to nucleoli, whereas mutant NSUN2 failed to do so, suggesting a mechanism for the observed lack of function in affected individuals. Additionally, deletion of the fly *NSUN2* ortholog results in severe short-term memory deficits, suggesting an evolutionary conserved role for tRNA methylation in normal cognitive development [16].

Intellectual disability associated with NSUN2 has also been observed in the form of the Dubowitz-like syndrome (DS). This disease is a rare genetic disorder characterized by microcephaly, growth and mental retardation, eczema, and peculiar facial features. Whole exome sequencing was used to identify a splicing mutation in NSUN2 in DS patients. Cells from these patients lacked NSUN2 protein and m^5C modification at positions 47 and 48 of $tRNA^{Asp}$(GTC) [26]. Another study also using whole exome sequencing found a disease-causing mutation in NSUN2 that resulted in Noonan-like syndrome [27]. This relatively common congenital disorder mainly affects the heart (pulmonary valve stenosis, atrial septal defects, hypertrophic cardiomyopathy, etc.), but also results in mental retardation in approximately 25% of affected individuals.

In yeast, the proteins Trm8p and Trm82p form the complex responsible for catalyzing the formation of

Table 1. Human diseases associated with tRNA modifications[a]

Disease category	Disease	Affected tRNA modification	Gene involved	Refs
Neurological	Intellectual disability	2′O-methylribose	*FTSJ1*[b]	[11–14,19]
		$m^2{}_2G$	*TRM1*	[15]
		m^5C	*NSUN2*	[16,25]
		m^7G	*WDR4*[c]	[10]
		A-to-I editing	*ADAT3*	[17]
	Familial dysautonomia	mcm^5s^2U	*IKBKAP*	[30–33]
	Amyotrophic lateral sclerosis	mcm^5s^2U	*ELP3*	[38]
	Rolandic epilepsy	mcm^5s^2U	*ELP4*	[39]
	Dubowitz-like syndrome	m^5C	*NSUN2*	[26]
Cardiac	Noonan-like syndrome[d]	m^5C	*NSUN2*	[27]
Respiratory	Bronchial asthma	mcm^5s^2U	*IKBKAP*	[36]
Cancer	Skin, breast, and colorectal	m^5C	*NSUN2*	[40,71]
	Breast	wybutosine	*TRMT12*	[41]
		m^5U	*TRMT2A*	[45]
	Colorectal	m^1G	*HRG9MTD2*[e]	[46]
	Urothelial	mcm^5U	*HABH8* (*HALKBH8*)	[42]
	Breast, bladder, colorectal, cervix, testicular	mcm^5U	*HTRM9L*	[49]
	Epigenetic cancer treatment	m^5C	*DNMT2*	[43]
Metabolic	Type 2 diabetes	ms^2t^6A	*CDKAL1*	[50–57]
Mitochondrial-linked	MELAS	τm^5U	*mt tRNA*^{Leu}(*UAA*)	[59,61,69]
	MERRF	τm^5s^2U	*mt tRNA*^{Lys}(*UUU*)	[60]
	Infantile liver failure	s^2U	*MTU1* (*TRMU*)	[64]
	Deafness associated with mutations in mitochondrial 12S ribosomal RNA	s^2U	*MTU1* (*TRMU*)	[65]

[a]Abbreviations: mt, mitochondrial; $m^2{}_2G$, N2,N2-dimethyl guanosine; m^5C, 5-methylcytosine; m^7G, 7-methylguanosine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; m^5U, 5-methyl uridine; m^1G, 1-methylguanosine; mcm^5U, 5-methoxycarbonylmethyluridine; ms^2t^6A, 2-methylthio-N6-threonyl carbamoyladenosine; τm^5U, 5-taurinomethyluridine; τm^5s^2U, 5-taurinomethyl-2-thiouridine; s^2U, 2-thiouridine.

[b]Linked to chromosome X.

[c]Might be involved in Down's syndrome but no direct link to the disease has been shown.

[d]Heart problems are one of the main features of the disease, but it is also characterized by specific morphological phenotypes (widely set eyes, low set ears, webbed neck, and chest deformity) and mental retardation in some cases.

[e]One of the three predicted potential homologs of yeast TRM10 [3].

7-methylguanosine (m^7G) at position 46 of several tRNAs. Human WD repeat domain 4 (*WDR4*) has been proposed as the closest homolog to yeast *TRM82* [3] (Figure 1). *WDR4* was identified in a search for genes linked to Down's syndrome phenotypes, although the direct association between this gene and the disease has not yet been made [10].

Finally, a recent report described a single missense mutation in human adenosine deaminase acting on tRNA 3 (*ADAT3*) present in families with individuals affected with intellectual disability and strabismus [17]. *ADAT3* encodes for one of the subunits of the heterodimeric adenosine deaminase acting on tRNAs (hetADAT), which catalyzes the conversion of adenosine-to-inosine at position 34 (I34) of tRNAs [28] (Figure 1). The activity of hetADAT has been proposed to be a major parameter in the evolution of eukaryotic genomes, and the enzyme has been shown to be essential in yeast [5,28]. However, very little is known about the functional importance of this enzyme in mammals, and the link to intellectual disability represents the first connection described between hetADAT function and human physiology.

Familial dysautonomia (FD) is a complex genetic neuropathy affecting the autonomic and sensory nervous system [29]. Numerous reports have linked mutations in genes encoding for subunits of the Elongator complex to this disorder [30–33]. Elongator is a six subunit protein complex that is highly conserved from yeast to humans. Defects in this complex were shown to affect a number of cellular pathways including histone acetylation, exocitosis, telomeric gene silencing, and transcriptional elongation. However, it is now becoming accepted that all of the phenotypes associated with defective Elongator actually derive from the lack of formation of 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) at position 34 of tRNAs [2] (Figure 1).

In 2002, the human Elongator complex was purified and was found to be composed of IκB kinase complex-associated protein [IKAP (yeast Elp1p)], Stat3-interacting protein [StIP1 (yeast Elp2p)], Elongator protein 3 homolog (ELP3), ELP4, and two unidentified polypeptides [34]. Two independent studies identified two mutations in the human *IKBKAP* gene, encoding IKAP, as causative of FD [30,31]. The most prevalent mutation (>99.5%), which was found in homozygosity, was a single nucleotide change that leads to exon skipping and an aberrant truncated protein. The other mutation, found only in heterozygosity, was an arginine-to-proline missense mutation that was predicted to abolish a threonine phosphorylation in IKAP. Both studies were performed in individuals from the Ashkenazi Jewish population. A third study detected a proline-to-leucine missense mutation in the *IKBKAP* gene in a non-Jewish individual with FD [35]. Interestingly, although the exon-skipping mutation is recessive (found in homozygosity), cells from patients were found to contain both normal and truncated *IKBKAP* mRNAs and IKAP protein at variable

levels. For example, lymphoblast cell lines primarily expressed the wild type mRNA, whereas brain cells primarily expressed the mutant mRNA [30]. This strongly suggests that control of exon skipping is the molecular mechanism for the tissue-specific expression of the disease. Notably, mutations in IKAP have also been associated with bronchial asthma in children [36], suggesting perhaps a role for this protein also in bronchi.

Because Elongator has also been shown to influence α-tubulin acetylation in neurons, it has been proposed that Elongator might be playing a role in other neurological disorders such as Huntington's disease, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) due to aberrant microtubule-dependent intracellular trafficking [37]. Indeed, allelic variants of *ELP3* detected in three human populations have been associated with ALS. Moreover, mutagenesis screens in flies for important genes in neuronal communication and survival identified two different loss-of-function mutations in *ELP3*, and knockdown of Elp3 in zebrafish resulted in motor axonal abnormalities [38]. Also, the human *ELP4* gene has been associated with Rolandic epilepsy, the most common form of human epilepsy [39].

Altogether, these results point towards an important role for Elongator in neuron biology. To the best of our knowledge, the role of α-tubulin acetylation in neurological diseases has not been directly demonstrated thus far. However, in the nematode *Caenorhabditis elegans*, neurological dysfunctions have been directly associated with defects in Elongator-mediated tRNA modifications caused by mutations in ELPC1 and ELPC3 (homologs of IKAP and ELP3, respectively). Further, mutations in thiolation of uridine in cytoplasmic tRNA 1 (TUC1), the protein responsible for the thiolation step that acts on the modification caused by Elongator to obtain mcm^5s^2U at position 34, lead to developmental defects when in combination with ELPC1 or ELPC3 mutants [33].

tRNA modifications and cancer

Cancer is among the most complex human diseases at the molecular level. Several reports propose a direct link between tRNA modifications and different types of cancer such as skin, breast, bladder, and colorectal [40–46] (Table 1). Therefore, aberrant expression of tRNA modification enzymes should be considered as one of the many factors that characterize the disease.

NSUN2 was first described as a downstream target of the proto-oncogene Myc and shown to be responsible for Myc-induced keratinocyte proliferation and cell cycle progression [40]. NSUN2 is expressed at low levels in normal tissues, but it is abundant in a range of human and mice tumor types, including squamous cell carcinoma, colorectal cancer, and breast cancer [40]. Indeed, knockdown of NSUN2 was shown to reduce the growth of human squamous cell carcinoma xenografts in nude mice [40]. Interestingly, *NSUN2* is localized in the genomic region 5p15, which is duplicated in 32% of breast cancer patients [44,47] and in several different breast cancer cell lines [44].

There are several other tRNA modification enzymes linked to cancer. tRNA methyltrasnferase homolog 12 (*TRMT12*) is the human homolog of yeast *TRM12*, one of the enzymes involved in formation of wybutosine at position 37 on $tRNA^{Phe}$ [3] (Figure 1). This gene was found amplified in several breast cancer cell lines and overexpressed in 26 out of 30 analyzed breast cancer tumors [41]. Mammostrat, a diagnosis tool for predicting breast cancer recurrence after tamoxifen treatment, uses TRMT2A (homolog of yeast Trm2p) expression as one of its five biomarkers [45]. In yeast, Trm2p catalyzes the formation of 5-methyluridine (m^5U) at positions 42 and 54 of tRNAs [3] (Figure 1). Human RNA (guanine-9-)methyltransferase domain containing 2 (*HRG9MTD2*) could potentially be the human homolog for yeast *TRM10*, which is responsible for methylation of guanine at position 9 (m^1G9) of several tRNAs [3] (Figure 1). *HRG9MTD2* mRNA was one of seven transcripts found to display significant differences between early-onset and late-onset colorectal cancer patients [46]. Azacytidine, a drug currently being developed for epigenetic cancer therapy, was shown to inhibit cytosine 38 methylation of $tRNA^{Asp}$ that is catalyzed by DNA methyltransferase 2 (DNMT2) [43,48] (Figure 1). Finally, knockdown of human AlkB homolog 8 (HABH8 or HALKBH8) in human urothelial carcinoma cell lines was shown to induce apoptosis and downregulation of NADPH oxidase 1 (Nox1)-dependent reactive oxygen species, whereas *in vivo* it suppressed angiogenesis, invasion, and growth of bladder cancers [42]. Strong evidence suggests that *HABH8* is the human homolog of the yeast *TRM9* that catalyzes the formation of 5-methoxycarbonylmethyluridine (mcm^5U) at position 34 on $tRNA^{Arg}$ and $tRNA^{Glu}$ [3] (Figure 1). A second potential homolog of yeast *TRM9* was recently reported and termed 'tRNA methyltransferase 9-like' (*HTRM9L*). *HTRM9L* mRNA was found to be downregulated in breast, bladder, colorectal, cervix, and testicular carcinomas. Further, re-expression of hTRM9L in two colon carcinoma cell lines dramatically suppressed tumor growth *in vivo* [49].

Type 2 diabetes and human mitochondrial diseases

T2D affects more than 200 million people worldwide. It is caused both by genetic and environmental factors and it is characterized by defects on insulin secretion of pancreatic β cells and insulin resistance. There are several reports linking the gene coding for CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) with T2D [50–57]. *CDKAL1* encodes for a methylthiotransferase involved in the complex 2-methylthio-N6-threonyl carbamoyladenosine (ms^2t^6A) modification at position 37 in $tRNA^{Lys}(UUU)$ [57] (Figure 1). Evidence suggests that lack of ms^2t^6A37 leads to mistranslation of several proteins, including proinsulin, thus triggering the endoplasmic reticulum (ER) stress response. The molecular pathogenesis is believed to be due to the synthesis of abnormal proinsulin, which accumulates and cannot be converted into insulin, leading, in turn, to glucose intolerance. In agreement with this model, the detection of gene variants of *CDKAL1* is associated with impaired insulin secretion in T2D patients, and not associated with insulin sensitivity or obesity (reviewed in [58]).

There are two human mitochondrial-linked diseases associated with aberrant tRNA modifications: (i) mitochondrial myopathy, encephalopathy, lactic acidosis, and

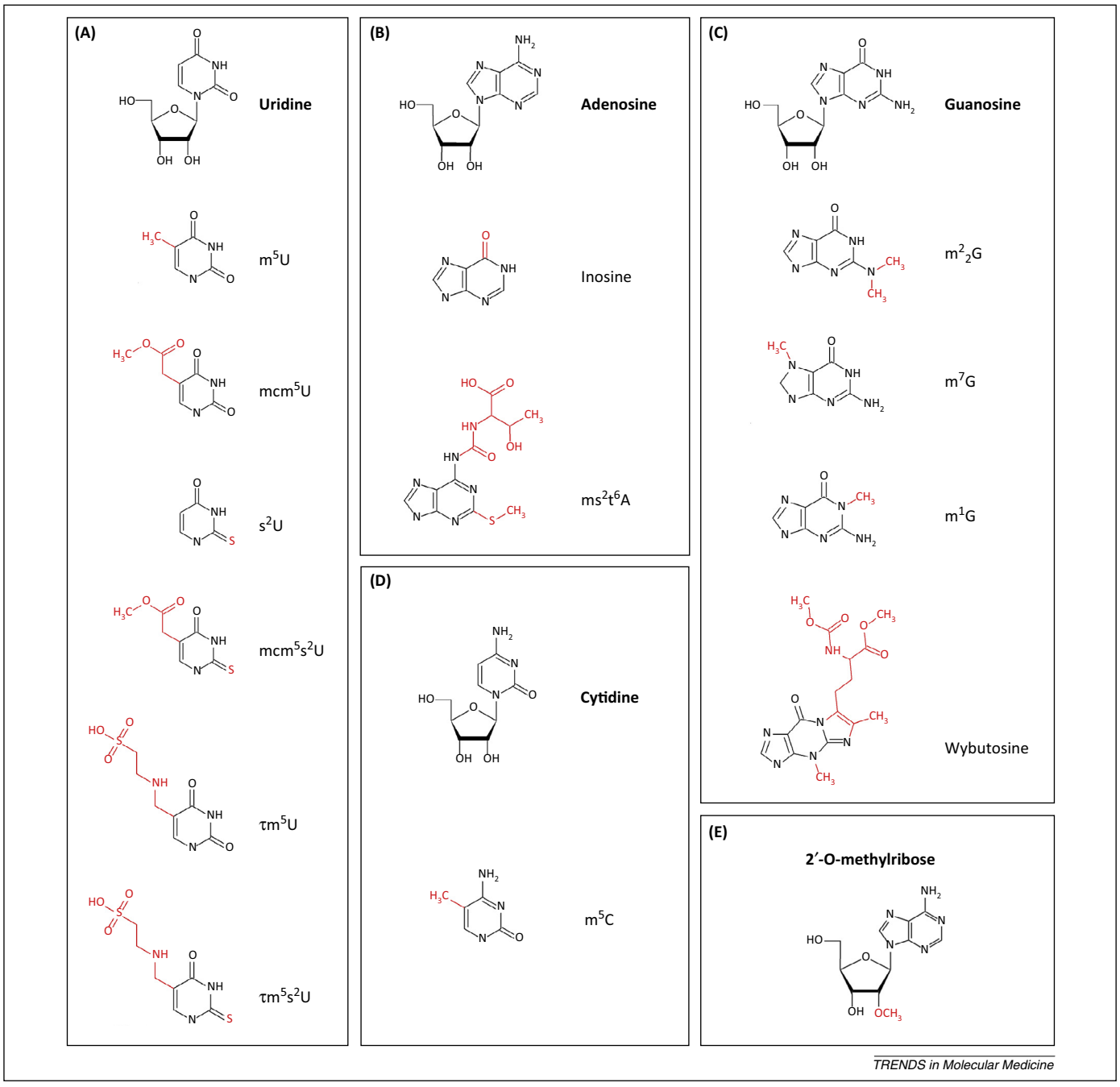

Figure 2. Chemical structures of tRNA modifications linked to human diseases. Unmodified nucleosides and nucleobases are depicted in black. Atomic changes for each chemical structure upon modification are shown in red. Nucleobase **(A–D)** and ribose **(E)** modifications are shown. Abbreviations: tRNA, transfer RNA; m^5U, 5-methyl uridine; mcm^5U, 5-methoxycarbonylmethyluridine; s^2U, 2-thiouridine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; τm^5U, 5-taurinomethyluridine; τm^5s^2U, 5-taurinomethyl-2-thiouridine; ms^2t^6A, 2-methylthio-N6-threonyl carbamoyladenosine; m^5C, 5-methylcytosine; $m^2{}_2G$, N2,N2-dimethyl guanosine; m^7G, 7-methylguanosine; m^1G, 1-methylguanosine.

stroke-like episodes (MELAS); and (ii) myoclonus epilepsy associated with ragged-red fibers (MERRF) [59–61]. In contrast to all the cases previously discussed, where cytosolic tRNAs are affected by aberrant modifications, both MELAS and MERRF are characterized by lack of modifications on mitochondrial tRNAs (mt tRNAs). The mutations in the mt tRNA genes that cause both diseases lie outside of the tRNA anticodon, but result in hypomodification of uridine 34. In particular, MELAS patients lack 5-taurinomethyluridine (τm^5U) on mt $tRNA^{Leu}$(UAA), whereas MERRF patients lack 5-taurinomethyl-2-thiouridine (τm^5s^2U) on mt $tRNA^{Lys}$(UUU) (Figure 1).

Modified mt $tRNA^{Leu}$(UAA) can read codons UUA and UUG, whereas modified mt $tRNA^{Lys}$(UUU) can read codons AAA and AAG. The absence of τm^5U specifically prevents mt $tRNA^{Leu}$(UAA) from reading UUG codons, whereas it does not affect reading of the UUA codons. However, the lack of τm^5s^2U impedes mt $tRNA^{Lys}$(UUU) to read both of its cognate codons (AAA and AAG). These effects correlate well with the marked reduction in overall mitochondrial translation observed for MERRF patients, and the specific reduction in ND6 observed in MELAS patients. ND6 is a component of the respiratory chain Complex I and it is encoded by a UUG codon enriched

transcript. A reduction of Complex I activity is characteristic of MELAS (reviewed in [62]).

As mentioned earlier, MERRF patients suffer from lack of $\tau m^5 s^2 U$ modification. It is unclear which is/are the enzyme(s) responsible for taurine modification in humans. However, the mitochondrial tRNA-specific-2-thiouridylase 1 (MTU1) was found to be responsible for s^2U modification in yeast and humans [63]. Therefore, this enzyme could have a role in the $\tau m^5 s^2 U$ modification absent in MERRF. Mutations in *MTU1* (also known as *TRMU*) have been associated with other human diseases, such as acute infantile liver failure [64], and deafness associated with mutations in mitochondrial 12S ribosomal RNA [65].

Towards novel tRNA modification-based therapeutics

These are early days to think of reliable approaches to develop therapeutics based on correcting hypomodified tRNAs or modulating the expression of tRNA modification enzymes. However, the current available literature does suggest potential ways forward in the form of specific gene therapies, splicing correction strategies, or tissue-specific drugs.

Gene therapy could be used to express normal tRNA modification enzymes in those cases where genetic mutations affect such enzymes. This could be feasible, for example, for correcting *ADAT3* mutations. Most tRNAs containing an A34 are found modified to I34. This suggests that perhaps the activity of hetADAT is saturated in cells. If this were true, overexpressing wild type ADAT3 to compensate for the mutated ADAT3 in principle should not have adverse effects. By contrast, this strategy might not be optimal to correct *FTSJ1* defects, considering that a male patient bearing a chromosomal duplication of normal *FTSJ1* (among other genes) showed moderate mental impairment, autistic-like behaviors, and mild dysmorphic features [13]. This might suggest that FTSJ1 levels in the brain need to be carefully regulated, and that therapies based on an overexpression of the enzyme might not be possible.

Existing literature indicates that modulating tRNA modification enzymes might be a promising therapeutic strategy to treat cancer. Such cases include the downregulation of NSUN2 [40] or HABH8 [42] for treatment of squamous cell carcinoma and bladder cancer, respectively, and the overexpression of HTRM9L [49] for treatment of colorectal cancer. Similar strategies could also be used for targeting TRMT12 against breast cancer [41].

The alternative splicing mechanism behind the IKAP phenotypes in FD is particularly interesting for developing therapeutic strategies. Here, splice correction therapies to modulate the expression of functional IKAP might be applicable. Indeed, exon-skipping strategies based on oligonucleotides conjugated to cell penetrating peptides are being developed for other diseases, such as Duchenne muscular dystrophy [66]. Alternatively, drugs that could modulate the selection of specific splice sites could be used. In FD, the exon-skipping mutation induces the preferential use of an intron distal 5′ splice site. The protein HNRNP A2/B1 promotes selection of such splice sites and downregulation of this protein by (–)-epigallocatechin gallate (EGCG) was shown to increase the levels of correct IKAP transcript in FD-derived cells [67]. Likewise, the plant cytokinin Kinetin has also proved promising for treatment of FD. Although the detailed mechanisms for which Kinetin induces the splicing correction in IKAP is unclear, initial evidence suggests a Kinetin responsive sequence element in IKAP that might also be present in other transcripts involved in other pathologies such as neurofibromatosis [68].

Lastly, a very interesting approach can be learned from the MELAS system. It was found that whereas the U34 could not be modified in the mutant mt $tRNA^{Leu}$(UAA), the other mt $tRNA^{Leu}$ isoacceptor (UAG) carrying a mutated anticodon to (UAA) could be modified to $\tau m^5 U34$. This modified mutated mt $tRNA^{Leu}$ isoacceptor restored the mitochondrial deficiencies of MELAS in a lung carcinoma cell line bearing 99% MELAS mutant mt DNA [69]. Therefore, expressing a modified closely related tRNA isoacceptor could potentially be an alternative strategy in those diseases where the target tRNA cannot be properly modified.

The therapeutic applications based on tRNA modification biology are not restricted to detection and modulation of the levels of the tRNA modification enzymes or overexpression of modifiable tRNAs. Detection of hypomodified tRNAs could be used as diagnostic tools against different diseases. HRG9MTD2 was found to be a good candidate gene to identify early-onset and late-onset colorectal cancer patients [46], thus measuring the levels of m^1G9 modification might also be useful for this purpose. Likewise, detecting the levels of m^5U at positions 42 and 54 of tRNAs might be an alternative to Mammostrat [45]. In this regard, direct RNA sequencing combined with direct detection of RNA modifications may allow the generation of personal 'epi-tRNAomes' in the future, similar to low cost personal genomes that are becoming a reality with the advent of next generation sequencing platforms. In the future, the determination of hypomodified tRNAs may be a new tool for medical diagnosis and treatment.

To take full advantage of the potential of tRNA modifications in therapeutics, it is imperative to develop the right tools to rapidly identify and quantify such modifications, as well as suitable mouse or non-human mammalian models that mimic disease states for preclinical research. Importantly, in the past years there have been significant technical improvements in the field of tRNA modification detection. For example, 'miCLIP' (methylation individual-nucleotide resolution crosslinking and immunoprecipitation) and 5-azacytidine-mediated RNA immunoprecipitation (Aza-IP) have been developed to detect m^5C modifications in transcriptomes [23,24]. Likewise, a mass spectrometry-based technique to monitor the levels of a whole battery of tRNA modifications has proved useful to monitor the dynamics of tRNA modifications in different stress-related scenarios [70].

Concluding remarks

We are only now realizing the important roles of tRNA modification enzymes in human diseases and many questions remain unanswered (Box 1). Central among those is the actual biological role of many of these modifications, which remain poorly understood. It has been proposed that

Box 1. Outstanding questions

- Are tRNA modifications involved in other complex human diseases?
- Are the predicted human homologs for tRNA modification enzymes truly carrying out their predicted function on tRNAs?
- How many more human proteins are involved in tRNA modification? Are these also involved in human pathologies?
- How is the protein translation affected in human diseases associated with aberrant tRNA modifications? Is protein efficiency (abundancy) and/or protein fidelity (accuracy) altered?
- Can modulation of the tRNA modification status in pathological scenarios be used as a tool to restore normal patterns of gene expression?
- Can detection of hypomodified tRNAs be used as a tool for early diagnosis and prognosis of different pathologies?

anticodon modifications may play an essential role in the fidelity and efficiency of mRNA translation. However, to what extent this is the case, and whether this affects all genes equally, or only specific genetic programs remains to be determined. In this regard, it appears that the physiological links between disease and tRNA modifications are being described at a faster rate than their molecular and cellular features.

It is now time to increase efforts on understanding the molecular mechanisms triggered upon production of hypomodified tRNAs and how this can affect protein translation in pathological scenarios. To address these issues, the improvement of current technologies to measure transcript-specific translation rates in a transcriptome-wide setting will be crucial. Such technologies may include RNA sequencing-based, ribosome profiling-based, mass spectrometry-based, or combined transcriptomics/proteomics-based approaches.

Lastly, we need to keep an open mind regarding the possibility that some other complex human diseases might bear at the core of known phenotypes a link to improper function of tRNAs, reflecting on specific patterns of gene expression. We hope that this opinion article stimulates future research in this area and broadens the spectra of ideas for novel therapeutics.

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References

1 Agris, P.F. *et al.* (2007) tRNA's wobble decoding of the genome: 40 years of modification. *J. Mol. Biol.* 366, 1–13
2 Phizicky, E.M. and Hopper, A.K. (2010) tRNA biology charges to the front. *Genes Dev.* 24, 1832–1860
3 Towns, W.L. and Begley, T.J. (2012) Transfer RNA methytransferases and their corresponding modifications in budding yeast and humans: activities, predications, and potential roles in human health. *DNA Cell Biol.* 31, 434–454
4 El Yacoubi, B. *et al.* (2012) Biosynthesis and function of post-transcriptional modifications of transfer RNAs. *Annu. Rev. Genet.* 46, 69–95
5 Novoa, E.M. *et al.* (2012) A role for tRNA modifications in genome structure and codon usage. *Cell* 149, 202–213
6 Rudinger, J. *et al.* (1994) Histidylation by yeast HisRS of tRNA or tRNA-like structure relies on residues -1 and 73 but is dependent on the RNA context. *Nucleic Acids Res.* 22, 5031–5037
7 Astrom, S.U. and Bystrom, A.S. (1994) Rit1, a tRNA backbone-modifying enzyme that mediates initiator and elongator tRNA discrimination. *Cell* 79, 535–546
8 Novoa, E.M. and Ribas de Pouplana, L. (2012) Speeding with control: codon usage, tRNAs, and ribosomes. *Trends Genet.* 28, 574–581
9 Ropers, H.H. (2010) Genetics of early onset cognitive impairment. *Annu. Rev. Genomics Hum. Genet.* 11, 161–187
10 Michaud, J. *et al.* (2000) Isolation and characterization of a human chromosome 21q22.3 gene (*WDR4*) and its mouse homologue that code for a WD-repeat protein. *Genomics* 68, 71–79
11 Freude, K. *et al.* (2004) Mutations in the *FTSJ1* gene coding for a novel S-adenosylmethionine-binding protein cause nonsyndromic X-linked mental retardation. *Am. J. Hum. Genet.* 75, 305–309
12 Ramser, J. *et al.* (2004) A splice site mutation in the methyltransferase gene *FTSJ1* in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). *J. Med. Genet.* 41, 679–683
13 Bonnet, C. *et al.* (2006) Pure de-novo 5 Mb duplication at Xp11.22-p11.23 in a male: phenotypic and molecular characterization. *J. Hum. Genet.* 51, 815–821
14 Takano, K. *et al.* (2008) A loss-of-function mutation in the *FTSJ1* gene causes nonsyndromic X-linked mental retardation in a Japanese family. *Am. J. Med. Genet.* 147B, 479–484
15 Najmabadi, H. *et al.* (2011) Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 478, 57–63
16 Abbasi-Moheb, L. *et al.* (2012) Mutations in *NSUN2* cause autosomal-recessive intellectual disability. *Am. J. Hum. Genet.* 90, 847–855
17 Alazami, A.M. *et al.* (2013) Mutation in *ADAT3*, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. *J. Med. Genet.* 50, 425–430
18 Feder, M. *et al.* (2003) Molecular phylogenetics of the RrmJ/fibrillarin superfamily of ribose 2′-O-methyltransferases. *Gene* 302, 129–138
19 Gong, P. *et al.* (2008) Genetic variations in *FTSJ1* influence cognitive ability in young males in the Chinese Han population. *J. Neurogenet.* 22, 277–287
20 Liu, J. and Straby, K.B. (2000) The human tRNA($m^2_2G_{26}$) dimethyltransferase: functional expression and characterization of a cloned *hTRM1* gene. *Nucleic Acids Res.* 28, 3445–3451
21 Vauti, F. *et al.* (2007) The mouse Trm1-like gene is expressed in neural tissues and plays a role in motor coordination and exploratory behaviour. *Gene* 389, 174–185
22 Brzezicha, B. *et al.* (2006) Identification of human tRNA:m5C methyltransferase catalysing intron-dependent m5C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). *Nucleic Acids Res.* 34, 6034–6043
23 Hussain, S. *et al.* (2013) NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. *Cell Rep.* 4, 255–261
24 Khoddami, V. and Cairns, B.R. (2013) Identification of direct targets and modified bases of RNA cytosine methyltransferases. *Nat. Biotechnol.* 31, 458–464
25 Khan, M.A. *et al.* (2012) Mutation in *NSUN2*, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. *Am. J. Hum. Genet.* 90, 856–863
26 Martinez, F.J. *et al.* (2012) Whole exome sequencing identifies a splicing mutation in *NSUN2* as a cause of a Dubowitz-like syndrome. *J. Med. Genet.* 49, 380–385
27 Fahiminiya, S. *et al.* (2013) Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. *Clin. Genet.* http://dx.doi.org/10.1111/cge.12280
28 Gerber, A.P. and Keller, W. (1999) An adenosine deaminase that generates inosine at the wobble position of tRNAs. *Science* 286, 1146–1149
29 Slaugenhaupt, S.A. and Gusella, J.F. (2002) Familial dysautonomia. *Curr. Opin. Genet. Dev.* 12, 307–311
30 Slaugenhaupt, S.A. *et al.* (2001) Tissue-specific expression of a splicing mutation in the *IKBKAP* gene causes familial dysautonomia. *Am. J. Hum. Genet.* 68, 598–605
31 Anderson, S.L. *et al.* (2001) Familial dysautonomia is caused by mutations of the IKAP gene. *Am. J. Hum. Genet.* 68, 753–758
32 Close, P. *et al.* (2006) Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. *Mol. Cell* 22, 521–531

33 Chen, C. *et al.* (2009) Defects in tRNA modification associated with neurological and developmental dysfunctions in *Caenorhabditis elegans* elongator mutants. *PLoS Genet.* 5, e1000561
34 Hawkes, N.A. *et al.* (2002) Purification and characterization of the human elongator complex. *J. Biol. Chem.* 277, 3047–3052
35 Leyne, M. *et al.* (2003) Identification of the first non-Jewish mutation in familial dysautonomia. *Am. J. Med. Genet. A* 118A, 305–308
36 Takeoka, S. *et al.* (2001) Amino-acid substitutions in the *IKAP* gene product significantly increase risk for bronchial asthma in children. *J. Hum. Genet.* 46, 57–63
37 Nguyen, L. *et al.* (2010) Elongator – an emerging role in neurological disorders. *Trends Mol. Med.* 16, 1–6
38 Simpson, C.L. *et al.* (2009) Variants of the elongator protein 3 (*ELP3*) gene are associated with motor neuron degeneration. *Hum. Mol. Genet.* 18, 472–481
39 Strug, L.J. *et al.* (2009) Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (*ELP4*). *Eur. J. Hum. Genet.* 17, 1171–1181
40 Frye, M. and Watt, F.M. (2006) The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. *Curr. Biol.* 16, 971–981
41 Rodriguez, V. *et al.* (2007) Chromosome 8 BAC array comparative genomic hybridization and expression analysis identify amplification and overexpression of TRMT12 in breast cancer. *Genes Chromosomes Cancer* 46, 694–707
42 Shimada, K. *et al.* (2009) A novel human AlkB homologue, ALKBH8, contributes to human bladder cancer progression. *Cancer Res.* 69, 3157–3164
43 Schaefer, M. *et al.* (2009) Azacytidine inhibits RNA methylation at DNMT2 target sites in human cancer cell lines. *Cancer Res.* 69, 8127–8132
44 Frye, M. *et al.* (2010) Genomic gain of 5p15 leads to over-expression of Misu (NSUN2) in breast cancer. *Cancer Lett.* 289, 71–80
45 Bartlett, J.M. *et al.* (2010) Mammostrat as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. *Breast Cancer Res.* 12, R47
46 Berg, M. *et al.* (2010) Distinct high resolution genome profiles of early onset and late onset colorectal cancer integrated with gene expression data identify candidate susceptibility loci. *Mol. Cancer* 9, 100
47 Pierga, J.Y. *et al.* (2007) Microarray-based comparative genomic hybridisation of breast cancer patients receiving neoadjuvant chemotherapy. *Br. J. Cancer* 96, 341–351
48 Goll, M.G. *et al.* (2006) Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science* 311, 395–398
49 Begley, U. *et al.* (2013) A human tRNA methyltransferase 9-like protein prevents tumour growth by regulating LIN9 and HIF1-α. *EMBO Mol. Med.* 5, 366–383
50 Saxena, R. *et al.* (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316, 1331–1336
51 Steinthorsdottir, V. *et al.* (2007) A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. *Nat. Genet.* 39, 770–775
52 Stancakova, A. *et al.* (2008) Single-nucleotide polymorphism rs7754840 of *CDKAL1* is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J. Clin. Endocrinol. Metab.* 93, 1924–1930
53 Omori, S. *et al.* (2008) Association of *CDKAL1*, *IGF2BP2*, *CDKN2A*/B, *HHEX*, *SLC30A8*, and *KCNJ11* with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* 57, 791–795
54 Groenewoud, M.J. *et al.* (2008) Variants of *CDKAL1* and *IGF2BP2* affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia* 51, 1659–1663
55 Kirchhoff, K. *et al.* (2008) Polymorphisms in the *TCF7L2*, *CDKAL1* and *SLC30A8* genes are associated with impaired proinsulin conversion. *Diabetologia* 51, 597–601
56 Ohara-Imaizumi, M. *et al.* (2010) Deletion of *CDKAL1* affects mitochondrial ATP generation and first-phase insulin exocytosis. *PLoS ONE* 5, e15553
57 Wei, F.Y. *et al.* (2011) Deficit of tRNA(Lys) modification by *Cdkal1* causes the development of type 2 diabetes in mice. *J. Clin. Invest.* 121, 3598–3608
58 Wei, F.Y. and Tomizawa, K. (2011) Functional loss of *Cdkal1*, a novel tRNA modification enzyme, causes the development of type 2 diabetes. *Endocrine J.* 58, 819–825
59 Yasukawa, T. *et al.* (2000) Modification defect at anticodon wobble nucleotide of mitochondrial $tRNAs^{Leu}$(UUR) with pathogenic mutations of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *J. Biol. Chem.* 275, 4251–4257
60 Yasukawa, T. *et al.* (2000) Defect in modification at the anticodon wobble nucleotide of mitochondrial $tRNA^{Lys}$ with the MERRF encephalomyopathy pathogenic mutation. *FEBS Lett.* 467, 175–178
61 Kirino, Y. *et al.* (2005) Specific correlation between the wobble modification deficiency in mutant tRNAs and the clinical features of a human mitochondrial disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7127–7132
62 Suzuki, T. and Nagao, A. (2011) Human mitochondrial diseases caused by lack of taurine modification in mitochondrial tRNAs. *Wiley Interdiscip. Rev. RNA* 2, 376–386
63 Umeda, N. *et al.* (2005) Mitochondria-specific RNA-modifying enzymes responsible for the biosynthesis of the wobble base in mitochondrial tRNAs. Implications for the molecular pathogenesis of human mitochondrial diseases. *J. Biol. Chem.* 280, 1613–1624
64 Zeharia, A. *et al.* (2009) Acute infantile liver failure due to mutations in the *TRMU* gene. *Am. J. Hum. Genet.* 85, 401–407
65 Guan, M.X. *et al.* (2006) Mutation in *TRMU* related to transfer RNA modification modulates the phenotypic expression of the deafness-associated mitochondrial 12S ribosomal RNA mutations. *Am. J. Hum. Genet.* 79, 291–302
66 Betts, C. *et al.* (2012) Pip6-PMO, a new generation of peptide-oligonucleotide conjugates with improved cardiac exon skipping activity for DMD treatment. *Mol. Ther. Nucleic Acids* 1, e38
67 Anderson, S.L. *et al.* (2003) EGCG corrects aberrant splicing of IKAP mRNA in cells from patients with familial dysautonomia. *Biochem. Biophys. Res. Commun.* 310, 627–633
68 Hims, M.M. *et al.* (2007) Therapeutic potential and mechanism of kinetin as a treatment for the human splicing disease familial dysautonomia. *J. Mol. Med. (Berl.)* 85, 149–161
69 Kirino, Y. *et al.* (2006) Acquisition of the wobble modification in mitochondrial tRNALeu(CUN) bearing the G12300A mutation suppresses the MELAS molecular defect. *Hum. Mol. Genet.* 15, 897–904
70 Chan, C.T. *et al.* (2010) A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. *PLoS Genet.* 6, e1001247
71 Vachon, C.M. *et al.* (2007) Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. *Cancer Res.* 67, 8412–8418