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Domestic pig as a model organism for in silico research on human hereditary diseases caused by variants in tRNA genes*

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Summary

The aim of the study was to evaluate the possibility of using the domestic pig (*Sus scrofa domesticus*) as an *in silico* model for analyzing diseases caused by selected mutations in mitochondrial tRNA and nuclear tRNA-Met encoding genes in humans. For this purpose, the reference sequences of analogous genes in both species and the secondary structures of the molecules were compared. Nucleotide positions whose mutations are associated with the occurrence of diseases in humans were compared with analogous positions in the domestic pig. The results revealed a high degree of conservation, with nuclear tRNA genes transporting methionine achieving an average of 92% homology, while initiator tRNA values ranged from 97% to 100%. In the case of mitochondrial tRNA genes, the degree of homology varied from 64% to 93% (with an average of 80%), with the highest similarity observed for *MT-TM* and *MT-TL2* and the lowest for *MT-TV*. Significantly, in nine out of the ten nucleotide positions analyzed, whose mutations in humans are associated with various disorders, the sequences in pigs were identical to their corresponding human sequences. The results obtained indicate that the high degree of tRNA sequence conservation, including the positions that determine the occurrence of diseases, may provide a basis for further research on genetic disorders associated with tRNA mutations using the domestic pig as a model organism.

Keywords: tRNA diseases, domestic pig, *in silico* model

Animal models currently play a crucial role in biomedical research on human diseases. One of the most commonly utilized model organisms for these studies is the domestic pig (*Sus scrofa domesticus*) (17). This is due to the similarities between humans and pigs in terms of organ anatomy, metabolism, signalling pathways and their dysfunctions, as well as physiological processes (10). In addition, the domestic pig is one of the species with a fully sequenced genome, in which 21,630 protein-coding genes have been identified (2130), compared to 19,969 in humans (20). The size of the domestic pig mitochondrial genome is around 16,613 base pairs (29), while that of humans is 16,569 base pairs (4). Moreover, both genomes consist of 37 genes that perform the same functions in organisms, i.e. encoding 12S and 16S rRNA, 13 proteins involved in the oxidative phosphorylation process, and 22 tRNA molecules. This high degree of similarity demonstrates the usefulness of pigs as a model organism for genetic disease research, including studies on disorders resulting from mutations in tRNA-encoding genes.

Molecules of tRNA play a key role in the process of translation by transporting the appropriate amino acids to the ribosomes, thus enabling protein biosynthesis. Mutations in tRNA-encoding genes may lead to serious cellular disturbances, such as mitochondrial dysfunction in the case of some variants of mitochondrial tRNA (mt-tRNA) genes (14), and may also be the cause of genetic diseases, including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibers), cataracts, or cardiomyopathy (1).

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The human nuclear genome encodes over 500 tRNA genes. Approximately half of them are poorly expressed or silent, and their expression is also cell-specific. In addition, there are also many tRNA pseudogenes, i.e., non-functional DNA segments with a sequence similar to functional genes (28). For this reason, the study only covers genes encoding tRNA that carry methionine, which is a special amino acid with a unique role in the translation process, being the first amino acid incorporated into each newly synthesized protein. The exceptional role of methionine is revealed in the existence of two distinct types of tRNA molecules that transport it: initiator tRNA (*tRNA-iMet*), which participates exclusively in the initiation of translation, and elongation tRNA (*tRNA-Met*), which is used during the elongation of the polypeptide chain (6).

The aim of the present study was to assess the possibility of using the domestic pig for *in silico* modeling and prediction of changes in tRNA genes without the necessity for intervention in a living organism, with reference to *Homo sapiens*. Modern genetic engineering technologies open prospects for enhancing both current and future biomedical models, for example, by employing humanized pig models in preclinical studies. Genetically modified domestic pigs have been utilized as model organisms in research on diseases, such as albinism, Alzheimer's disease, Parkinson's disease, diabetes, as well as breast, colon, and lung cancers (17).

In this study, an evaluation of the possibility of using the domestic pig as a model organism for *in silico* researches was carried out on the basis of a comparative analysis of pig and human tRNA-encoding genes.

Material and methods

Sequences of mitochondrial tRNA-encoding genes of the domestic pig and the human were retrieved from the GenBank database, from the human mitochondrial genome reference sequence, NCBI RefSeq: NC_012920.1 (4) and from the porcine mitochondrial genome reference sequence, NCBI RefSeq: NC_012095.1. The exact positions of the genes in the sequences are given in Table 1. The nuclear tRNA and initiator tRNA methionine transporting genes were selected for comparative analyses on the basis of the highest prediction scores from the GtRNAdb 2.0 database (7) based on the human reference genome assembly GRCh38.p14 sequence, NCBI RefSeq: GCF_000001405.40 (24) and domestic pig genome assembly Sscrofa11.1 sequence, NCBI RefSeq: GCF_000003025.6 (30). The exact positions of the genes in the sequences are given in Table 2. Sequence alignments for all homologs of human and pig mt-tRNA were performed separately for each pair of genes using the CLUSTALW algorithm in the Unipro UGENE v. 51.0 program (21). On the basis of the alignments obtained, the degree of gene homology was determined for each type of tRNA transporting a given amino acid, and the corresponding nucleotides in the sequences were identified, taking into account their position in the gene and the genome. In cases where differences in sequences between humans and pigs were observed, changes were classified as mutual transversions, transitions, or indel mutations. For each tRNA molecule, the secondary structure was generated using the tRNAscan-SE 2.0 tool (16), and differences in their folding were compared. Additionally, using the Online Mendelian Inheritance in Man (OMIM) database (2, 3), the *loci* in human tRNA-encoding genes associated with disease-causing mutations were compiled and compared with pig genes to assess the convergence of nucleotide sequences at the corresponding positions.

Tab. 1. List of gene positions in reference sequences used in the analysis of mitochondrial genes

Gene	Abbreviation	*Sus scrofa domesticus* mitochondrial genome NCBI RefSeq: NC_012095.1	*Homo sapiens* mitochondrial genome NCBI RefSeq: NC_012920.1
		Position	
tRNA-Phe	*MT-TF*	1-70	577-647
tRNA-Val	*MT-TV*	1031-1098	1602-1670
tRNA-Leu (UUR)	*MT-TL1*	2669-2743	3230-3304
tRNA-Ile	*MT-TI*	3701-3769	4263-4331
tRNA-Gln	*MT-TQ*	3767-3839	4329-4400
tRNA-Met	*MT-TM*	3841-3908	4402-4469
tRNA-Trp	*MT-TW*	4953-5020	5512-5579
tRNA-Ala	*MT-TA*	5027-5094	5587-5655
tRNA-Asn	*MT-TN*	5096-5170	5657-5729
tRNA-Cys	*MT-TC*	5203-5268	5761-5826
tRNA-Tyr	*MT-TY*	5268-5333	5826-5891
tRNA-Ser (UCN)	*MT-TS1*	6883-6951	7446-7514
tRNA-Asp	*MT-TD*	6959-7026	7518-7585
tRNA-Lys	*MT-TK*	7715-7781	8295-8364
tRNA-Gly	*MT-TG*	9408-9476	9991-10058
tRNA-Arg	*MT-TR*	9824-9892	10405-10469
tRNA-His	*MT-TH*	11561-11629	12138-12206
tRNA-Ser (AGY)	*MT-TS2*	11630-11688	12207-12265
tRNA-Leu (CUN)	*MT-TL2*	11689-11758	12266-12336
tRNA-Glu	*MT-TE*	14091-14160	14674-14742
tRNA-Thr	*MT-TT*	15303-15371	15888-15953
tRNA-Pro	*MT-TP*	15371-15435	15956-16023

Results and discussion

The average similarity of nuclear genes encoding tRNA molecules transporting methionine was 92% (Tab. 3). The highest similarity of 100% was observed between the genes representing the pig isodecoder group 3, i.e., genes encoding tRNA molecules with the same anticodon, but differing

Tab. 2. List of gene positions in reference sequences used in the analysis of nuclear tRNA-Met and tRNA-iMet genes

Isodecoder ID	*Sus scrofa domesticus* Genome assembly Sscrofa11.1 NCBI RefSeq: GCF_000003025.6			*Homo sapiens* Genome assembly GRCh38.p14 NCBI RefSeq: GCF_000001405.40		
	Transcript ID	Chromosome	Position	Transcript ID	Chromosome	Position
tRNA-Met 1	1-1	7	21164856-21164928	1-1	8	123157230-123157302
tRNA-Met 2	2-1	2	13342707-13342779	2-1	1	71426493-71426565
tRNA-Met 3	3-1	4	16203143-16203215	3-1	6	28944575-28944647
				3-2		28953265-28953337
tRNA-Met 4	4-1	Unplaced scaffold	120140-120212	4-1	6	26735370-26735442
	4-2		125108-125180	4-2		26743263-26743335
	4-3		134795-134867	4-3		26766234-26766306
tRNA-Met 5	5-1	7	22247730-22247802	5-1	6	26701483-26701555
				5-2		26800113-26800185
tRNA-Met 6	6-1	7	21158237-21158309	6-1	16	87384022-87384094
tRNA-Met 7	7-1	7	21169304-21169376	7-1	6	57842214-57842286
tRNA-Met 8	8-1	6	14444347-14444419			
tRNA-iMet 1	1-1	3	28290820-28290891	1-1	1	153671250-153671321
	1-2	4	95980578-95980649	1-2	6	26286526-26286597
	1-3	7	20912465-20912536	1-3		26313124-26313195
	1-4		20922437-20922508	1-4		26330301-26330372
	1-5		20936554-20936625	1-5		27332985-27333056
	1-6		21343887-21343958	1-6		27592821-27592892
	1-7		21505542-21505613	1-7		27902493-27902564
	1-8		21639917-21639988	1-8	17	82494721-82494792
tRNA-iMet 2	2-1	7	21311986-21312057	2-1	6	27777885-27777956
	2-2		21316204-21316275			
	2-3		21468948-21469019			

Tab. 3. Summary of homology of nuclear methionine transporter tRNA genes

Homo sapiens / *Sus scrofa domesticus*	Met 1	Met 2	Met 3	Met 4	Met 5	Met 6	Met 7
Met 1	88%	97%	95%	96%	96%	90%	92%
Met 2	92%	95%	99%	93%	92%	95%	88%
Met 3	100%	86%	92%	85%	84%	97%	79%
Met 4	92%	93%	100%	92%	90%	93%	86%
Met 5	88%	95%	96%	93%	92%	89%	88%
Met 6	88%	99%	95%	97%	95%	90%	90%
Met 7	86%	96%	95%	97%	96%	88%	92%
Met 8	86%	97%	92%	96%	96%	86%	92%
	Mean: 92%						

in the sequences of the other domains of tRNA (11), and human isodecoder group 1, as well as between pig isodecoder group 4 and human isodecoder group 3. The lowest similarity was 79% for the genes of pig isodecoder group 3 compared to human isodecoder group 7. The high degree of conservation of these genes in both species results from their key role in the process of translation.

For nuclear genes encoding initiator tRNA transporting methionine, the homology reached 100% for human iMet 1 and pig iMet 1, while for human iMet 2 compared to pig iMet 2 it was 97%. This indicates an exceptional conservation of the initiator tRNA transporting methionine, which is crucial for translation and may suggest significant evolutionary pressure to maintain the gene unchanged (32), thereby confirming the close phylogenetic relationship between humans and pigs (13).

The sequence homology between pigs and humans in the mitochondrial tRNA-encoding genes ranged from 64% to 93%, with an average value of 80%. These results confirm the similarity between the two species regarding the genes under analysis. Moreover, the predominant type of mutation observed were transitions, which may be due to the lesser structural destabilization they cause compared to other types of changes. Detailed analysis results are presented in

Tab. 4. Results of comparative analysis of mitochondrial tRNA-encoding genes in humans and domestic pigs

Gene	Abbreviation	Transitions	Transversions	Ins/del	Identical positions	Sequence length	Homology
tRNA-Phe	*MT-TF*	8	5	5	54	72	75%
tRNA-Val	*MT-TV*	17	7	1	44	69	64%
tRNA-Leu (UUR)	*MT-TL1*	12	3	0	60	75	80%
tRNA-Ile	*MT-TI*	5	1	0	63	69	91%
tRNA-Gln	*MT-TQ*	8	7	1	58	73	79%
tRNA-Met	*MT-TM*	2	0	3	63	68	93%
tRNA-Trp	*MT-TW*	5	2	4	58	69	84%
tRNA-Ala	*MT-TA*	13	1	1	54	69	78%
tRNA-Asn	*MT-TN*	8	2	2	63	75	84%
tRNA-Cys	*MT-TC*	5	4	0	57	66	86%
tRNA-Tyr	*MT-TY*	4	0	2	61	67	91%
tRNA-Ser (UCN)	*MT-TS1*	10	6	0	53	69	77%
tRNA-Asp	*MT-TD*	8	5	2	53	68	78%
tRNA-Lys	*MT-TK*	15	4	3	48	70	69%
tRNA-Gly	*MT-TG*	11	3	1	53	68	78%
tRNA-Arg	*MT-TR*	5	4	4	52	65	80%
tRNA-His	*MT-TH*	14	6	0	49	69	71%
tRNA-Ser (AGY)	*MT-TS2*	13	1	0	45	59	76%
tRNA-Leu (CUN)	*MT-TL2*	1	3	1	66	71	93%
tRNA-Glu	*MT-TE*	6	2	1	61	70	87%
tRNA-Thr	*MT-TT*	12	5	7	47	69	68%
tRNA-Pro	*MT-TP*	10	1	3	54	68	79%
							Mean: 80%

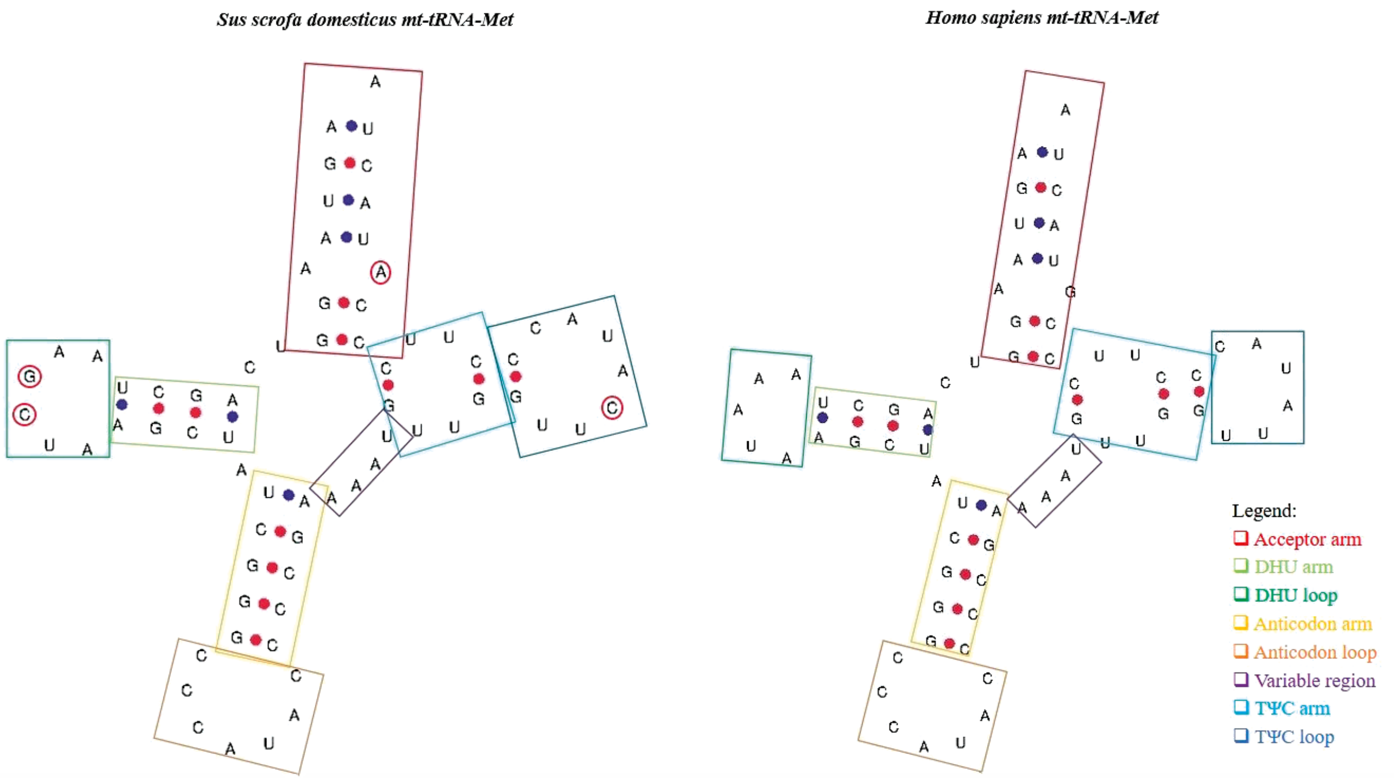

Fig. 1. Comparison of the secondary structures of human and domestic pig tRNA-Met molecules, highlighting sequence differences (substitutions and insertions) with red circles (models of molecules were generated using tRNAscan-SE 2.0)

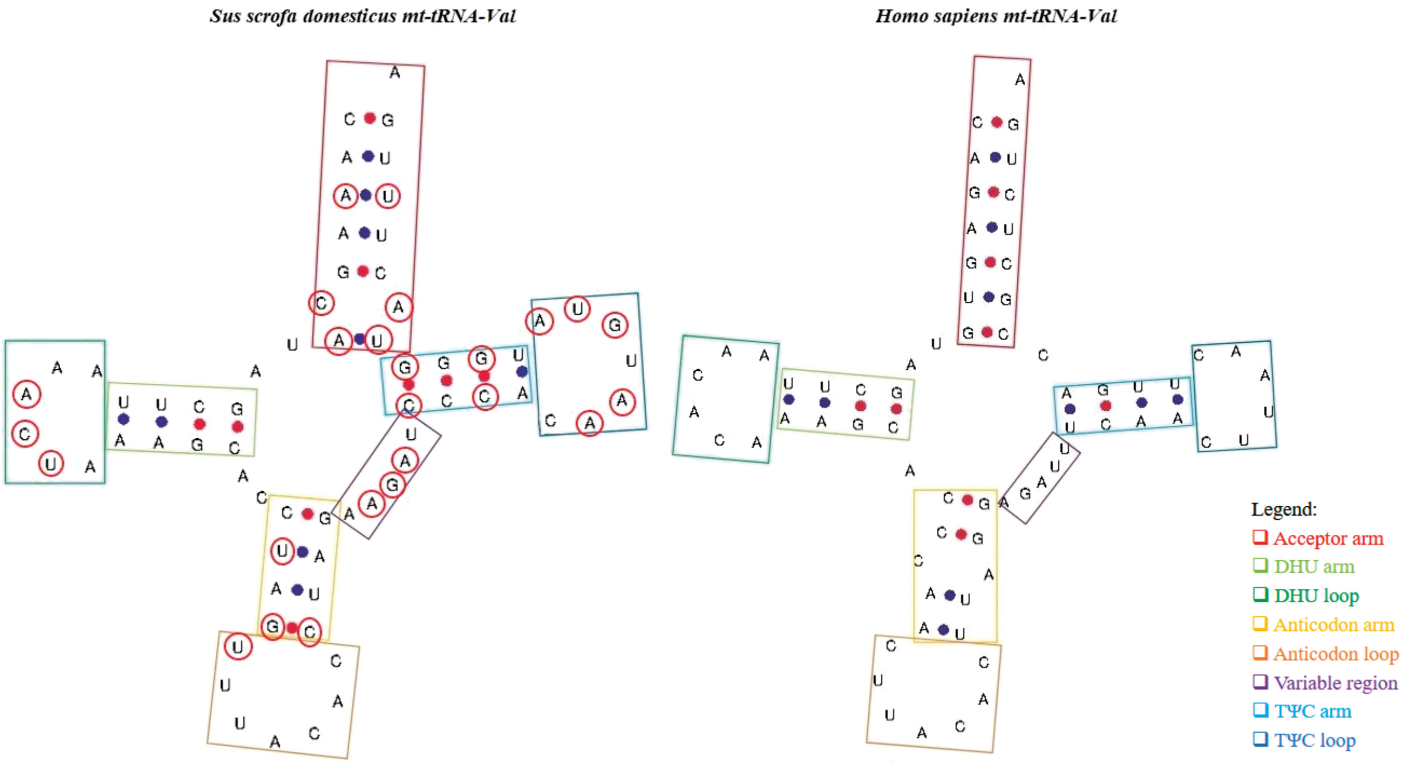

Fig. 2. Comparison of the secondary structures of human and domestic pig tRNA-Val molecules, highlighting sequence differences (substitutions and insertions) with red circles (models of molecules were generated using tRNAscan-SE 2.0)

Table 4. The highest interspecies similarity was observed for the tRNA transporting methionine (*MT-TM*) (Fig. 1) and the tRNA transporting leucine (*MT-TL2*), where the degree of homology was 93% in both cases. Differences in human and pig *MT-TM* genes comprised two transitions and three indel mutations, while for *MT-TL2* there was one transition, three transversions, and one indel. This suggests that the aforementioned tRNA molecules play fundamental roles in mitochondrial metabolism and are highly conserved throughout mammalian evolution.

The lowest similarity was observed in the genes encoding tRNA transporting valine (*MT-TV*) (Fig. 2). The degree of homology for *MT-TV* was 64%, with 17 transitions, seven transversions, and one indel mutation, indicating a greater evolutionary variability of this gene, which may be due to specific metabolic adaptations in humans and pigs (27). Comparisons of the secondary structures of the remaining molecules are attached to the supplementary material.

So far, pathogenic tRNA mutations have been identified only in their mitochondrial forms, which suggests that the mechanisms underlying tRNA-related diseases are closely linked with mitochondrion functioning. Mutations of nuclear tRNA-encoding genes, despite being found to occur and causing dysfunction in cell metabolism, have not yet been linked to any disease entity (22). Mitochondrial DNA is inherited exclusively from the mother and can occur in thousands of copies in a single cell. A condition in which different variants of mitochondrial DNA are present in a cell, tissue or organism is called heteroplasmy. Signs of the disease appear when the percentage of mutated mitochondria in a given tissue exceeds a critical threshold, which can range from 50% to 90%, depending on the mutation or cell type (1).

The two most common and best-known diseases associated with mitochondrial tRNA mutations are MELAS and MERRF. Their occurrence is closely related to changes in mitochondrial tRNA genes transporting leucine (*MT-TL1*) and lysine (*MT-TK*). In 80% of confirmed cases in humans, the occurrence of MELAS is attributed to the m.3243A>G heteroplasmic mutation in the dihydrouridine (DHU) loop region of the *MT-TL1* gene (12). This transition is also the most commonly encountered mitochondrial DNA mutation associated with type 2 diabetes mellitus (9). In the pig *MT-TL1* gene, nucleotide position 2682, which corresponds to the human position 3243, also contains adenine and is located in the DHU loop region.

In the case of MERRF, the m.8344A/G heteroplasmic mutation in the *MT-TK* gene in the pseudouridine (TΨC) loop region accounts for 80% to 90% of confirmed cases in humans (25). The corresponding position m.7761 in the pig *MT-TK* molecule was not identical to the human one, as it contains guanine, indicating that the pig reference sequence exhibits a variant that in humans is responsible for pathogenic cellular changes associated with MERRF. Among patients with MELAS, which evolved into MERRF, a heteroplasmic transition m.12147G/A was identified in the gene encoding tRNA transporting histidine (*MT-TH*) (18, 25), and the corresponding nucleotide position m.11570 in the pig *MT-TH* gene was identical.

Tab. 5. Comparison of the nucleotide positions of tRNA genes whose mutations were pathogenic with analogous positions of domestic pig genes (the authors' elaboration based on Abott et. al 2014 (1))

Gene	Mutation causing disease in humans	Analogous position in the domestic pig	Disease	Structural location – in both human and pig tRNA molecule	OMIM#
MT-TL1	m.3243A/G	m.2682A	MELAS	DHU loop	540000
			Type 2 diabetes		125853
MT-TK	m.8344A/G	m.7761G	MERRF	TΨC loop	545000
MT-TI	m.4295A>G	m.3733A	Hypertrophic cardiomyopathy	DHU loop	192600
	m.4300A>G	m.3738A		Anti-codon arm	
	m.4308G>A	m.3745G	CPEO	TΨC stem	590045
	m.4302A>G	m.3740A		Variable region	
MT-TH	m.12183G>A	m.11606G	Sensorineural hearing loss, pigmentary retinopathy	TΨC stem	590040
	m.12201T/C	m.11624T	Non-syndromic hearing impairment	Acceptor stem	590040
	m.12147G/A	m.11570G	MELAS	DHU stem	540000
			MERRF		545000
MT-TE	m.14685G>A	m.14102G	Cataract, peripheral retinal degeneration, pigmentary retinopathy	TΨC stem	590025

Diseases caused by tRNA gene mutations also include hypertrophic cardiomyopathy, caused by transitions m.4295A>G (19) or m.4300A>G (26) in the gene encoding tRNA transporting isoleucine (*MT-TI*). In both cases, the analogous nucleotide positions in the reference sequence, m.3733 and m.3738 respectively, were identical, both containing adenine.

In addition to MELAS, MERRF, and cardiomyopathy, mutations in mitochondrial tRNA genes have been associated with sensory organ defects. In one study, non-syndromic hearing impairment was linked to the m.12201T>C heteroplasmic mutation in the acceptor stem of *MT-TH*, although the severity of the hearing loss was associated with the degree of heteroplasmy (31). In patients with a heteroplasmic *MT-TH* mutation – m.12183G/A – visual impairment developed at around 7 years of age (8). The corresponding positions in the domestic pig were identical, with position m.11624 containing thymine corresponding to human position m.12201 and position m.11606 containing guanine analogous to human position m.12183. The same *MT-TH* mutation is responsible for retinitis pigmentosa as well as sensorineural hearing loss. One study showed that the pathogenic transition m.14685G>A in the mitochondrial gene encoding tRNA transporting glutamic acid (*MT-TE*) is associated with cataracts, peripheral retinal degeneration, and retinitis pigmentosa (15). In the case of porcine *MT-TE* gene, the corresponding position m.14102 was also identical, containing guanine.

Chronic progressive external ophthalmoplegia (CPEO) is another disease with a mitochondrial tRNA mutation background. CPEO has been linked to transitions m.4308A>G (23) and m.4302A>G (5) within the variable region of *MT-TI*. In this case, the corresponding nucleotide positions in the pig *MT-TI* gene, m.3745 and m.3740, were identical as well.

Among the ten nucleotide positions, whose mutations in humans are associated with diseases, nine were found to be identical when compared with the corresponding positions in the pig genes. In one case, the pig sequence contained a nucleotide corresponding to the pathogenic human variant. Detailed analysis results are presented in Table 5.

The nucleotide positions whose mutations in humans are linked with diseases were identical to their pig counterparts in cases causing MELAS, MELAS/MERRF, hypertrophic cardiomyopathy, CPEO, cataracts, peripheral retinal degeneration, *retinitis pigmentosa*, and hearing defects. These mutations occur in the *MT-TH*, *MT-TI*, *MT-TL1*, and *MT-TE* genes, which indicates the particular importance of these molecules in mitochondrial metabolism, as well as the significance of the affected nucleotide positions for the proper function of these molecules. Moreover, in all cases these changes were transitions, which may suggest that transversions or insertion/deletion mutations are less frequent or less tolerated due to their lethality.

The results obtained may serve as a basis for further research on the utility of the domestic pig as a model organism in the context of diseases caused by mutations in tRNA-encoding genes.

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