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Review article

MELAS as an example of a mitochondrial disease

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Abstract. MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is a disease mainly due to a mutation at position 3243 (A → G) in the leucine tRNA gene in mitochondrial DNA. Symptoms of the disorder are complex and the exact pathogenesis is not understood. A review of the literature on the subject is presented.

Key words: mitichondrial diseases, MELAS.

MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is one of the most common mitochondrial encephalomyopathies. The first symptoms are usually observed in childhood or in early adolescence. The most frequent symptoms are: myopathy, encephalopathy, vomiting, seizures, migraine-like headaches, dementia, and recurrent stroke-like episodes causing hemiparesis, hemianopia or cortical blindness. Stroke-like episodes appear at first in the parieto-occipital cortex, but the remaining cortex may be affected subsequently. Their appearance is caused by cerebral ischaemia associated with the abnormal functioning of the smooth muscle vasculature. Basal ganglia calcifications are also commonly observed (DIMAURO et al. 1999, JAMES et al. 1999, LEONARD, SHAPIRA 2000).

MELAS is a maternally inherited genetic disorder. All mutations associated with MELAS have been found in mitochondrial DNA (mtDNA). The transition A3243G in the $tRNA^{Leu(UUR)}$ gene is found in 80% of the subjects. Almost all known mutations appear in tRNA genes, but mutations in genes encoding respiratory chain proteins – ND4 (A11084G) (subunit 4 of NADH : ubiquinone

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oxidoreductase) (LERTRIT et al. 1992) and CO3 (T9957C) (cytochrome oxidase subunit 3) (MANFREDI et al. 1995) have also been reported.

Heterogeneity of symptoms in patients with the A3243G mutation

The heterogeneity of clinical symptoms is a characteristic feature of mutations in mtDNA. For instance, transition A3243G may cause non-insulin-dependent diabetes and deafness. Symptoms characteristic for different mitochondrial disorders may overlap as well. Patients harbouring the A3243G mutation may exhibit mixed features observed in other mitochondrial disorders: MERRF (myoclonous epilepsy with ragged red fibres) (FABRIZI et al. 1996), MERRF/MELAS (CAMPOS et al. 1996), MELAS/PEO (progressive external opthalmoplegia) (PETRUZZELLA et al. 1994), MERRF/PEO (VERMA et al. 1996). The same effect is observed in patients with other mutations (NAKAMURA et al. 1995, ZEVIANI et al. 1993). Frequently the MELAS phenotype is not completely expressed: some patients may exhibit only partial symptomatology of the syndrome. Severity of the existing symptoms can vary dramatically.

The differences in symptoms are explained by the random segregation of mutated mtDNA molecules, which causes different mutation loads in various tissues. CHINNERY et al. (1997) investigated 150 individuals harbouring A3243G (MELAS) and A8344G (MERRF) mutations. They showed that the level of mutant mtDNA in skeletal muscle was highly correlated with the frequency of the major neurological features associated with each mutation. The same correlation was observed between the level of brain lactate and the mutation load in individuals carrying this mutation, whether they were symptomatic or not (DUBEAU et al. 2000). The mutation load is usually lower in blood in comparison with post-mitotic tissues such as the brain or muscle. In some cases the mutation was not detectable in blood despite a high level of mutated mtDNA in muscles (CHINNERY et al. 1999).

The threshold effect is a frequently observed phenomenon for mutations in mtDNA. The cell develops biochemical defects when the proportion of mutated mtDNA exceeds a certain critical level. The threshold of mutation is 60% mutated mtDNA for deletions and 95% for tRNA mutations (LEONARD, SHAPIRA 2000).

Histochemical analysis of muscle biopsy is critical for the diagnosis of mitochondrial disorders. Gomori trichrome stain makes it possible to reveal red ragged fibers (RRFs). RRFs are associated with abnormal subsarcolemmal accumulation of mitochondria in muscle fibers (CHINNERY et al. 1999). Muscle histochemistry may also show cytochrome oxidase deficiency. RRFs from MELAS subjects are usually COX positive (DIMAURO et al. 1999). Investigations revealed that the level of mutated mtDNA in RRFs (90-95%) is significantly higher in comparison with the surrounding fibers (~50% of mutated mtDNA) (PETRUZZELLA et al. 1994, SCHON 2000).

Tissue specificity of some mtDNA mutations

It is worth mentioning that many mutations in mtDNA are tissue-specific. For example, mutations in mitochondrial $tRNA^{Ile}$ gene commonly cause hypertrophic cardiomyopathies, whereas mutations in $tRNA^{Ser(UCN)}$ are associated only with deafness, even if a high level of mutation is present in other tissues. The same specificity is documented for mutations in mitochondrial rRNA genes. Four of them cause cardiomyopathies, and one is associated with aminoglycoside-induced deafness (SCHON 2000). In LHON patients (Leber's hereditary optic neuropathy), mutations in protein coding genes are often homoplasmic (100% of mutated mtDNA in all tissues). Despite that, only the optic nerve is affected; the functions of remaining tissues are not disturbed. These observations indicate that the phenotypic expression of a mutation depends on the type of tissue and on the particular nuclear background. This in turn suggests that faulty interactions leading to pathological symptoms would arise from the interactions of mitochondria containing a mutation with a protein or proteins present only in a given tissue. For example, in adPEO patients deletions in mtDNA are present in tissues, where an isoform of the adenine nucleotide translocator ANT1 is expressed (KAUKONEN et al. 2000).

It is possible that the nuclear background influences the mutation load. EMMERSON et al. (2001) constructed 4 different cell lines P1 and P2 obtained by fusing enucleated cells with the A3243G mutation with two different ρ_0 cell lines (rho zero, i.e. cell lines not containing any mitochondrial DNA): 206 and B2, respectively. The products of such fusions are called cybrids. In cybrids derived from ρ_0 line 206 the mutation load increases gradually. On the contrary, in cybrids derived from ρ_0 line B2 the mutation load decreases. A preferential replication of the mutated mtDNA in the P1 line was shown. In the P2 line the mutated mtDNA was under-represented among the replicated mtDNA molecules.

Biochemical defects in cells of MELAS patients

Mitochondrial disorders are believed to be the result of an oxidative phosphorylation defect and energy deficiency. In patients with a mitochondrial dysfunction, the phosphocreatine : inorganic phosphate ratio in mitochondria is lower when compared to normal subjects, it decreases excessively during exercise and returns to baseline values more slowly than normally (DIMAURO et al. 1999). The notion that mitochondrial diseases affect long-living postmitotic tissues with high oxidative requirement is at least incomplete. In some cases tissues with low energy requirement (such as bone, connective tissue or skin) are affected as well. On the contrary, there are only a few reports of lung and liver defects despite the fact that these tissues require a high level of ATP production (SCHON 2000).

Biochemical defects in cells harbouring MERRF and MELAS mutations are very similar. Both syndromes are caused by mutations in tRNA genes that affect the overall synthesis of mitochondrially encoded proteins. The analysis of fibroblasts carrying A3243G (MELAS) and A8344G (MERRF) mutations revealed that in both cases the cells exhibit a significantly decreased ATP synthesis rate and mitochondrial transmembrane potential (JAMES et al. 1996). Despite the respiratory chain defect, the levels of intracellular ATP are not decreased (BRINI et al. 1999, JAMES et al. 1999). The maximum rate of mitochondrial ATP synthesis is decreased by 60-88% as a consequence of a decrease in the proton electrochemical potential gradient. However, the ATP/ADP ratio reflecting the energetic load of the cell remains unchanged (JAMES et al. 1999).

As a result of a decreased mitochondrial transmembrane potential, MERRF cells exhibit changes in mitochondrial calcium homeostasis. Histamine agonist stimulation of MERRF cybrids causes a much lower increase in the mitochondrial calcium concentration in comparison with control. Such amplification is an important feature of the mitochondrial Ca^{2+} homeostasis necessary for the prompt stimulation of the mitochondrial effector systems. In consequence, mitochondria are unable to increase ATP synthesis after the Ca^{2+} stimulation. The defect in signal transduction of the higher energy requirement directly affects the level of cytoplasmic ATP (BRINI et al. 1999).

Calcium plays a critical role in the initiation of muscle contraction as well as the initiation and transduction of signals in the neuronal system and receptor cells. Induced biochemical processes (muscle contraction, plasma depolarisation) demand large quantities of energy and should be connected with increased ATP synthesis. It seems that the cell runs out of energy during the period of the highest energy requirement. The amount of ATP necessary for the functioning of ion channels and pumps is so high that its usage is limited by ATP diffusion, whereas glycolysis could never provide enough energy. Mitochondria produce ATP exactly in points of its highest requirement. Moreover, glycolysis produces large quantities of lactate. High lactate levels may cause brain injury (DIMAURO et al. 1999, DUBEAU et al. 2000). Frequent energy deficiencies could be compensated by an increased mitochondrial proliferation observed in RRFs.

Evidence supporting this hypothesis may be provided by the studies of JAMES et al. (1999). They simulated increased energy requirements in fibroblasts by the addition of gramicidine. This ionophor stimulates ATP hydrolysis by an ion pump: Na^+/K^+-ATPase. These authors found a threshold gramicidine concentration in control cells, at which both the ATP/ADP ratio and the plasma membrane potential decreased dramatically due to the ATP demand outstripping mitochondrial ATP synthesis. In MELAS and MERRF fibroblasts the corresponding threshold concentrations were 2-20-fold lower than those for control cells. In all types of quiescent fibroblasts ATP levels were not affected. These results suggest that MERRF and MELAS cells are sensitive to increased energy requirements.

Effects of the MELAS mutation on mitochondrial translation and transcription

Almost all mutations causing MELAS are localised in tRNA genes. This type of mutation called syn⁻ causes various defects in the translation system. Some of them are: the inhibition of mitochondrially encoded protein synthesis, the synthesis of defective proteins, or the synthesis of proteins in incorrect mutual ratios. Mutations in tRNA genes may influence the tRNA function by affecting: (i) a proper addition of –CCA at the 3' end of the tRNA (the –CCA sequence is not encoded in human tRNA genes); (ii) the recognition of tRNA molecules by enzymes involved in posttranscriptional modification of the tRNA; (iii) the recognition of the tRNA by aminoacyl-tRNA synthetases (a lower rate of charging or mischarging of tRNAs with incorrect aminoacids); (iv) the recognition of tRNAs by the ribosome or elongation factors; (v) the stability of tRNA molecules resulting in their increased degradation (KAUKONEN et al. 2000). Some of these defects have been observed in cell lines harbouring the A3243G mutation.

In most biopsies from patients with A3243G mutation (BORNER et al. 2000), mutant tRNA is under-represented among the processed and/or aminoacylated tRNAs. CHOMYN et al. (2000) proved that cell lines harbouring almost homoplasmic A3243G mutation exhibited a strong (70-75%) reduction in the level of aminoacylated $tRNA^{Leu(UUR)}$ molecules. YASUKAWA et al. (2000), while investigating cells with the A3243G mutation, revealed: (i) a decreased life-span of $tRNA^{Leu(UUR)}$ resulting in the 70% decrease in the amount of the tRNAs in the steady state; (ii) a slight decrease in the ratios of aminoacyl-$tRNA^{Leu(UUR)}$ versus uncharged $tRNA^{Leu(UUR)}$; (iii) charging tRNA with leucine (a lack of misaminoacylation). These studies suggest that an increased degradation of $tRNA^{Leu(UUR)}$ molecules results in a decreased level of this aminoacylated tRNA species.

Additionally, YASUKAWA et al. (2000) revealed a decreased rate of uridine modification in the first position of the anticodon. The lack of this modification may lead to the mistranslation of leucine into non-cognate phenylalanine codons as a result of mispairing mutated tRNAs with UUU or UUC codons. Despite these results, there is no correlation between the quantity of the UUR codons and the degree of protein synthesis inhibition. KING et al. (1992) suggested a correlation between the protein length and the degree of its synthesis inhibition.

Another effect of the A3243G mutation is conceivable. This mutation appears in a transcription termination site located on the boundary of 16S rRNA and $tRNA^{Leu(UUR)}$ genes. The transcription termination site is recognised by a protein factor called mtTERM. The main function of the mtTERM is a partial termination of transcription downstream from the rRNA genes. This mechanism is responsible for the maintenance of proper ratios of rRNAs versus the remaining RNA molecules. *In vitro* investigations revealed that the A3243G transition significantly

decreases the mtTERM affinity for the target tridecamer sequence resulting in decreased transcription termination (HESS et al. 1991, SHANG, CLAYTON 1994). *In vivo*, in cybrids harbouring A3243G mutation, the steady state levels of rRNA and mRNA are not affected. However, it could not be excluded that the proper rRNA/mRNA ratio is maintained by an increased degradation of mRNA (KING et al. 1992).

A defect in the processing of large polycistronic RNA transcribed from the H strand is another possible effect of mutations in mitochondrial tRNA genes. Probably tRNA gene sequences, flanking almost every rRNA and mRNA gene, serve as sites for recognition by enzymes that precisely cleave transcripts producing three kinds of RNA molecules. Cybrids harbouring the A3243G mutation exhibit increased levels of RNA 19, consisting of uncut 16S rRNA, $tRNA^{Leu(UUR)}$ and ND1 (mitochondrial subunit 1 of NADH : ubiquinone oxidoreductase) mRNA molecules. Genes encoding these RNAs are localised next to each other in the mtDNA. The RNA 19 level is increased 2-fold and is equal to 2% of the amount of mature rRNA and 28% of the ND1 mRNA (KING et al. 1996). An increased level of RNA 19 is also observed in biopsies derived from patients harbouring the A3243G mutation (KAUFMANN et al. 1996). SCHON et al. (1992) observed a strong inverse correlation between the levels of RNA 19 per cell and the rates of respiration in the cybrid cells. The mechanism affecting the translation is unknown. Since RNA 19 contains a 16S rRNA fragment, it could be inserted into the ribosome. Such defective ribosomes could bind mRNA and inhibit its translation (ribosome stalling hypothesis) (SCHON et al. 1992). On the other hand, since RNA 19 also contains the ND1 mRNA fragment, this fragment may associate with the ribosome and the attempts of its translation might occur. However, the 5' end of ND1 mRNA is probably protected by the rRNA + tRNA tertiary structure lying upstream from the start codon. A ribosome being on its way to localise the start codon could become blocked. Occasionally, the ribosome could start translation using an internal methionine codon as the start codon (placed at position 3, 17, or 21). In such a case, the protein synthesised is slightly smaller. This fact could explain the slightly increased mobility of ND1 protein on SDS-PAGE gels described by KING et al. (1992) and SCHON et al. (1992).

The example of the A3243G mutation shows that mutations in tRNA genes may display a pleiotropic effect and can affect various processes taking place in mitochondria. Despite a steadily increasing amount of data we still do not completely understand the many pathological symptoms caused by this most common MELAS mutation. Indeed, it can affect the translation and transcription in mitochondria, perturb energy production and calcium homeostasis, and probably cause a variety of other hitherto undetected defects that together can manifest themselves clinically as a serious disease.

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REFERENCES

BORNER G.V., ZEVIANI M., TIRANTI V., CARRARA F., HOFFMANN S., GERBITZ K.D., LOCHMULLER H., PONGRATZ D., KLOPSTOCK T., MELBERG A., HOLME E., PAABO S. (2000). Decreased aminoacylation of mutant tRNAs in MELAS but not in MERRF patients. Hum. Mol. Genet. 9: 467-475.

BRINI M., PINTON P., KING M.P., DAVIDSON M., SCHON E.A., RIZZUTO R. (1999). A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency. Nat. Med. 5: 951-954.

CAMPOS Y., MARTIN M.A., LORENZO G., APARICIO M., CABELLO A., ARENAS J. (1996). Sporadic MERRF/MELAS overlap syndrome associated with the 3243 $tRNA^{Leu(UUR)}$ mutation of mitochondrial DNA. Muscle Nerve 19: 187-190.

CHINNERY P.F., HOWELL N., ANDREWS R.M., TURNBULL D.M. (1999). Clinical mitochondrial genetics. J. Med. Genet. 36: 425-436.

CHINNERY P.F., HOWELL N., LIGHTOWLERS R.N., TURNBULL D.M. (1997). Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain 120: 1713-721.

CHOMYN A., ENRIQUEZ J.A., MICOL V., FERNANDEZ-SILVA P., ATTARDI G. (2000). The mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode syndrome-associated human mitochondrial $tRNA^{Leu(UUR)}$ mutation causes aminoacylation deficiency and concomitant reduced association of mRNA with ribosomes. J. Biol. Chem. 275: 19198-19209.

DIMAURO S., BONILLA E., DE VIVO D.C. (1999). Does the patient have a mitochondrial encephalomyopathy? J. Child Neurol. 14 Suppl 1: S23-S35.

DUBEAU F., DE STEFANO N., ZIFKIN B.G., ARNOLD D.L., SHOUBRIDGE E.A. (2000). Oxidative phosphorylation defect in the brains of carriers of the tRNAleu(UUR) A3243G mutation in a MELAS pedigree. Ann. Neurol. 47: 179-185.

EMMERSON C.F., BROWN G.K., POULTON J. (2001). Synthesis of mitochondrial DNA in permeabilised human cultured cells. Nucleic Acids Res. 29: E1.

FABRIZI G.M., CARDAIOLI E., GRIECO G.S., CAVALLERO T., MALANDRINI A., MANNESCHI L., DOTTI M.T., FEDERICO A., GUAZZI G. (1996). The A to G transition at nt 3243 of the mitochondrial $tRNA^{Leu(UUR)}$ may cause an MERRF syndrome. Neurol. Neurosurg. Psychiatry 61: 47-51.

HESS J.F., PARISI M.A., BENNETT J.L., CLAYTON D.A. (1991). Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 351: 236-239.

JAMES A.M., SHEARD P.W., WEI Y.H., MURPHY M.P. (1999). Decreased ATP synthesis is phenotypically expressed during increased energy demand in fibroblasts containing mitochondrial tRNA mutations. Eur. J. Biochem. 259: 462-469.

JAMES A.M., WEI Y.H., PANG C.Y., MURPHY M.P. (1996). Altered mitochondrial function in fibroblasts containing MELAS or MERRF mitochondrial DNA mutations. Biochem. J. 318: 401-407.

KAUFMANN P., KOGA Y., SHANSKE S., HIRANO M., DIMAURO S., KING M.P., SCHON E.A. (1996). Mitochondrial DNA and RNA processing in MELAS. Ann. Neurol. 96: 172-180.

KAUKONEN J., JUSELIUS J.K., TIRANTI V., KYTTALA A., ZEVIANI M., COMI G.P., KERANEN S., PELTONEN L., SUOMALAINEN A. (2000). Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 289: 782-785.

KING M.P., KOGA Y., DAVIDSON M., SCHON E.A. (1996). Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the $tRNA^{Leu(UUR)}$ mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes. Mol. Cell Biol. 12: 480-490.

LEONARD J.V., SCHAPIRA A.H. (2000). Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. Lancet 355: 299-304.

LERTRIT P., NOER A.S., JEAN-FRANCOIS M.J., KAPSA R., DENNETT X., THYAGARAJAN D., LETHLEAN K., BYRNE E., MARZUKI S. (1992). A new disease-related mutation for mitochondrial encephalopathy lactic acidosis and strokelike episodes (MELAS) syndrome affects the ND4 subunit of the respiratory complex I. Am. J. Hum. Genet. 51: 457-468.

MANFREDI G., SCHON E.A., MORAES C.T., BONILLA E., BERRY G.T., SLADKY J.T., DIMAURO S. (1995). A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. Neuromuscul. Disord. 5: 391-398.

NAKAMURA M., NAKANO S., GOTO Y., OZAWA M., NAGAHAMA Y., FUKUYAMA H., AKIGUCHI I., KAJI R., KIMURA J. (1995). A novel point mutation in the mitochondrial $tRNA^{Ser(UCN)}$ gene detected in a family with MERRF/MELAS overlap syndrome. Biochem. Biophys. Res. Commun. 214: 86-93.

PETRUZZEELLA V., MORAES C.T., SANO M.C., BONILLA E., DIMAURO S., SCHON E.A. (1994). Extremely high levels of mutant mtDNAs co-localize with cytochrome c oxidase-negative ragged-red fibers in patients harboring a point mutation at nt 3243. Hum. Mol. Genet. 3: 449-454.

SCHON E.A. (2000). Mitochondrial genetics and disease. Trends Biochem. Sci. 25: 555-560.

SCHON E.A., KOGA Y., DAVIDSON M., MORAES C.T., KING M.P. (1992). The mitochondrial $tRNA^{Leu(UUR)}$ mutation in MELAS: a model for pathogenesis. Biochim. Biophys. Acta 1101: 106-109.

SHANG J., CLAYTON D.A. (1994). Human mitochondrial transcription termination exhibits RNA polymerase independence and biased bipolarity in vitro. J. Biol. Chem. 269: 2911-2920.

VERMA A, MORAES C.T., SHEBERT R.T., BRADLEY W.G. (1996). A MERRF/PEO overlap syndrome associated with the mitochondrial DNA 3243 mutation. Neurology 46: 1334-1336.

YASUKAWA T., SUZUKI T., UEDA T., OHTA S., WATANABE K. Modification defect at anticodon wobble nucleotide of mitochondrial $tRNA^{Leu(UUR)}$ with pathogenic mutations of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. J. Biol. Chem. 275: 4251-4257.

ZEVIANI M., MUNTONI F., SAVARESE N., SERRA G., TIRANTI V., CARRARA F., MARIOTTI C., DIDONATO S. (1993). A MERRF/MELAS overlap syndrome associated with a new point mutation in the mitochondrial DNA $tRNA^{(Lys)}$ gene. Eur. J. Hum. Genet.1: 80-87.