COMMENT

A de novo mutation in mitochondrial ATPsynthase subunit α causes a life threatening disease in neonates which heals in infancy

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Mitochondrial (mt) ATP is produced by oxidative phosphorylation (OXPHOS) through the mt-ATP synthase, or complex V (CV; EC 3.6.3.14). CV is embedded in the inner mitochondrial membrane (IMM) and synthetises ATP in the mitochondrial matrix, using the energy provided by the proton-motive force generated by respiration [1]. To date, numerous mutations have been found in mtDNA-related subunits a (encoded by *MT-ATPase6*) and A6L (encoded by *MT-ATPase8*), as well as a handful of mutations in nuclear-encoded gene products related to CV, i.e., a structural subunit, subunit epsilon [2], an assembly factor, ATP12 [3], and an ancillary factor, TMEM70 [4], which seems to play a double role in the assembly of both CI and CV [5]. Most of these mutations give rise to severe mitochondrial disorders. For instance several 60–70% heteroplasmic mutations in *MT-ATPase6* (and more rarely *MT-ATPase8*) are associated with adult-onset NARP (Neuropathy, Ataxia, and Retinitis Pigmentosa, OMIM #551500) whereas infantile MILS (Maternally Inherited Leigh Syndrome, OMIM #256000) occurs when >70% heteroplasmy affects the same genes; neonatal mitochondrial encephalo(cardio)myopathy and dysmorphic features are reported in patients with *ATP12* (OMIM *608918) or *TMEM70* (OMIM #614052) nuclear gene mutations [3, 4]. Neonatal lactic acidosis 3-methylglutaconic aciduria, mild mental retardation, and peripheral neuropathy were reported in a patient with a homozygous mutation of the structural epsilon subunit [2].

ATPsynthase consists of the F_1 particle, projecting in the mitochondrial matrix, and the F_0 particle, bound to the IMM. F_1 is composed of a flapping dome made of three copies, each consisting of a heterodimer formed by one subunit α and one subunit β, in contact to each other. The dome is in contact with an asymmetric central stalk composed of subunits γ, δ and ε. F_0 consists of a subunit c-ring (eight copies in bovine) [6], and one copy each of subunits a, b, d, F_6 and the oligomycin sensitivity-conferring protein (OSCP). Subunits b, d, F_6 and OSCP form the peripheral stalk (the "stator"), which lies to one side of the complex and "fixates" the rotary engine to the cristae membrane. A number of additional subunits (e, f, g, and A6L), all spanning the membrane, are associated with F_0 [7, 8].

The energy sustaining the condensation of ATP from ADP and $_{Pi}$ in F_1 is derived from the dissipation of a proton-based electrochemical gradient (ΔP) formed by respiration. Protons pass from the intermembrane space into the matrix through an obliquely oriented channel largely formed by subunit a in the F_0 [9]. The electrochemcal gradient provides a proton-motive force formed by a chemical differential (ΔpH) and an electrical membrane potential ($\Delta\psi_m$) [10]. The released energy provided by the cross of protons through the subunit a channel causes rotation of the c-ring [11], transmitted to the central stalk [12], to which it is attached. Rotation of subunit γ within the F_1 $\alpha_3\beta_3$ hexamer provides energy for ATP synthesis ("rotary catalysis") [8] through a "binding-change" mechanism [13]. Either ATP synthesis or ATP hydrolysis occur at the three catalytic sites, at the interface between each β subunit with an adjacent α subunit. For ATP synthesis, each site switches cooperatively through conformations in which ADP and $_{Pi}$ bind, ATP is formed, and then released. ATP hydrolysis uses the same pathway, but in reverse [14]. These transitions are caused by rotation of the γ subunit. Every full rotation produces 3 ATPs.

In addition to the very few mutations in nuclear genes associated with impaired CV [15], Lines et al. [16] describe, in the present issue of the EJHG, three unrelated infants with the same de novo, heterozygous mutation, c.206 G > A [p.(Arg207His)], in *ATP5F1A*, encoding the α-subunit of CV. Affected neonates showed very severe lactic acidosis and multiorgan failure. The identification of sporadic cases due to de novo heterozygous mutations is becoming increasingly frequent with the systematic utilization of deep exome screening. An example reported by Lines et al are several heterozygous de novo mutations in *ANT1*, encoding the muscle-brain specific ATP/ADP mitochondrial translocator, recently reported in unrelated neonates with fatal multiorgan failure. The presence of three α subunits in F_1 may perhaps explain the pathogenicity of the heterozygous Arg207His change, but more work is warranted to establish whether this change is permissive for extra-uterine survival, vs. other changes that are embryonic lethal, or additional missense mutations could be identified in the same *ATP5F1A* gene.

According to Lines et al. [16], the residue Arg207 has a positively charged sidechain impinging the α-β interface, close to the β-subunit active site. At pH 7.8, which is similar to the pH in the matrix interface of the IMM, the replaced His207 would create a negatively-charged side chain interfering with the stability of the α-β interface. This change causes reduction of both CV activities and

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amount. Thus, a combination of negative dominance and instability of the mutant structures may concur to the overall failure of CV activity due to the Arg207His change However, the most surprising result of this study is perhaps that all three patients underwent a rapidly and robust improvement, until virtual normalization of the clinical features within infancy. The paper does not report whether this very relevant clinical observation was associated with the recovery of the biochemical proficiency and structural normalization of the initial profound decrease of CV amount through correction or exclusion of the aberrant α-subunit containing structures observed in the initial natural history. Additional investigation may, for instance, show that the mutant gene expression becomes suppressed, or the normal gene is overexpressed, or other mechanisms have produced the spontaneous clinical improvement, starting from potentially fatal neonatal conditions. As mentioned by Lines et al. [16] at least two additional disorders are associated with spontaneous recovery in mitochondrial disease. The first (OMIM *610230) are missense homozygous or compound heterozygous mutations associated with transient liver failure in nucleus-encoded TRMU, involved in mt-tRNA modification, important for mitochondrial translation [17]. The second is a transient, neonatal mitochondrial myopathy (OMIM #500009) characterized by severe COX deficiency in muscle with lactic acidosis, which recovers spontaneously after 1 year of age. This is due to homoplasmic 14674 T>C transition in *MTTE*, encoding the mitochondrial $tRNA^{Glu}$ [18]. The still unknown mechanistic events leading to the intriguing reversibility of these conditions warrants future experimental work based on suitable in vitro, cellular and animal models.

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COMPETING INTERESTS

The author declares no competing interests.

ADDITIONAL INFORMATION

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