

Mitochondria in Down Syndrome: From Organelle Abnormalities to Clinical Phenotype

Nunzia Mollo¹, Antonella Izzo¹, Maria Nitti¹, Simona Paladino¹, Gaetano Cali², Rita Genesio¹, Ferdinando Bonfiglio³, Rita Cicatiello¹, Anna Conti^{1*} and Lucio Nitsch¹

¹Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico, Italy

²Institute of Experimental Endocrinology and Oncology, National Research Council, Italy

³Department of Biosciences and Nutrition, Karolinska Institutet, Sweden

***Corresponding author:** Anna Conti, Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via Pansini 5, Naples 80131, Italy, Tel: 0039 0817463621; Fax: 0039 0817463656; Email: anconti@unina.it

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ABSTRACT

Down syndrome (**DS**), caused by either full or partial trisomy of chromosome 21 (**TS21**), is a multiorganic disorder. Its phenotype is highly complex with constant features, such as mental retardation, dysmorphic traits and hypotonia, and variable features including heart defects, susceptibility to Alzheimer's disease (**AD**), type 2 diabetes, obesity and immune disorders. Accumulating evidences suggest that the mitochondrial dysfunction, which is consistently observed in TS21 cells and tissues, might contribute to the severity of the DS phenotype.

This chapter aims at presenting the most recent evidences supporting possible links between mitochondrial defects and clinical features in DS. We first provide a basic overview of mitochondrial alterations in terms of mitochondrial bioenergetics, biogenesis and morphology in DS. We then discuss how mitochondrial malfunction may contribute to the pathogenesis of clinical manifestations of DS with a special focus on intellectual disability, neurodegeneration, hypotonia, heart defects, type 2 diabetes, obesity and immune disorders. We do believe that understanding the molecular mechanisms responsible for mitochondrial dysfunction in DS could provide the bases to develop novel and effective therapies to improve and/or prevent some aspects of the DS phenotype.

Keywords: Down Syndrome; Mitochondrial Dysfunction; Mitochondrial Dynamics; Down Syndrome/Physiopathology

Abbreviations: Alzheimer's Disease (**AD**); Adenine Nucleotide Translocator (**ANT**); Down Syndrome (**DS**); Human Fibroblasts from Down Syndrome Fetuses (**DS-HFFs**); Chromosome 21(**Hsa21**) ; Mitochondrial DNA (**mtDNA**); Nuclear-Encoded Mitochondrial Genes (**NEMGs**); Human Non Trisomic Fetal Fibroblasts (**N-HFFs**); Oxygen Consumption Rate (**OCR**); Oxidative Phosphorylation (**OXPHOS**); Intracellular Reactive Oxygen Species (**ROS**); Mitotropic probe Tetramethylrhodamine Methyl Ester (**TMRM**); Hsa21 Tisomy (**TS21**).

INTRODUCTION

Down syndrome (**DS**) is a human genetic disorder caused by the trisomy of chromosome 21 (**TS21**), which affects many organs and tissues during embryogenesis and later on in adult life. The mechanism by which an extra copy of chromosome 21 (**Hsa21**) produces the DS phenotype is complex and still largely unknown. It has been proposed that the phenotypic alterations of DS could result from an interplay between Hsa21 over expressed genes and genes dysregulated elsewhere in the genome. Furthermore epigenetic, environmental and stochastic influences may overlap with the genomic events thus justifying the high variability in the manifestation of clinical features among DS individuals [1,2].

Mitochondrial abnormal activity and structure have been well documented in DS both in human subjects, animal models and in cultured neurons, astrocytes, pancreatic b cells, endothelial cells, smooth muscle cells, lymphocytes and fibroblasts. Significant decrease in respiratory capacity, mitochondrial membrane potential, ATP production, oxido-reductase activity as well as an impairment in mitochondrial dynamics were demonstrated [3-10]. Mitochondrial alterations in DS cells have been attributed to changes in the abundance and the activity of the transcriptional co activator *PGC-1 α* (peroxisome proliferator-activated receptor gamma co activator 1-alpha), a key modulator of mitochondrial biogenesis and respiratory function [7,11-13]. This protein, which was down regulated at both the mRNA and protein level in DS fetal fibroblasts [7], is known to act by interacting with transcriptional partners such as *ERR α* , *NRF1* and *NRF2* that regulate the expression of nuclear-encoded mitochondrial genes (**NEMGs**) [14]. Its transcriptional partners and most of NEMGs have been found down regulated in DS fetal heart tissue [15].

Hsa21 Genes and Mitochondrial Dysfunction

A key issue of DS pathogenesis is to understand how up regulation of Hsa21 genes might influence pathogenetic mechanisms. A known repressor of *PGC-1 α* activity is the nuclear receptor interacting protein 1 (*NRIP1/RIP140*), a gene which maps to Hsa21. A pivotal finding was that it is consistently over expressed in DS cells and tissues [15-17]. *NRIP1* attenuation in DS fetal fibroblasts increases transcription levels of *PGC-1 α* and *NEMGs* and restores mitochondrial function [17]. In addition to *NRIP1*, two other Hsa21 genes, namely *DYRK1A* and *DSCR1/RCAN1*,

involved in the calcineurin/*NFAT* pathway, might affect mitochondrial activity [18]. Even modest over-expression of *DYRK1A* decreases *NFATc* protein activity and levels. Knock-out of *Nfatc3* and *Nfatc4* was demonstrated to decrease enzymatic activity of complex II and IV of the respiratory chain and mitochondrial oxidative activity in cardiomyocytes [19].

RCAN1, also known as calcipressin, promotes mitochondrial dysfunction through altered Ca^{2+} buffering. It is also responsible for changes in mitochondrial fission/fusion dynamics via phosphorylation of *DRP-1* and altered expression of mitochondrial factors through inhibition of nuclear translocation of transcription factors *NFKB/NFAT* [20,21]. *RCAN1* is a stress-inducible factor that is modulated by oxidative stress [22]. Transient over expression of *RCAN1* appears to be protective but chronic up regulation is deleterious to cells likely through the expression of *SOD1*, another Hsa21 gene [23]. *RCAN1* also alters cellular susceptibility to oxidative stress as neurons with no *RCAN1* expression display an increased resistance to damage by ROS [24].

Altered mitochondrial number and size, increased mitochondrial ROS production, and altered mitochondrial membrane potential were demonstrated in response to ROS in cells over expressing *RCAN1* [25].

The over expression of another gene on Hsa21, *SOD1*, may be considered as a further cause of increased oxidative stress [26,27]. The gene dosage effect leads to an imbalance in the ratio of *SOD1* to catalase and glutathione peroxidase activity in DS [28], resulting in the accumulation of H_2O_2 [29]. Transgenic mouse strains over expressing wild-type human *SOD1* showed learning and memory deficits and mitochondrial swelling and vacuolization [30]. Mitochondrial ATP synthase alpha/beta chain and elongation factor Tu were aberrant [31]. Mitochondrial abnormalities and a decrease of COX activity might also be induced by overproduction of beta *APP* [32], a gene mapping on Hsa21, although the TS1Cje mouse model, in which APP is not triplicated, also shows decreased mitochondrial function and ATP production [3]. Last, it was recently reported that the Hsa21 miR-155-5p regulates mitochondrial biogenesis by targeting mitochondrial transcription factor A (*TFAM*) [33].

Mitochondrial Dynamics is Altered in DS

It has been suggested that the equilibrium between mitochondrial fusion and fission determines the ultra structural and cellular morphology of the organelle. Changes in mitochondrial shape appear to regulate crucial mitochondrial and cellular functions [34].

A substantial alteration in mitochondrial morphology was observed in primary cultures of TS21 astrocytes and neurons with increased fragmentation of the mitochondrial network [35]. Alterations were demonstrated also in trisomic fetal fibroblast (**DS-HFFs**) which present an increased number of shorter mitochondria and a smaller average mitochondrial volume if compared with euploid cells (N-HFFs) (Figure 1) [8].

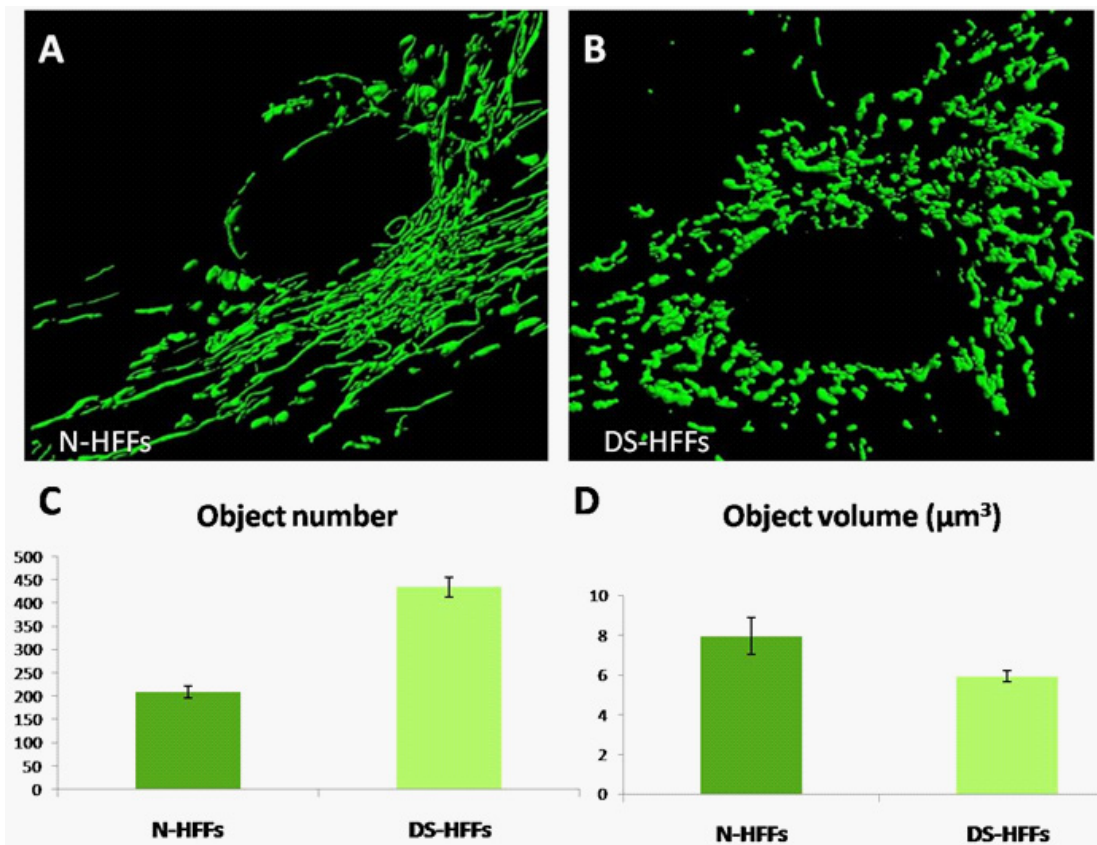


Figure 1: DS-HFFs mitochondrial network appears fragmented. Representative images showing that the mitochondrial network is less fragmented in N-HFFs (A) than in DS-HFFs (B). The number of mitochondria is significantly higher in trisomic cells compared with non-trisomic cells (C). The mitochondrial volume is significantly lower in DS cells compared with non-trisomic cells (D). The bars show mean values \pm SEM of two non-trisomic and five trisomic cell cultures.

In agreement with these results two genes involved in the fusion machinery *MFN2* and *OPA1* are down-regulated in trisomic cells [8]. Electron microscopy of the same DS fetal fibroblasts revealed a significant number of damaged mitochondria which were broken and shorter, with concentric and/or highly swollen cristae (Figure 2) [7].

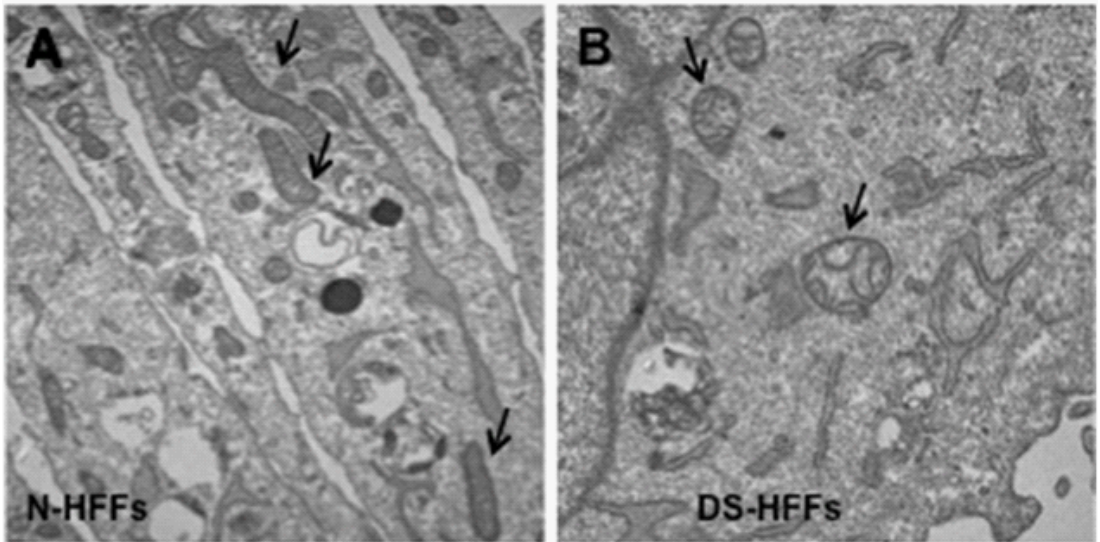


Figure 2: Mitochondrial ultra structure is altered in DS-HFFs. Representative ultra structural images of N-HFFs showing mitochondria with an intact morphology (arrowheads), without any particular changes in the structure (A), and of DS-HFFs showing damaged mitochondria characterized by a range of mitochondrial alterations (arrowheads). Scale bar 1 μm.

These mitochondrial cristae abnormalities of trisomic cells are in agreement with the decrease in the expression of *OPA1*, which is involved in the maintenance and remodeling of cristae morphology in addition to its role in mitochondrial fusion [36,37].

The down regulation of OXPHOS genes, together with the altered mitochondrial dynamics and morphology, accounts for a global deficiency of the mitochondrial energy production apparatus which contributes to ROS overproduction in DS mitochondria. This situation is worsened by the contemporary dysregulation of ATP translocators (*ANT1*, *ANT2* and *ANT3*), ATP synthase and adenylate kinase [7,11].

The lack of balance in the metabolism of reactive oxygen species, as a consequence of mitochondrial dysfunction, might play a role in the development of DS pathology [27]. The presence of oxidative stress in DS has been documented early in embryonic life as well as in human trisomic fetal fibroblasts [7,38].

Even though impaired mitochondrial function and dynamics, and the relative unbalance of reactive oxygen species, may not be considered the main cause for many phenotypic anomalies, they might play a role in the pathogenesis of clinical features of DS as described below.

MITOCHONDRIAL ROLE IN DS ASSOCIATED PATHOLOGIES

Intellectual Disability, Neurodegeneration and Mitochondria

Development of the DS brain is associated with decreased neuronal number and abnormal neuronal differentiation. Accordingly, a constant feature of patients with DS is the intellectual disability and an increased risk for Alzheimer's disease (**AD**). Clinical signs of AD are manifest in 75% of DS individuals starting from 40 years of age [39,40]. It is known that the calcineurin-*NFAT* signaling pathway, in which the Hsa21 genes *DYRK1A* and *RCAN1* are deeply involved, plays fundamental role in both neuronal development and degeneration. *DYRK1A* affects synaptic plasticity and memory consolidation. Its over expression causes neurofibrillary degeneration and cell loss in the hippocampus area [41,42]. Moreover, increasing evidences are demonstrating that mitochondrial function is a key actor in the neuronal drama. Substantial alteration in mitochondrial morphology was observed in primary cultures of TS21 astrocytes and neurons with increased fragmentation of the mitochondrial network [35]. In addition to mitochondrial alterations, patients with DS show higher levels of oxidative stress at all ages if compared with the general population [43]. Apoptosis and generation of reactive oxygen species are increased in human fetal DS neurons compared with non trisomic controls [44]. Mitochondrial function, fission-fusion mechanisms, biogenesis and degradation are critical for neuronal function. In particular mitochondrial dynamics are essential for synaptogenesis, Ca^{2+} buffering, axonal transport, and bioenergetics [45]. Functionally and structurally damaged mitochondria do not produce sufficient ATP and are more prone in producing proapoptotic factors and ROS [46], which could represent an early stage in neurodegenerative process [47]. Energy depletion and oxidative stress can also induce amyloidogenic changes in A β PP processing [48-51], suggesting a potential link between mitochondrial dysfunction, oxidative stress, and A β production. Busciglio et al. [52] demonstrated that there is a marked alteration in A β PP processing and A β trafficking in cortical DS astrocytes and neurons. Similar alterations can be induced in normal human astrocytes by inhibition of mitochondrial energy metabolism. Moreover, mitochondrial function is impaired in DS astrocytes, as indicated by reduced mitochondrial red ox activity and membrane potential. The authors suggested that impaired energy metabolism in DS cells gives rise to increased β -secretase cleavage of A β PP and altered A β trafficking resulting in intracellular accumulation of aggregated A β 42. Similar patterns of A β PP processing were detected in the DS brain. These results raise the possibility that impaired mitochondrial energy metabolism in the DS brain may contribute to the pathogenesis of AD [52].

Neurodegenerative diseases such as AD, Parkinson's (**PD**) and Huntington's diseases, show alterations of mitochondrial function and fusion and/or fission processes very similar to those observed in DS (47, 53-56). By comparing the list of NEMGs down regulated in DS fetal hearts and the list of genes belonging to Alzheimer's disease KEGG pathway hsa05010, we found a significant overlapping of 20 genes (Figure 3). Most of them are target of the *NRIP1/PGC-1 α* axis [17].

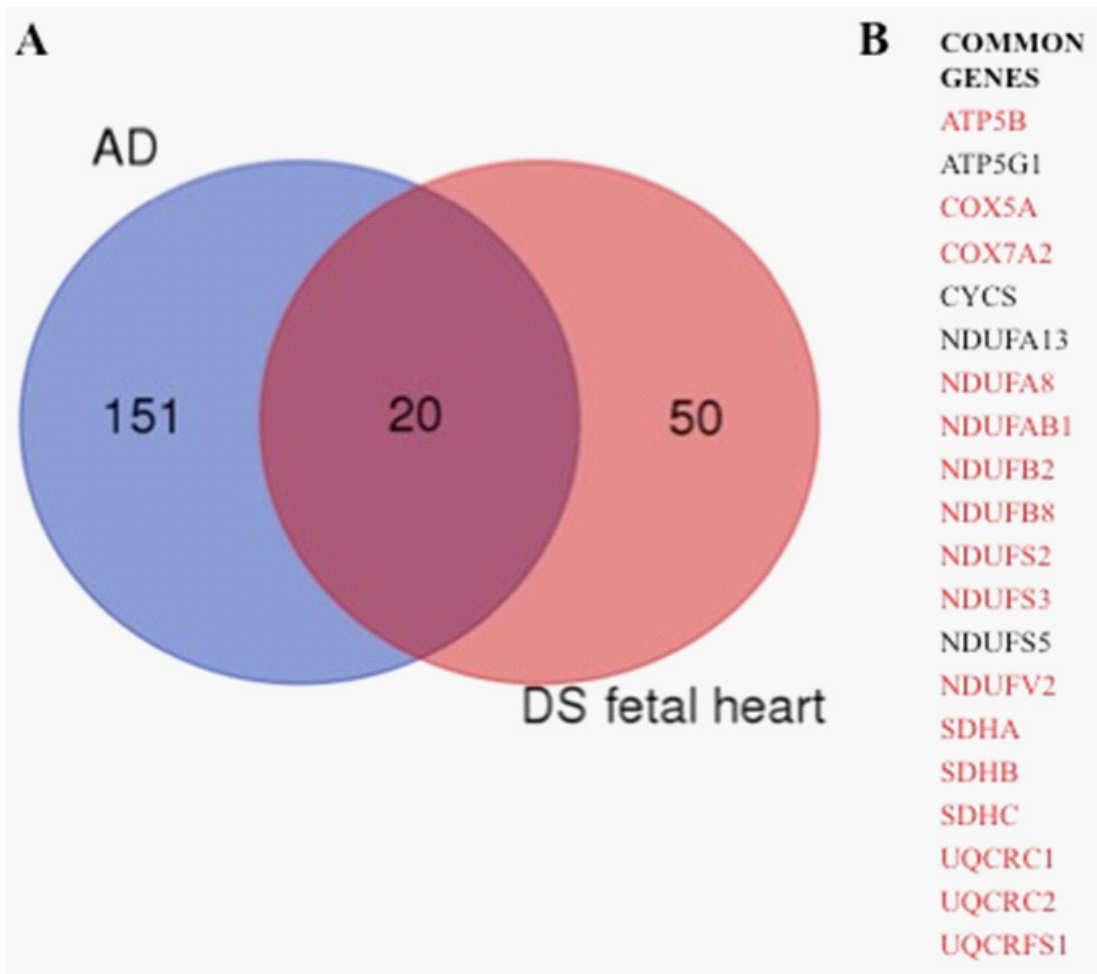


Figure 3: Venn diagram showing overlapping genes between NEMGs down regulated in DS fetal hearts and genes from the Alzheimer's disease pathway hsa05010. (A). Twenty mitochondrial genes out of the 70 down regulated in DS fetal hearts (15) overlap to the list of 170 genes belonging to the Alzheimer's disease (**AD**) KEGG pathway hsa05010. Over-representation analysis (**ORA**) detects highly significant enrichment ($p < .000001$) of overlapping genes (20 observed versus 1.25 expected genes). (B) List of the 20 overlapping genes. In red are shown genes regulated by the *NRIP1/PGC-1 α* axis (17).

Hypotonia and Mitochondria

DS patients suffer from muscle hypotonia and altered motor coordination whose basic mechanisms are still largely unknown. Many studies provide evidence of the involvement of mitochondria in the hypotonia.

Skeletal muscle is particularly vulnerable to oxidative stress. This is due, in part, to the rapid and coordinated changes in energy supply and oxygen flux that occurs during contraction, resulting in increased electron flux and leakage from the mitochondrial electron transport chain. It has been recently shown that over-expression of *PGC1 α* inhibits muscle atrophy during fasting and denervation [57]. Furthermore the over expression of the Hsa21 miR-155 leads to the repression of endogenous myogenic enhancer factor 2 (*MEF2A*) expression and the inhibition of myoblast differentiation [58]. The disruption of the mitochondrial network, together with mitochondrial dysfunction, is an essential amplificatory loop of the muscular atrophy programme. Romanello et al. [59] demonstrated that the induction of mitochondrial dysfunction and fission activates a muscular atrophy programme in adult animals. Conversely, inhibition of the mitochondrial fission inhibits muscle loss during fasting and after FoxO3 over expression [60]. Furthermore, changes in mitochondrial morphology have been implicated in apoptosis as well as in the regulation of muscle metabolism [61].

Ts65Dn mice, a widely used model of DS, exhibit DS-like motor dysfunctions [62]. The ultra-structural analysis of myofibrils of the trisomic mice showed evident structural changes in the mitochondria [63,64]. Furthermore microarray analysis revealed that numerous pathways are altered in Ts65Dn muscle, including ATP biosynthesis, proteolysis, glucose and fat metabolism, and neuromuscular transmission [63].

It is worth noting that patients with DS have features of premature aging [65,66], and exhibit a decrement in muscle strength compared to euploid subjects as it occurs in aged versus young persons [67]. It is, therefore, possible that muscle hypotonia and motor dysfunction in DS share some basic mechanisms with the progressive age-related decrease in skeletal muscle mass, strength and quality known as sarcopenia [68].

Heart Defects and Mitochondria

DS is a major cause of congenital heart defects (**CHD**), mainly endocardial cushion defects [69,70], the most frequent being atrioventricular septal defects followed by ventricular septal defects and tetralogy of Fallot [70].

Transcriptome analysis of human fetal heart tissues from DS subjects show a global down regulation of NEMGs, especially enzymes involved in the oxidative phosphorylation from all five respiratory chain complexes [71].

The Hsa21 genes *DYRK1A* and *RCAN1*, which play a role in the calcineurin/*NFAT* pathway [18], are believed to affect mitochondrial activity and morphology during heart development [19,72]. *NFATc*-null mice show phenotypic anomalies that resemble those observed in human DS and 65% of *NFATc1-4*-null mice have endocardial cushion defects [18]. Even modest over expression of *DYRK1A* decreases *NFATc* protein activity and levels and may induce vascular and cardiac defects [18]. The inhibition of the mitochondrial activity in *Nfatc3*^{-/-}; *Nfatc4*^{-/-} cardiomyocytes [19] suggests that the calcineurin/*NFAT* pathway affects mitochondrial activity

during heart development. In human DS fetal fibroblasts and hearts, *NFATc3* and *NFATc4* were found significantly down regulated while *DYRK1A* and *RCAN1*, involved in regulating the levels of *NFATc* phosphorylation, were over expressed due to dosage effect [7,15].

When mitochondrial phenotype was analyzed, fibroblasts from DS fetuses with congenital heart defects showed a chronic pro-oxidative state more pronounced with respect to fetuses without cardiopathy [7]. Significant differences in mitochondrial respiration, complex I activity and reactive oxygen species production were observed, suggesting a relationship between mitochondrial function and cardiac phenotype [7].

NRIP1-dependent repression of genes involved in mitochondrial function may be linked also to the ventricular hypertrophy, which occurs in DS after birth, possibly as a result of reduced mitochondrial electron-transport chain activity and oxygen consumption. *NRIP1* over expression in a transgenic mouse results in cardiac hypertrophy [73]. Alterations in mitochondrial function observed in right ventricular cardiac hypertrophy are mainly attributed to complex I dysfunction [74].

Also the Hsa21 miR-155, a known repressor of *TFAM* gene [33], has been hypothesized to be an inducer of cardiac hypertrophy. The authors suggest that its inhibition might have clinical potential to counteract this pathology [75].

Complications of CHD in patients with DS are the development of pulmonary hypertension **(PH)** [76] and other common associated lesions namely patent ductus arteriosus and pulmonary stenosis [70]. Both functional and structural alterations of mitochondria occur in PH. Rafikov et al. [77] reported that smooth muscle cells isolated from the pulmonary vessels of rats with PH show deficiencies in the activities of complexes I-III, an increase in mitochondrial ROS generation and altered mitochondrial membrane potential. In muscle cells from patients with PH, a disruption of the normal mitochondrial filamentous reticulum have been observed [78].

Type 2 Diabetes and Obesity and Mitochondria

Children with DS demonstrate an increased risk of developing various endocrine disorders such as type 2 diabetes and childhood obesity [79]. Emerging evidence supports the potentially unifying hypothesis that prominent features of type 2 diabetes and the related condition of obesity are caused by mitochondrial dysfunction and by an impaired bioenergetics capacity [80]. Given the important role that subsarcolemmal mitochondria have for bioenergetics support of signal transduction, fat oxidation, and substrate transport, an impairment of electron transport chain activity in this sub cellular location may have particular relevance to the pathogenesis of insulin resistance in type 2 diabetes [81]. A disproportionately large reduction of electron transport chain activity was observed in the subsarcolemmal mitochondrial fraction in type 2 diabetic and obese subjects compared with unaffected volunteers. Mitochondria from human skeletal muscle were found to be smaller and to have reduced activity of complex I in both type 2 diabetes and obesity [82]. Interestingly *NRIP1* and *PGC-1 α* both play key roles in the transcriptional regulation of genes

involved in energy homeostasis. The expression and promoter activity of *CIDEA*, an important regulatory factor in adipose cell function and obesity, is repressed by *NRIP1* and induced by *PGC-1 α* , through *ERR α* and *NRF-1* activity. *NRIP1* and *PGC-1 α* are also involved in glucose uptake and therefore in the physiopathology of diabetes through the regulation of the insulin sensitive glucose transporter *GLUT4* expression and its sub-cellular localization [83]. The depletion of *NRIP1* improves the metabolic parameters in both oxidative muscles and glycolytic processes thus suggesting that *NRIP1* might be a potential therapeutic target in the treatment of insulin resistance in obese and type 2 diabetic patients [83]. Furthermore, mice lacking *Nrip1* are lean, show resistance to high-fat diet-induced obesity and have increased oxygen consumption [84].

Fusion machinery expression is crucial in metabolism through the maintenance of the mitochondrial network architecture. Indeed, it is known that obesity in both humans and mice is associated with reduced *Mfn* expression [85]. In particular *Mfn2* reduced expression may explain some of the metabolic alterations associated with obesity [85]. Furthermore an altered proteolytic processing of the GTPase *OPA1*, in humans is associated with insulin resistance [86]. Additionally loss of mitochondrial protease *OMA1*, which alters processing of *OPA1*, causes obesity and defective thermo-genesis in mice [87].

Immune Disorders and Mitochondria

Children with DS have increased susceptibility to infections, usually of the upper respiratory tract [88-90], and autoimmune disorders, including hypothyroidism [91] and celiac disease [92,93]. The abnormalities of the immune system associated with DS include: alteration of B and T-cell number, with marked decrease of naive lymphocytes, abnormal thymus functions and development, impaired mitogen-induced T cell proliferation, reduced specific antibody responses to immunizations and defects of neutrophil chemo taxis [94,95]. The rates of lymphocyte respiration in the children with DS were found slower than in the control group [96]. These differences could reflect a relatively lower rate of mitochondrial energy conversion in DS children that may be linked to some pathological findings pertinent to this disorder, such as defects in the inner mitochondrial membrane potential [9]. Campello et al. [97] demonstrated that mitochondria transportation during lymphocyte migration requires mitochondrial fission. Recently Buck et al. [98] demonstrated that mitochondrial remodeling is a signaling mechanism that instructs T cell metabolic programming. The authors showed that T_{Effector} (**T_E**) cells have punctuate mitochondria, while T_{Memory} (**T_M**) cells maintain fused networks. Additionally the loss of *OPA1* decreased survival of T_M lymphocytes, which was associated with altered cristae structure and decreased spare respiratory capacity. TE cells could be shifted to a T_M fate according to changes of mitochondrial dynamics. These data suggest that, by altering cristae morphology, fusion in T_M cells configures electron transport chain (**ETC**) complex associations favoring OXPHOS and fatty acid oxidation, while fission in T_E cells leads to cristae expansion, reducing ETC efficiency and promoting aerobic glycolysis [98].

CONCLUDING REMARKS

An increasing number of studies provides evidence that mitochondrial abnormalities play a role in the major clinical manifestations of DS such as neural defects and hypotonia. Furthermore, mitochondrial dysfunction may contribute to increase susceptibility of individuals with DS to clinical conditions in which altered energy metabolism may play a role, such as heart defects, hypotonia, Alzheimer's disease, type 2 diabetes, obesity and immune disorders. Counteracting the mitochondrial defects might improve and/or prevent some aspects of the DS phenotype providing a better quality of life for DS individuals and their families.

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