



## Review article

Mitochondria as pharmacological targets in Down syndrome<sup>☆</sup>

Daniela Valenti<sup>a</sup>, Nady Braidy<sup>b</sup>, Domenico De Rasmò<sup>a</sup>, Anna Signorile<sup>c</sup>, Leonardo Rossi<sup>d</sup>,  
A.G. Atanasov<sup>e,i,j</sup>, Mariateresa Volpicella<sup>f</sup>, Alexandra Henrion-Caude<sup>g</sup>, S.M. Nabavi<sup>h</sup>,  
R.A. Vacca<sup>a,\*</sup>



<sup>a</sup> Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Council of Research, Bari, Italy

<sup>b</sup> Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Australia

<sup>c</sup> Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari, Italy

<sup>d</sup> Department of Clinical and Experimental Medicine, University of Pisa, Italy

<sup>e</sup> Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, 05-552 Jastrzebiec, Poland

<sup>f</sup> Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy

<sup>g</sup> INSERM U1163, Université Paris Descartes, Sorbonne Paris Cité, Institut Imagine, GenAtlas Platform, 24 Boulevard du Montparnasse, 75015 Paris, France

<sup>h</sup> Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>i</sup> Department of Pharmacognosy, University of Vienna, 1090 Vienna, Austria

<sup>j</sup> Department of Vascular Biology and Thrombosis Research, Center for Physiology and Pharmacology, Medical University of Vienna, 1090 Vienna, Austria

## ARTICLE INFO

## Keywords:

Mitochondrial dysfunction

Down syndrome

Neurodevelopmental diseases

Neurodegeneration diseases

Polyphenols

Mitochondrial signalling pathways

Oxidative stress

## ABSTRACT

Mitochondria play a pivotal role in cellular energy-generating processes and are considered master regulators of cell life and death fate. Mitochondrial function integrates signalling networks in several metabolic pathways controlling neurogenesis and neuroplasticity. Indeed, dysfunctional mitochondria and mitochondrial-dependent activation of intracellular stress cascades are critical initiating events in many human neurodegenerative or neurodevelopmental diseases including Down syndrome (DS). It is well established that trisomy of human chromosome 21 can cause DS. DS is associated with neurodevelopmental delay, intellectual disability and early neurodegeneration. Recently, molecular mechanisms responsible for mitochondrial damage and energy deficits have been identified and characterized in several DS-derived human cells and animal models of DS. Therefore, therapeutic strategies targeting mitochondria could have great potential for new treatment regimens in DS. The purpose of this review is to highlight recent studies concerning mitochondrial impairment in DS, focusing on alterations of the molecular pathways controlling mitochondrial function. We will also discuss the effects and molecular mechanisms of naturally occurring and chemically synthesized drugs that exert neuroprotective effects through modulation of mitochondrial function and attenuation of oxidative stress. These compounds might represent novel therapeutic tools for the modulation of energy deficits in DS.

## 1. Introduction

Down syndrome (DS) is the most common chromosomal defect leading to intellectual disability. The majority of DS individuals (95%) have a free trisomy of human chromosome 21 (Hsa21) i.e. a full extra

copy of Hsa21 in all cell types. Triplication of part of Hsa21 or mosaic DS, i.e. the presence of a mixture of cell types some with a normal set of chromosomes or trisomy 21, are rare forms (3–4%) [1].

From a clinical point of view, DS is a neurodevelopmental disorder characterized by specific patterns of learning and short-term memory

**Abbreviations:** AD, Alzheimer's disease; AGO, Argonaute proteins; AMPK, AMP-activated protein kinase; ANTs, adenine nucleotide translocators; AK, adenylate kinase; APP, amyloid- $\beta$  precursor protein; ASD, autism spectrum disorder; CAT, catalase; CNS, central nervous system; CoQ10, coenzyme Q10; Drp1, dynamin-related protein 1; DS, Down syndrome; DSCR, Down syndrome critical region; DYRK1A, dual-specificity tyrosine (Y)-phosphorylation regulated kinase 1A; EGCG, epigallocatechin-3 gallate; ERRs, estrogen-related receptors; FOXO1, forkhead box O1; FOXO3, forkhead box O3; GPX, glutathione peroxidase; GSH, reduced glutathione; Hsa21, human chromosome 21; L-OPA, long OPA1; mtDNA, mitochondrial DNA; miRNAs, microRNAs; Mfn-1, mitofusin 1; Mfn-2, mitofusin 2; mPTP, mitochondrial permeability transition pore; MQC, mitochondrial quality control; MRC, mitochondrial respiratory chain; NRF-1, nuclear respiratory factor 1; NRF-2, nuclear respiratory factor 2; NPCs, neural progenitor cells; OPA1, optic atrophy 1; OXPHOS, mitochondrial oxidative phosphorylation; PGC-1 $\alpha$ , peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 alpha; RCAN1, calcineurin 1; PKA, protein kinase A; PPARs, Peroxisome Proliferator Activated Receptors; SOD1, superoxide dismutase 1; S-OPA, short OPA1; Sirt1, sirtuin 1; ROS, reactive oxygen species; RSV, resveratrol; TFAM, mitochondrial transcription factor A; TFBM, mitochondrial transcription factor B

<sup>☆</sup> This article is part of a special issue entitled: Down Syndrome: From Development to Adult Life to Alzheimer Disease, edited by Allan D. Butterfield and Marzia Perluigi.

\* Correspondence to: Institute of Biomembranes, Bioenergetics and Molecular Biotechnology, IBIOM - CNR, Via Amendola 165/A, 70126 Bari, Italy.

E-mail address: [r.vacca@ibiom.cnr.it](mailto:r.vacca@ibiom.cnr.it) (R.A. Vacca).

<http://dx.doi.org/10.1016/j.freeradbiomed.2017.08.014>

Received 28 June 2017; Received in revised form 16 August 2017; Accepted 18 August 2017

Available online 31 August 2017

0891-5849/© 2017 Elsevier Inc. All rights reserved.

difficulties that lead to delay in many areas of development, including acquisition of language, motor skills and reading. These cognitive impairments are associated with mild to severe mental retardation, with IQ of patients between 25 and 50 [2]. Other clinical features of DS are characterized by atypical craniofacial profiles, congenital malformations, which include heart and gastro-enteric defects, immune disorders, increased susceptibility to infection, lymphoblastic and myeloid leukaemia, diabetes mellitus and obesity [3,4]. Most individuals with DS currently reach the age of sixty years. However, in the middle age, they develop neurochemical features of Alzheimer's disease (AD) and early aging. Accordingly, DS has been enclosed among the progeroid and neurodegenerative diseases [5].

A great number of genetic and molecular studies have been carried out in trisomic cellular and animal models of DS with the aim to identify mechanisms responsible for the clinical phenotype (aneuploidy-phenotype correlation) and intellectual disability. A part of the long arm of chromosome 21 represents the “Down syndrome critical region (DSCR)”. Overexpression of genes in the DSCR, and the consequent down- or up-regulation of their targets, have long been proposed to mediate the majority of DS phenotypic features [6]. These include the dual-specificity tyrosine (Y)-phosphorylation regulated kinase 1 A (DYRK1A), a major modulatory protein for brain development [7]; the regulator of calcineurin 1 (RCAN1), which controls cell growth and immune responses [8]; the cystathionine beta-synthase, a key enzyme in the homocysteine/folate/transulfuration pathways [9], and superoxide dismutase 1 (SOD1), which is involved in the regulation of redox homeostasis [10]. The overexpression of chromosome 21-encoded microRNAs (miRNAs), in particular miR-155, a multifunctional microRNA involved in a plethora of biological and pathological processes [11], may contribute to the neurochemical anomalies, mitochondrial deficits, and overall pathophysiology observed in DS individuals, as well [12,13]. The relevance of genes comprised in the DSCR (including DYRK1A and RCAN1) has been recently questioned [14] and the trisomy of a very restricted DSCR, containing no known genes, has been proposed as a candidate for typical DS features [15].

Several studies have shown that alterations of mitochondrial structure and function associated with an impairment of reactive oxygen species (ROS) homeostasis are critically linked to DS pathogenesis and are inherent features of the etiology of intellectual disability. This has been demonstrated in DS and in other neurodevelopmental diseases, including Rett's syndrome and autism (for refs. see [16,17]), and in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases (for refs. see [18]). Indeed, neural developmental processes including cellular proliferation and differentiation, axonal and dendritic growth, and generation of synaptic spine and pre-synaptic compartments are strongly supported by mitochondrial ATP production and redox homeostasis in brain mitochondria [19]. Deficits in energy metabolism due to mitochondrial dysfunctions negatively affect neuronal function, survival and central nervous system (CNS) development and occur as an early event in intellectual disability-linked diseases and several forms of dementia [16,20–22]. In this line, targeting mitochondria represents a potential therapeutic strategy to correct DS clinical phenotypes associated with mitochondrial energy deficits.

Herein, we will first review signalling pathways controlling mitochondrial functions in physiological/pathological conditions, as a prerequisite to discuss our recent studies and those available in the literature on the molecular basis of mitochondrial dysfunctions and the origin of oxidative stress in DS. Finally, we propose the use of chemically synthesized drugs, as well as bioactive natural products targeting mitochondria and mitochondrial signalling pathways as therapeutic tools to advance treatment of some clinical signs of DS, which, as yet, remains untreated.

## 2. Integral pathways controlling mitochondrial functions

The mitochondria are highly complex, metabolically active and

motile organelles that play an important role in the maintenance of cellular energy production and redox homeostasis. Optimal mitochondrial efficiency is fundamental to cell life, and therefore, alterations in mitochondrial function might lead to irreversible cellular damage and loss of function, culminating in the development of several diseases including neurodegenerative and neurodevelopmental diseases, as well as the aging process [16]. Mitochondria represent the main site of oxidative phosphorylation (OXPHOS), and are key regulatory organelles in many cell processes [23,24]. Investigating the molecular mechanisms for mitochondrial dysfunction is of major significance to determine the biology of aging and neurodegenerative disorders including DS. In this section, we will provide recent insight in the pathways controlling mitochondrial function, whose impairments are particularly harmful for the CNS.

### 2.1. Regulation of mitochondrial bioenergetics

In most catabolic tissues, mitochondria represent the main source of energy production required for cell growth and other important biochemical processes, consuming about 90% of mammalian oxygen to generate ATP via OXPHOS [25]. Mitochondria are also involved in catabolism of major macromolecules into smaller important metabolites such as pyruvate, fatty acids and amino acids, which are subsequently converted by  $\beta$ -oxidation and Krebs cycle, into NADH and/or FADH<sub>2</sub>. The oxidation reaction involves the donation of electrons to the mitochondrial electron transport chain in the mitochondrial inner membrane [26].

The exchange of electrons between complex I (and/or II) to complex IV in the mitochondrial respiratory chain stimulates the translocation of protons from the matrix to the inter-membrane space. This produces an electrochemical proton gradient required for F<sub>1</sub>-F<sub>o</sub> ATP synthase (i.e. complex V) activity and ATP synthesis. Specific adenine nucleotide translocators (ANTs) facilitate the exchange of mitochondrial ATP into the cytosol. Specific mitochondrial “shuttle” systems, which facilitate the transport of NADH, have also been identified [27].

Given the importance of mitochondria in cellular energy production, mitochondrial bioenergetics is regulated by several cellular effectors including specific kinases and transcription factors. Changes to the cellular AMP/ATP ratio are known to activate AMP-activated protein kinase (AMPK). Once activated, AMPK enhances phosphorylation reactions that culminate in the activation of catabolic pathways associated with ATP synthesis, and inhibition of anabolic pathways that utilize ATP (for review see [28]). Recently, it has been shown that AMPK influences energy metabolism via activation of the nuclear sirtuin 1 (Sirt1) [29,30] and subsequent alteration to downstream Sirt1 targets, such as the peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 alpha (PGC-1 $\alpha$ ), and the transcription factors, forkhead box O1 (FOXO1) and O3 (FOXO3).

### 2.2. Control of mitochondrial biogenesis

Mitochondrial function is strictly dependent on the efficiency of mitochondrial biogenesis, which is an important biological process associated with the maintenance and survival of pre-existing mitochondria. It includes the synthesis of nuclear DNA- and mitochondrial DNA (mtDNA)-encoded proteins, together with membrane biosynthesis, mtDNA replication and the import into mitochondria of nuclear-encoded proteins (for review see [31]).

Mitochondrial biogenesis is spatiotemporally coordinated in response to cell demand, such as cell division, differentiation and aging, or to environmental stresses including cold exposure, caloric restriction, exercise and oxidative stress. Coordinate expression of the two genomes is achieved thanks to a regulatory circuit that integrates signalling pathways and cellular energy demand to the activation of nuclear-encoded transcriptional complexes. Several transcriptional factors that regulate the nuclear genome are implicated in modulating the genomic

expression of mitochondrial proteins. These include, but are not limited to, the nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2), which were the first transcription factors to be identified [32]. NRF-1/2 regulate the expression of several proteins of the five respiratory complexes, mitochondrial import and heme biosynthesis [31,33]. Moreover, both NRFs activate the expression of the mitochondrial transcription factors TFAM, TFB1M and TFB2M [34]. As TFAM and TFBs are required for basal transcription of mammalian mtDNA [35], their regulation through NRF transcriptional activity is responsible for nucleo-mitochondrial interactions.

Additional transcriptional factors, belonging to subclasses of the nuclear hormone receptor superfamily, are involved in the control of mitochondrial biogenesis. These include the Peroxisome Proliferator-Activated Receptors (PPARs), activated by several fatty-acid ligands. PPARs are master regulators of whole body and liver metabolism and stimulate the expression of mitochondrial  $\beta$ -oxidation genes (reviewed in [36]). Estrogen-related receptors (ERRs) ERR- $\alpha$ , ERR- $\beta$  and ERR- $\gamma$  are also involved in mitochondrial biogenesis. Functional genomics analysis has revealed that the ERRs bind to hundreds of genes involved in mitochondrial functions [37], such as oxidative phosphorylation, fatty acid oxidation, Krebs cycle and mitochondrial fission/fusion. Other nuclear transcription factors including CREB [38], myocyte enhancer factor-2 [39], the GLI-Kruppel class of zinc finger protein YY1 [40,41] and c-Myc [42] have been demonstrated to bind mitochondrial gene promoters and control mitochondrial biogenesis in response to different signalling pathways.

The common denominator to most nuclear transcription factors regulating mitochondrial gene expression is that their transcriptional activity is potentiated by PGC-1 $\alpha$ , PGC-1 $\beta$  and PRC. It has become clear that PGC-1 $\alpha$  and  $\beta$  control any aspect of mitochondria function and biogenesis [43] through their ability to bind and potentiate the transcriptional activity of NRFs, ERRs, PPARs and YY1 [31,44]. PGC-1 coactivators act as inducible nuclear receptor “boosters” to maintain energy demands under different environmental conditions [44]. For instance, the transcriptional activity of PGC-1 family is controlled by environmental signals regulating pathways of gluconeogenesis, thermogenesis, muscle differentiation and cell growth [43–45]. Numerous studies suggest that PGC-1 $\alpha$  and  $\beta$  mediate the response to increased metabolic demands during starvation, exercise or cold by promoting the activity of the same pool of transcription factors [46,47]. Among the PGC-1 family of coactivators, the role of PGC-1 $\alpha$ , in connecting cellular bioenergetics to mitochondrial biogenesis, is well established. Indeed, several signalling pathways are thought to converge upon PGC-1 $\alpha$  [48]. For example, calcium-dependent phosphatase calcineurin controls PGC-1 $\alpha$  expression at the transcriptional level in muscle, providing a link between increased contractile activity and the increase in mitochondria content [49]. On the other hand, the AMPK/Sirt1/PGC-1 $\alpha$  pathway is thought to play a crucial role in metabolic programs that are activated during caloric restriction and exercise [30,50].

### 2.3. Mitochondrial dynamics for quality control of mitochondria

*De-novo* mitochondrial biogenesis and clearance are strictly coordinated events for proper mitochondrial homeostasis. These organelles are indeed essential, but potentially also highly toxic; thus, their quality must be well controlled to avoid activation of cell death processes. Mitochondria quality control (MQC) is achieved by activation of several interdependent surveillance mechanisms [51]. Mitochondrial proteases are involved in proteolytic activities comprising removal of damaged, misfolded and surplus proteins, as well as regulatory processing, maturation cleavage and assembly of polypeptides (for a comprehensive review please see [52]).

Additionally, the maintenance of mitochondrial dynamics by fission/fusion mechanisms allows segregation of damaged organelle components via the fission process and exchange of mitochondrial content via the fusion process. This led to the introduction of the

paradigm of asymmetrical mitochondrial fission as a central event in MQC [53]. Asymmetrical fission might be indeed considered the initial step of a highly regulated process that maintains functional mitochondrial network by sequestering and eliminating damaged organelle components. Photo-labelling experiments have demonstrated that mitochondria undergo continuous cycles of fission/fusion and that two distinct sets of mitochondria, with different transmembrane potential, are created in the cell [54]. Following asymmetrical division, the daughter mitochondrion with the higher transmembrane potential proceeds to fusion, while in the one with the lower transmembrane potential, inner and outer membrane fusion proteins might be degraded as a result of depolarization [55]. Thus, if transmembrane potential is not recovered, this latter daughter mitochondrion will be less likely involved in a fusion event and might become a solitary mitochondrion targeted for degradation through autophagy [54]. Partly damaged mitochondria, with moderate drop in transmembrane potential, might still maintain a certain possibility to be reincorporated into the mitochondrial network. In this view, fusion represents a selective mechanism by which partly impaired mitochondria are rescued by diluting damaged organelle components in the network.

Mitochondria contain a unique residual genome (about 16 kilobases), which encodes 13 proteins required for the maintenance of mitochondrial respiratory chain function [56]. This autonomous DNA has lower repair efficiency compared to nuclear DNA, leading to a greater risk of mutations in mtDNA. Therefore, mitochondrial fusion/fission represents an effective quality control process to maintain homogeneous mitochondrial population [57].

Mitochondrial outer membrane fusion is modulated by dynamin-related GTPases, mitofusin 1 (Mfn-1) and mitofusin 2 (Mfn-2), while optic atrophy 1 (OPA1) is responsible for inner membrane fusion [53,58,59]. Apart from its role in fusion, OPA1 governs normal cristae morphology, and assembling of electron transport chain complexes, and thus cooperates in maintaining proper mitochondria function [60,61].

Mitochondrial fission relies on the GTPase dynamin-related protein 1 (Drp1), a functionally inert cytoplasmic protein that is translocated to the outer mitochondrial membrane when the fission process is commenced [62]. When located in the outer mitochondrial membrane, Drp1 polymerizes into spirals and constricts, in a GTP-dependent manner, both inner and outer membranes promoting fission. Drp1 binds to outer mitochondrial membrane through interaction with four mitochondrial receptors: Fis1, Mff, MID49 and MID51 [63–65]. Different conditions, such as apoptosis, cell division, mitophagy and mitochondria remodelling, promote specific Drp1-dependent post-translational modifications, such as phosphorylation/dephosphorylation, S-nitrosylation, ubiquitination and SUMOylation/deSUMOylation (reviewed in [66]) to stimulate its translocation from cytosol to mitochondria. The activity of Drp1 appears to be regulated by post-translational modification operated by several enzymes including calcium/calmodulin-dependent protein kinase 1 $\alpha$ , protein kinase A or Ca<sup>2+</sup> calcineurin [67]. Mutations in Drp1 and fission protein 1 can lead to non-uniform mtDNA distribution, reduced ATP production, increased ROS production, and increased vulnerability of cells to apoptotic cell death [68,69].

### 2.4. microRNA regulation of mitochondrial functions

In the course of the last two decades, the discovery of miRNAs has established a new paradigm for the regulation of gene expression of most fundamental biological pathways in eukaryotic cells. miRNAs also provide a multi-level regulatory system particularly relevant for optimal brain function, and offer a great range of novel targets [70]. Small non-coding regulatory RNAs act on the expression of genes at a post-transcriptional level. Best-known regulation occurs through imperfect base pairing, most commonly in the 3' untranslated region (UTR) of key mRNAs, where together with Argonaute proteins (AGO) miRNAs act as effectors to recruit factors and lead mostly to translational repression,

mRNA deadenylation and mRNA decay. Of the four AGO proteins known in human, only AGO2 exhibits endonucleolytic activity that can slice perfectly matched mRNAs.

miRNA are abundantly expressed in the CNS, and their dysregulation is observed in DS [70,71] as in other neurodegenerative disorders, such as Friedreich ataxia, Huntington's disease and Alzheimer's diseases [72,73], suggesting their notable involvement in the mechanisms of neurodegeneration (reviewed in [72]). The idea that some miRNAs could consist of new vectors to fine-tune mitochondrial activity with the rest of the cellular machinery is of considerable interest.

It is well established that miRNAs may localize and function in the cytosol where they associate with targets, such as nuclear-encoded mitochondrial mRNAs. A few miRNAs are known to regulate important mitochondrial factors, such as miR-141, which is involved in the regulation of the mitochondrial phosphate carrier (Slc25a3) [74]. Importantly, for the understanding of axonal regulation, miR-338 exerts its modulation on COXIV [75] and ATP5G1 [76], leading to a locally marked effect on axonal radical levels, as well as on axonal growth. ATP synthase has also been reported as a target for two other miRNAs: miR-101-3p and miR-127-5p, whose activity is responsible for over 50% reduction in ATP synthase activity [77,78] while some miRNAs, such as miR-210 and miR-155 are well established key regulators of mitochondrial activity and biogenesis [13,79].

Following transcription, miRNAs are also known to expand their action in mitochondria by targeting mitochondrially-encoded mRNAs [80,81], known as “mitomiRs” [80]. Initially, the Henrion-Caude's team reported the mitochondrial localization of those specific miRNAs and AGO2 protein [80], suggesting that miRNA-mediated processes may directly regulate mitochondrial homeostasis and function [82]. The view that mitomiRs could indeed exert a mitochondrial regulatory function on gene expression has been further established by various experimental approaches and in different mammalian species. A typical example is provided by miR-494, a mitomiR, which is common to several species [80,83], and that was shown to regulate mitochondrial biogenesis, notably through the downregulation of the mitochondrial transcription factor, TFAM [84]. Das et al. observed that nuclear miR-181c translocates into the mitochondria to selectively regulate mitochondrial complex IV expression through targeting of mt-COX1 [81]. Taken together, these observations provide renewed insight to our understanding of mitochondrial dynamics, and the role of miRNAs in mitochondria.

### 3. Mitochondrial energy metabolism and oxidative imbalance in Down syndrome

Impaired mitochondrial energy metabolism and altered homeostasis of radical species are implicated in the DS pathogenesis, and are critically linked to several phenotypical features of DS, such as defective neurogenesis and neural plasticity leading to cognitive impairment [85], accelerated aging and early neurodegenerative processes [86].

Mitochondrial phenotype in DS is characterized by a reduced efficiency to produce ATP through OXPHOS, a decreased respiratory capacity and ability to generate mitochondrial membrane potential, as well as impaired mitochondrial dynamics. Interestingly, these mitochondrial defects are present in all DS cell types analyzed so far ranging from peripheral to CNS cells [87–91]. Therefore, mitochondrial dysfunction is in most cases proposed to be an inherent feature of the syndrome [16,86]. However, in one study, impaired mitochondrial function was considered as an adaptive response to protect against injury and maintain optimal cellular functions [92].

In this section, we will highlight and discuss the molecular determinants responsible for impaired ATP synthesis, alterations in mitochondrial biogenesis, structure and dynamics, and the origin of oxidative stress in DS (see Fig. 1).

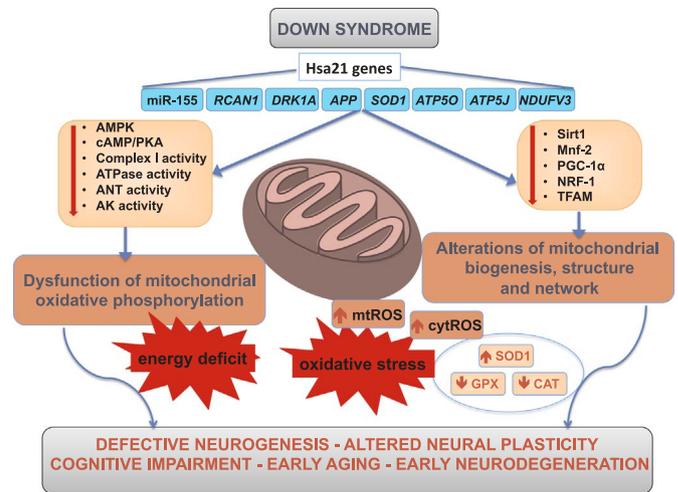


Fig. 1. Overview of the molecular targets and signalling pathways deregulated in DS and associated with mitochondrial dysfunction and oxidative stress. The scheme shows certain Hsa21-genes overexpressed in DS and the molecular determinants inactivated (↓) or inhibited (⊥) as the result of their overexpression. These alterations, leading to dysfunction of mitochondrial oxidative phosphorylation, impairment of mitochondrial biogenesis and oxidative stress, contribute to DS phenotype (indicated in the gray box). For the references relative to the alterations indicated in the figure see the chapter “Mitochondrial energy metabolism and oxidative imbalance in Down syndrome”.

#### 3.1. Dysfunction of mitochondrial oxidative phosphorylation machinery in DS

Mitochondrial OXPHOS machinery consists of five assembled complexes organized across the inner mitochondrial membranes in a super molecular organization to form the mitochondrial respiratory chain (MRC). Some mitochondrial transporters such as ANTs, which export ATP from mitochondria exchanging the cytosolic ADP, and adenylate kinase (AK), to catalyze the interconversion of adenylate nucleotides, are metabolically interconnected with the MRC contributing to the synthesis of ATP from mitochondria [89]. In DS the OXPHOS apparatus is selectively affected at the molecular level. Indeed, down-regulation of genes encoding for MRC subunits (complexes I, III and V) and the increase of certain enzymes of Krebs cycle (i.e. aconitase and NADP-linked isocitrate dehydrogenase) have been reported in the heart of DS fetuses and brain regions of subjects with DS [93,94]. Analysis of mitochondrial function performed by our group showed that fibroblasts and lymphoblastoid cells from DS subjects have multilevel deficits in the OXPHOS machinery, including the mitochondrial respiratory chain complex I, the ATP synthase, the ADP/ATP translocator, and the AK enzyme, leading to cell energy deficit, and increase in free radical production from mitochondria [89–91]. A severe bioenergetic deficit was also found in neural progenitor cells (NPCs) in which a deficit in cell proliferation was observed in hippocampal tissue derived from Ts65Dn mice, a commonly used DS murine model [85,95]. Despite an apparent increase in glycolytic compensation, as revealed by the increase in the basal levels of L-lactate in NPCs, a significant depression of the whole cellular ATP content was reported. The drop in ATP cellular content could account for reduced NPCs proliferation. The bioenergetic impairment of the MRC complex activity (in particular complex I and ATP synthase), can affect both respiration-mediated and general mitochondrial ATP production.

Our group previously demonstrated that the bioenergetic deficit correlates with alteration of cAMP/PKA signalling pathway due to decreased basal levels of cAMP and reduced protein kinase A (PKA) activity. It should be noted that a down-regulation of PKA activity and cAMP signalling also occurs in the hippocampus of Ts65Dn mice [96]. Depression of cAMP-dependent PKA activity leading to reduced cAMP-dependent phosphorylation of the NDUFS4 subunit of complex I, and

the consequent decrease in the complex I activity [90,91]. MRC complex I represents the rate-limiting step for ATP synthesis and respiration, and responsible for the control of mitochondrial bioenergetics, therefore its deficit is considered deleterious for the bioenergetic function of mitochondria [97]. Recently, we further showed that AMPK, the key cell energy sensor and central regulator of cellular metabolism in eukaryotes [98] as described above, failed to be activated despite the strong reduction of cell energy in DS cells [95], and could be involved in the bioenergetic impairment of DS.

Gene expression imbalance of some Hsa21 genes were considered relevant for OXPHOS alteration; these include the ATP5O and ATP5J subunits of ATP synthase and NDUFV3 subunit of MRC complex I [99], RCAN1 a key regulatory in protein calcineurin/NFAT pathway [100], and DRK1A which regulate CREB/PKA signalling pathways [101] could also play important roles in affecting mitochondrial activity.

### 3.2. Alterations of mitochondrial biogenesis, structure and dynamics in DS

The decline of OXPHOS efficiency in DS could be linked to the severe impairment of mitochondrial biogenesis found to occur both in CNS cells, such as NPCs from the hippocampus of Ts65Dn mice [95], and in skin fibroblasts [88]. The entire mitochondrial biogenic system was found to be compromised in Ts65Dn cells, including a reduced content in mtDNA as well as reduced protein levels of PGC-1 $\alpha$ , NRF-1 and TFAM [95]. The expression and activity of PGC-1 $\alpha$ , the master regulator of mitochondrial biogenesis, was down-regulated in DS cells [88,95,102]. Overexpression of the chromosome 21 gene *RCAN1*, which negatively regulates PGC-1 $\alpha$  [103], could account for lower PGC-1 $\alpha$  protein levels. In addition, miR-155, which is also encoded by chromosome 21 and overexpressed in DS brain, targets the mitochondrial transcription factor TFAM [13] reducing its protein level and concurring to reduced mitochondrial biogenesis. Post-translational modifications of PGC-1 $\alpha$ , such as direct phosphorylation by AMPK [104] and deacetylation by Sirt 1 [105] seem to be affected in DS cells [95,102]. Altogether, these pathway alterations concurred with the impairment of PGC-1 $\alpha$  activity and correlate with the defective mitochondrial biogenesis.

Furthermore, PGC-1 $\alpha$  regulates the expression of the mitofusin Mfn-2; thus, in DS fibroblasts, PGC-1 $\alpha$  down-regulation causes a reduced Mfn-2 expression with a decrease in mitochondrial fusion [102]. Several alterations in mitochondrial morphology and dynamics have been observed both *in vivo* in DS mouse models and *in vitro* in human cell culture studies. Ultrastructural abnormalities in cerebellar neurons derived from the Ts16 mice, have been early reported, including irregular shaped mitochondria [106]. Electron microscopy of DS human fetal fibroblasts also showed that a large number of mitochondria have an irregular shape, evident breaks, predominantly within the inner membranes, and changes in the pattern of cristae with an increased number of shorter mitochondria, and a smaller mitochondrial volume [102]. Morphological alterations were also observed in primary cultures of DS astrocytes and neurons, with increased mitochondrial fragmentation and breaking of mitochondrial network [92]. Alteration in mitochondrial dynamics strongly influences the proliferation of adult neurons, which has been associated with the maintenance of neuronal function, differentiation and survival (for refs. see [107]). Therefore, we postulate that alterations in mitochondrial structure and dynamics could account for impairment of neural function in DS.

### 3.3. Origin of oxidative stress in DS: contribution of mitochondrial dysfunction

The accumulation of oxidative stress is an essential part of the pathobiology of DS and an early event in DS phenotype [108]. Oxidative stress is generated by an imbalance in cellular redox status, due to either overproduction of radical species, and/or decreased antioxidant response as well as insufficient clearance of the oxidized proteins [109].

Recently, the role of iron metabolism deregulation into DS redox homeostasis alteration has been reviewed and discussed [110].

Oxidative stress in DS is partly due to an imbalance between the Hsa21-encoded *SOD1* and glutathione peroxidase (GPX) activities. Increased *SOD1* expression and activity have been reported in DS [111,112]; catalase (CAT) and GPX are expressed at lower levels in DS [113], and this may account for reduced antioxidant defenses reported in DS patients. An increase in the oxidized glutathione/reduced glutathione (GSH) ratio and elevated peroxide production have been previously observed in human fibroblast cultures from DS fetuses and correlated with reduced endogenous antioxidant capacity [114]. As well, the Hsa21 amyloid- $\beta$  precursor protein (APP) is believed to be another player involved in the origin of oxidative stress and in brain damage of DS patients [86].

However, the association between oxidative stress and mitochondrial dysfunction is relevant, given the pivotal role of mitochondria not only as the major cellular site for free radical generation, but also as targets of ROS [115]. Upregulation in ROS generation and mitochondrial dysfunction has been identified in DS cells during early development and commencing of embryonic life [116]. Indeed, markers of oxidative stress are already present in the amniotic fluid of trisomic fetuses during pregnancy [117]. In addition, primary cultured astrocytes and neurons from Ts1CJe mouse model of DS showed increased ROS levels, that was associated with mitochondrial dysfunction [111].

Oxidative stress and metabolic mitochondrial deficits have been observed in different DS-derived primary cells, including fetal and adult fibroblasts [89–91], cortical neurons, astrocytes, and pancreatic  $\beta$  cells [92]. These alterations are related to the presence of increased basal levels of ROS, deficits in electron transport chain components [90,91], and a downregulation of mitochondrial activity [92]. Moreover, complex I represents a key target for alterations in ROS homeostasis in Ts16 neurons [118,119]. Accordingly, we previously demonstrated that the reduced catalytic activity of complex I is the primary cause for increased mitochondrial ROS in DS human peripheral cells [90,91], thus providing additional support for the mitochondrial origin of oxidative stress in DS.

## 4. Mitochondrial dysfunctions in some DS-associated diseases

Down syndrome is often associated with some congenital defects or diseases, including among others, autism spectrum disorders, AD, congenital cardiomyopathy and early aging, in which mitochondrial dysfunctions occurred early in the pathogenesis of these diseases. Piccoli and co-authors have even suggested that changes to the bioenergetic background in trisomy 21 fetuses may represent an important factor associated with a more severe phenotype [88]. In this section, the implication of mitochondrial dysfunctions in the pathogenesis of some DS-related diseases (see Fig. 2) is reviewed and discussed.

### 4.1. Autism spectrum disorder

A subset of DS children develop autism spectrum disorder (ASD), characterized by deficits in social communication and interaction, as well as repetitive and restricted behavior [120]. ASD is associated with intellectual disability, reduced motor coordination, attention difficulties, and other selected health issues, such as epilepsy, sleep disorders and gastrointestinal problems [121–124]. ASD is recognized as a complex genetic condition that plays an important role in its etiology [125]. Both *de-novo* and inherited genetic factors involve more than 15 loci. Therefore, abnormalities in gene-gene and gene-environment interactions, contribute to the development of ASD [126]. Increased exposure to environmental factors is likely to account for the increased incidence of ASD due to epigenetic-dependent gene regulation alterations [127]. Mitochondrial dysfunctions in ASD have been of major focus in the last few years, and an increasing body of evidence suggests a role in the etiology of ASD. It has been estimated that 50–80% of

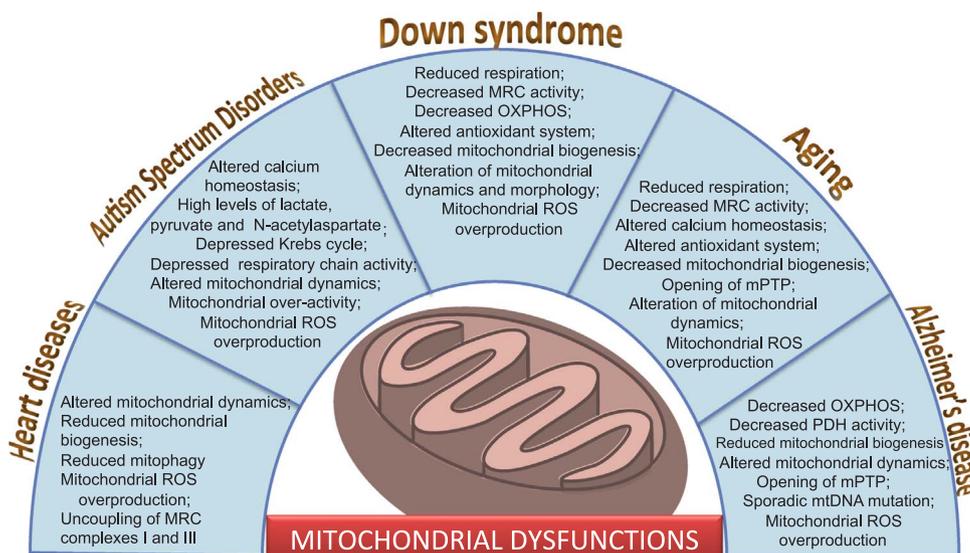


Fig. 2. Summary of mitochondrial alterations in DS and DS-related diseases. For references of the indicated mitochondrial alterations see: heart diseases [145–149,154]; autism spectrum disorders [124,128–130,133–139]; Down syndrome [16,85,86,89–96,102]; aging [181,185–187]; Alzheimer's disease [164,169,170,172–176].

children with autism exhibit mitochondrial dysfunction [128,129]. Moreover, a higher prevalence of mitochondrial dysfunction has been reported in individuals with ASD [124]. Indeed, subjects affected by ASD show abnormal levels of metabolites associated with mitochondrial function such as serum lactate and pyruvate [124,130], as well as altered N-acetylaspartate, mitochondrial aspartate/glutamate carrier 1 and calcium homeostasis [130–132].

Furthermore, reduced activity of enzymes in the MRC [124,134,135] and Krebs cycle [136,137] have been reported to be depressed in different brain areas and in muscle tissue [124]. In addition, increased amount of mitochondrial fission proteins and decreased amount of fusion proteins that control mitochondrial dynamics have been reported in the temporal lobe of ASD children [134]. Interestingly, a recent work showed that ASD lymphoblastoid cell lines exhibited greater ATP-linked respiration, and mitochondrial activity compared to cells from siblings [138]. Consistently, reduced intracellular glutathione redox capacity and increased *UCP2* gene expression has been reported in ASD children compared to controls. The authors found evidence of mitochondrial over-activity and greater susceptibility to oxidative stress in ASD [138]. A mitochondrial over-activity in some children with autism has also been reported in other studies [133,139].

In addition, two variants of interest in the MT-ND5 gene known to disrupt mitochondrial complex I function have been reported following analysis of mtDNA sequence collected from whole-exome sequencing in 10 multiplex families. Other variants of interest in mitochondrial *ATP6* and nuclear *NDUFS4* genes were also identified [140]. The *NDUFS4* gene, which encodes for a subunit of the MRC complex I regulates cAMP/PKA pathway activation and is associated with increased complex I activity [141,142]. Particularly, reduced phosphorylation level of the complex I subunit *NDUFS4* in both fibroblast and lymphoblastoid cells from a DS subject has been associated with reduced complex I activity [91].

#### 4.2. Cardiovascular diseases

Congenital heart defects have been reported in over 50% of newborns with Hsa21 trisomy. Similarly, many studies have established the critical role of mitochondrial dysfunctions and oxidative stress in a wide range of cardiovascular diseases including congenital heart defects [for refs. see [143,144]]. Changes to mitochondrial dynamics can lead to alterations in the proliferation of vascular smooth muscle cells, impaired cardiac development and differentiation, de-regulated stem cell differentiation, cardiomyocyte hypertrophy, increased vulnerability to myocardial ischemia-reperfusion injury, and congestive heart failure

[145,146].

Reduced OPA1, in parallel to an increase in small mitochondria, has been reported in *in vivo* models of heart failure [147]. The constitutive processing of OPA1 results in the conversion of the uncleaved long OPA1 (L-OPA) to a cleaved short OPA1 (S-OPA) form [148]. Accelerated OPA1 proteolysis triggers mitochondrial fragmentation and alters cardiac metabolism causing dilated cardiomyopathy and heart failure [149]. ROS-dependent apoptosis is associated with changes in mitochondrial cAMP/PKA signalling. This causes degradation/proteolysis of Sirt3, a mitochondrial sirtuin, which ultimately enhances acetylation and proteolytic processing of OPA1 [135].

Dysfunctional mitochondria segregated by fission are removed through autophagy/mitophagy [53,149]. The major checkpoint of mitochondrial autophagy is the mammalian target of rapamycin, AMPK which also enhances Sirt1 activity and downstream PGC-1 $\alpha$ , thus promoting mitochondrial biogenesis [29,150,151]. Interestingly, dysfunctional AMPK signals are thought to underlie several cardiovascular pathologies [152], thus confirming also the involvement of the deregulation of mitochondrial biogenesis in these pathologies [153].

Oxidative stress is a major cause of heart diseases. The majority of ROS in the heart are generated by uncoupling of MRC complexes I and III [154]. However, other mechanisms such as monoamine oxidases [155], NADPH oxidase [156], xanthine oxidoreductase [157], or NOS generate ROS and participate to oxidative damage in heart tissue. Mitochondria are highly vulnerable to increased oxidative stress that they generate. In particular, mtDNA does not have protective histones and an efficient repair system [158]. The important role of mtDNA in cardiac disorders is also evidenced by the fact that congenital heart disease is frequently associated with mtDNA mutations [159]. In heart diseases, the increase of ROS generation together with reduced endogenous antioxidant function [160] resulted in further accumulation of impaired mitochondria. Damaged mitochondria can be isolated from the existing mitochondrial network through fission and are removed by autophagy. Therefore, impaired autophagy is likely to be a contributing factor to cardiovascular disorders [161].

#### 4.3. Alzheimer's disease

Individuals with DS over 55 years of age have high risk to develop amyloid neuropathology and AD-like symptoms. This is believed to be due to overexpression of the Hsa21 *APP* gene and potentially associated with increased risk of dementia [5,162]. Since mitochondrial dysfunctions and oxidative stress play a central role in AD pathogenesis [163], it is likely that the entity of mitochondrial impairment is a risk factor to

early development of this deleterious neurodegenerative disease within DS population.

AD is an age-related progressive neurodegenerative disorder characterized by progressive neuronal loss, and the presence of neurofibrillary tangles and senile plaques in the brain. The neurodegenerative processes are characterized by deposition of amyloid  $\beta$ -peptide (A $\beta$ ) [164], and neurofibrillary degeneration, associated with abnormal formation of intracellular tangles containing phosphorylated tau protein [165]. Although the majority of AD cases are sporadic, less than 10% of AD cases are due to genetic mutations in three genes including APP, presenilin 1, and presenilin 2, which regulate A $\beta$  processing.

The “mitochondrial cascade hypothesis” suggests that mitochondrial dysfunction is a primary major event in the pathogenesis of AD [166]. Studies have shown that, as well as extracellular localization, A $\beta$  is also found in different subcellular compartments including mitochondria [167,168] where its accumulation proceeds prior to extracellular deposition. A $\beta$  is associated with a decrease in enzymatic activity of respiratory chain complexes, and a reduction in the rate of oxygen consumption [169]. Reduced OXPHOS, pyruvate dehydrogenase, and cytochrome c oxidase activities, and increased oxidative stress and lipid peroxidation have also been reported in an AD mouse model [170]. Another study showed the interaction of APP with the inner mitochondrial membrane proapoptotic protein, Apoptosin, which is involved in the heme biosynthesis. This protein was found to be upregulated in brain samples collected from AD patients and has been shown to regulate intrinsic caspase-dependent apoptosis [171].

Expression levels of PGC-1  $\alpha$ , NRF-1, NRF-2, and TFAM were significantly decreased in both AD hippocampal tissue and APPswe M17 cells [172], suggesting that alterations in mitochondrial biogenesis may also contribute to mitochondrial abnormalities in AD. Abnormal mitochondrial dynamics, such as increased fission and decreased fusion, associated with unusual changes in mitochondria structure have been reported in AD. It has been demonstrated that the Drp1 protein interacts with A $\beta$  and phosphorylates tau, leading to mitochondrial fragmentation and impaired axonal transport of mitochondria, culminating in neuronal damage and cognitive decline [173]. Moreover, a reduction in mitochondrial fusion proteins (OPA1, Mfn1, and Mfn2) and an increase in Fis1 levels has also been reported [173].

Another mechanism of mitochondrial dysfunction has been related to the interaction of A $\beta$  with various components of the mitochondrial permeability transition pore (mPTP). In fact, studies have shown that, in APP transgenic mice, A $\beta$  acts to upregulate VDAC1, a component of the mPTP, to stimulate blockade of mPTP [174]. Moreover, the interaction between A $\beta$  and CypD, a mitochondrial matrix protein, causes the opening of mPTP, leading to reduced mitochondrial membrane potential, increased oxidative stress, apoptosis, and deficits in axonal mitochondrial transport [175].

Mitochondrial dysfunction in AD induces an increase in ROS production by defective mitochondria. The increased oxidative stress has been shown to further damage mitochondrial components such as mtDNA and critical mitochondrial enzymes. A three-fold increase in oxidative damage in mtDNA has been reported in the AD brain [175–177].

#### 4.4. Aging

DS individuals are more vulnerable to accelerated aging, which may involve mitochondrial dysfunction and intracellular ROS accumulation. The “free-radical theory of aging”, which was first proposed by Harman [178], suggests that the aging process and the development of age-related degenerative diseases are due to increased free-radical damage to cells and tissues. This is likely due to an imbalance between the formation of ROS and the body’s endogenous antioxidant defense mechanisms. This essential process culminates in the production of superoxide anion radicals by complexes I and III [179]. Under normal physiological conditions, ROS serve as important immune mediators

during inflammation, and are involved in the maintenance of synaptic plasticity, learning and memory processes. However, excessive levels of free radicals can induce oxidative damage to macromolecules, and thus altering and impairing vital cellular processes.

Impairments in cellular bioenergetics and disruption to cellular redox homeostasis have been shown to increase in the CNS with advanced age. For instance, an age-related increase in protein nitration and oxidation, parallel to a decline in endogenous antioxidant components has been documented in the hippocampus and frontal cortex of post-mortem human brains between 0.01 and 80 years old [180]. Similarly, antioxidant enzymes and GSH levels were reduced in the aged brain [181]. This effect was also reported in several animal models. Recently, we demonstrated reduced mitochondrial bioenergetics (oxygen consumption and mitochondrial complex activities) in physiologically aged female Wistar rats (24 months old) compared with young animals (3 months old) [182]. Age-related mitochondrial impairments have been also correlated to reduced locomotor function in Rhesus monkeys [183]. Importantly, treatment with Ginkgo biloba extract (EGb 761), which has demonstrated potent antioxidant properties, attenuated oxidative stress and age-related mitochondrial impairments in another study [184].

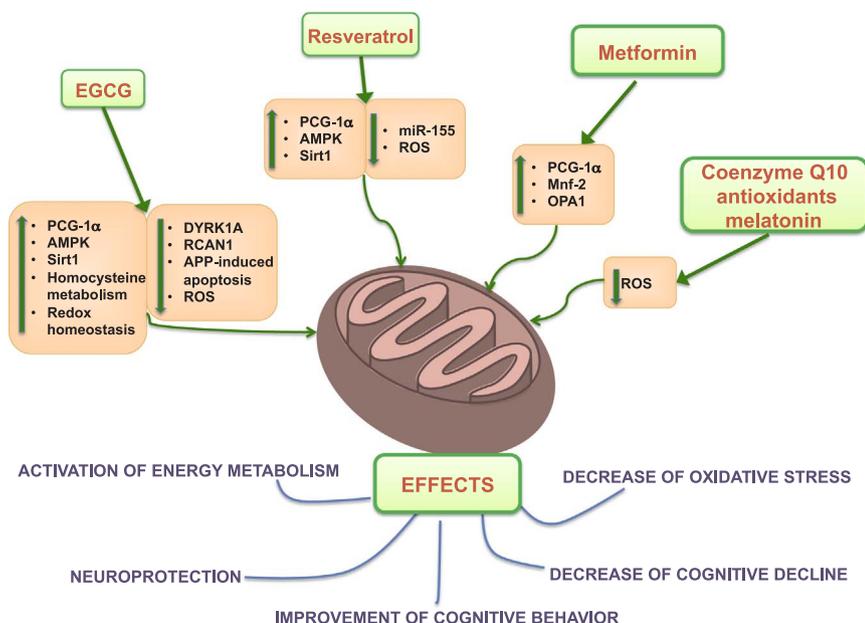
Reduced threshold concentration of Ca<sup>2+</sup> which facilitates the opening of mPTP has also been reported in mitochondria isolated from 18 month-old rats compared to young (3 months old) rats [185]. Similarly, senescence accelerated mice strains, which are a useful model to study aging, display impairments in the brain antioxidant system including reduced GSH level, down-regulation of SOD and catalase activity (MnSOD and Cu/Zn-SOD), and age-related impairments in learning and memory [186,187].

Therefore, it is likely that increased ROS generation can induce oxidative insult leading to mitochondrial dysfunction and cell death via apoptosis and energy restriction. However, whether increased free radical production represents the main cause of mitochondrial dysfunction or occurs in response to irregular respiratory function remains unclear. Indeed, additional sources of endogenous ROS production can contribute to ROS-induced toxicity. The distribution of mitochondria is an important regulator of spatial and temporal demands of neuronal energy in the adult brain [188,189]. Thus, alteration in mitochondrial dynamics represents another facet in the pathobiology of age-related diseases associated with synaptic/neuronal degeneration.

A recent study observed that exogenous cAMP increased the activity of Sirt3, a sirtuin protein involved in age-related diseases [190], that finely regulates mitochondrial function [191], and increases lifespan in mice. The authors suggest that Sirt3 may be affected by mitochondrial proteases under reduction by cAMP levels [192]. Consistently, we have shown that oxidative stress reduces mitochondrial cAMP levels leading to activation of mitochondrial proteases and reduced Sirt3 activity [135]. It should be recalled that analysis of cAMP/PKA pathway in skin fibroblasts from Down syndrome subjects, that present in early aging, showed reduced cAMP levels and PKA activity compared to normal cells [91] suggesting a role of cAMP signalling in the aging process.

#### 5. Targeting mitochondrial dysfunctions in Down syndrome: challenges and pitfalls

Mitochondria, for their unique structure and functions, represent primary or secondary targets of various drugs used for pharmacotherapy in many diseases, including neurodegenerative disorders, cancer, cardiovascular diseases, diabetes and obesity (for refs. see [193]); all these diseases are associated with mitochondrial dysfunction but also, to a various extent, to DS phenotypes. Mitochondrial drugs and their targets, which include those affecting mitochondrial electron transport chain complexes, intermediates of mitochondrial metabolism, mitochondrial transporters and mtDNA, are widely reviewed in [193]. Targeting of signalling pathways controlling mitochondrial functions offers a great potential for new therapeutic approaches aimed to



**Fig. 3.** Overview of bioactive drugs and their molecular targets shown to reverse mitochondrial impairment, oxidative stress, and some DS clinical phenotypes. The scheme reports identified proteins and pathways traced back to the levels of normal cells by reactivation (↑) or inhibition (↓) as results of treatment with EGCG, resveratrol, metformin, CoQ10, antioxidants and melatonin. The resulting effects of the treatments positively modulate mitochondria and improve DS phenotype. For references see the chapter “Targeting mitochondrial dysfunctions in Down syndrome: challenges and pitfalls” and Table 1.

attenuate mitochondrial dysfunction.

Herein, we will focus on several bioactive compounds, which have demonstrated beneficial protective effects on mitochondrial signalling pathways. These agents can reduce mitochondrial impairment and ROS overproduction in DS, and are likely to be promising therapeutic tools to improve DS phenotype (Fig. 3 and Table 1).

### 5.1. Polyphenols

Plant-derived polyphenols are interesting candidates for the management of DS, for their multimodal action in pathways altered in DS. Polyphenols have been shown to modulate: i) gene expression of different genes (in particular for DS, *DYRK1A* and *RCAN1*), and several miRNAs; ii) lipid oxidation; iii) homocysteine metabolism; iv) signalling pathways which regulate mitochondrial functions and v) redox homeostasis (for Ref. [194]). Polyphenols are naturally-occurring secondary metabolites of plants largely found in fruits, vegetables and cereals [195] whose regular consumption is inversely associated to the developmental risk of chronic human diseases. Polyphenols have been

shown to protect against different diseases such as cancer, neurodegenerative, neurologic, cardiovascular and metabolic pathologies [196,197]. Two polyphenols, epigallocatechin-3 gallate (EGCG) and resveratrol (RSV) are of particular interest in DS.

EGCG is a polyphenol belonging to the class of flavonoids and is the main and most bioactive polyphenol present in the *Camellia sinensis* (green tea) leaves extract [198]. It has been shown to be a multipotent therapeutic agent with beneficial neuroprotective effects [199]. EGCG has been reported to be about ten time more effective as a free radical scavenger than vitamin E and C, which rely on the presence of more steric structures than EGCG to stabilize the phenoxy radical structure [194]. EGCG can readily cross the blood-brain barrier, making it an attractive compound for therapy. Abd El Mohsen and colleagues [200] and Zhang and colleagues [201] reported that after oral administration of EGCG for a period of 5 days in mice, the polyphenol could be detected in brain tissue samples.

The strong antioxidant properties, the capability to counteract mitochondrial dysfunctions and to target several signalling pathways controlling neural function, make EGCG a potent disease-modifying

**Table 1**  
Therapeutic use of bioactive drugs targeting mitochondrial dysfunctions in Down syndrome.

Drugs	Dose		Therapeutic actions		Refs.
	in vitro	in vivo	Involving mitochondria	Others	
EGCG	20 μM	10 mg/kg/die	Mitochondrial ROS scavenging; Aβ-induced mitochondrial apoptosis; Rescue of MRC complex I and V activities; Activation of mitochondrial biogenesis; Promotion of ATP synthesis; Rescue of mitochondrial membrane potential	Modulation of homocysteine metabolism; Reduction of glutamate cytotoxicity	[87,91,95,194,203–205,209,210]
RSV	10 μM	n.t.	Activation of mitochondrial biogenesis; Promotion of ATP synthesis; Promotion of respiratory chain activity	Reduction of Aβ peptides; Reduction of tau phosphorylation	[95,194,211]
Metformin	0.05 mM	n.t.	Promotion of mitochondrial network; Activation of mitochondrial bioenergetics; Activation of mitochondrial biogenesis	Control of blood sugar level	[102]
CoQ10	n.t.	4 mg/kg/die	Activation of mitochondrial OXPHOS apparatus; Promotion of ATP synthesis; Mitochondrial ROS scavenging	Modulation of DNA repair mechanisms	[215–217,220]
Melatonin	n.t.	100 mg/L/die	ROS scavenging; Reduction of oxidative damage	Regulation of sleep and wake cycles	[223–225]

Drugs tested in DS able to target mitochondria are indicated. Therapeutic actions relevant for DS not involving mitochondria are indicated with “Others”. n.t., not tested in DS.

agent for DS and neurodegenerative diseases [202]. Extensive studies carried out in several mouse and cell models of DS and neurological diseases, have shown that EGCG can act by a number of mechanisms: i) controlling mitochondrial homeostasis and ROS scavenging [203]; ii) protecting against  $\beta$ -amyloid-induced mitochondrial apoptosis [55]; iii) protecting against glutamate cytotoxicity [204] and iv) activating mitochondrial bioenergetics and biogenesis [91,95,205]. AMPK is a target of EGCG. Indeed neural hippocampal progenitor cells from Ts65Dn mouse model of DS treated with 20  $\mu$ M EGCG showed AMPK activation, which correlated with an increased mitochondrial ATP synthesis and attenuation of deficit in hippocampal neural progenitor cell proliferation [95]. Several reports have shown that EGCG modulates Sirt1 activity (for refs. see [196]). Sirt1 is a master regulator in maintaining genomic integrity and stability as well as a key modulatory target in aging and neurodegeneration [206]. EGCG works synergistically with AMPK, influencing the phosphorylation/acetylation status and the activity of the PGC-1 $\alpha$  [207]. In a recent paper, we reported that 20  $\mu$ M EGCG promoted respiratory chain function, mitochondrial biogenesis, and neural progenitor cell proliferation in Ts65Dn mice by up-regulating AMPK/Sirt1/ PGC-1 $\alpha$  axis [95]. We also reported that stimulation of complex I activity occurred as result of 20  $\mu$ M EGCG treatment in fibroblasts of DS patients [91]. In this case, EGCG modulates the levels of the key signalling molecule, cAMP [208] and increases the activity of cAMP-dependent PKA which enhances phosphorylation of the NDUF54 subunit of mitochondrial complex I. This in turn facilitates the rescue of complex I activity and a reduction of ROS production from the impaired complex I [91]. EGCG can also inhibit kinase activity of DRK1A, which is hyperactivated in DS and possibly responsible for the impairment in brain development and cognitive deficit in DS [209]. Very relevant for DS subjects suffering of recurrent sleep apnea disturb is a study reporting that oral supplements of EGCG reduces the neural susceptibility to intermittent hypoxia during sleep due to oxidative stress and neuroinflammation in rodents [210].

Recently, two clinical studies were performed in order to demonstrate the safety and the efficacy of EGCG in DS. A double-blind, randomized, placebo-controlled, phase 2 trial demonstrated that EGCG (9 mg/kg per day) supplementation for 12 months potentiates the effect of the cognitive training in young adults with DS [211]. Recently, in a case study, we reported the safety and the positive effect of dietary supplementation of EGCG (10 mg/kg per day) plus fish oil omega-3 in a DS child in which, following the treatment, we observed a rescue of the mitochondrial dysfunction and improvement of some behavioral deficits [87].

Apart from EGCG, the efficacy of resveratrol (RSV) has also been recently tested in DS. RSV is a polyphenol belonging to the class of stilbenoids. It is found in grapes, red fruits, peanuts, and some plants such as *Polygonum cuspidatum*. We previously showed that 10  $\mu$ M RSV was able to attenuate deficits in mitochondrial bioenergetics and biogenesis in NPCs from Ts65Dn mice by targeting PGC-1 $\alpha$ /Sirt1/AMPK axis, and leading to the rescue of impaired neurogenesis in vitro. It should also be noted that RSV does not interact with DRK1A but down-regulates the chromosome 21-encoded miR-155 [212], which controls TFAM and regulates mitochondrial biogenesis [13]. Thus, the normalization of miR155 by RSV could be a very interesting strategy to prevent the impairment of mitochondrial biogenesis and other metabolic alterations found in DS.

The major pitfall for supplementation with polyphenols is represented by the absence of correlation between the dose of polyphenols and their bioavailability in the human body, due to their rapid metabolism and extensive modifications occurring during absorption. Moreover, the polyphenols are found in lower concentrations in food compared to their effective dose in humans. In this perspective, different strategies have been adopted to enhance the delivery and bioavailability of polyphenol extracts, such as combination with other polyphenols, combination with other nutrients, encapsulation, prodrugs, polyphenol analogues, and alternative methods to oral

administration [194]. In addition, prior to clinical translation, the dose-response relationship should be considered in view of a recent study reporting that when the dosage was increased from 10 to 50 mg/kg per day, EGCG supplementation is harmful to skeletal DS phenotypes and fails to improve behavioral deficits in Ts65Dn mouse model of DS [213].

## 5.2. Metformin

Recently, metformin has been also proposed as a potential therapeutic strategy in DS for its ability to counteract impairment of mitochondrial network and correct mitochondrial alterations [102]. Metformin is normally used as therapy for type 2 diabetes controlling blood sugar level. Izzo and co-worker demonstrated, in fibroblasts of subjects with DS, that treatment with metformin treatment enhances PGC-1 $\alpha$  expression and increases mitochondrial biogenesis. Additionally, metformin promotes mitochondrial network organization, inducing the expression of the fusion-inducing genes Mfn-2 and OPA1.

However, recent studies revealed that chronic use of metformin increased the risk of developing AD by inducing mitochondrial dysfunction and aggregation of A $\beta$  and tau proteins in the brain cortex [214,215]. Thus, caution should be warranted for application of metformin as chronic therapy in DS, since the syndrome is already tightly associated with high risk to develop AD in the middle age.

## 5.3. Coenzyme Q10, antioxidants and melatonin

Other supplementations such as coenzyme Q10 (CoQ10) and some vitamins and antioxidants were discussed as possible therapies for DS phenotype due to their ability to target mitochondrial dysfunctions [216,217]. CoQ10 is a bioenergetic enzyme cofactor central to mitochondrial OXPHOS apparatus. It is responsible for electron transfer from complexes I and II to complex III. It also provides potent antioxidant protection for the OXPHOS complex machinery, and is a potent ROS scavenger [218]. CoQ10 has been administered in patients with Friedreich's ataxia Huntington's and Parkinson's diseases with favorable results in decreasing disease progression [219]. In DS, a recent study comparing forty-three young DS children and forty-three controls revealed a significant decrease of plasma CoQ10 levels, which positively correlated with a decrease of IQ score [220]. In addition, a clinical trial aimed at assessing the impact of CoQ10 supplementation following a prolonged treatment regimen (4 mg/kg CoQ10 per day) in DS patients revealed a potential role of CoQ10 in modulating DNA repair mechanisms [221].

Antioxidants supplementation, including  $\alpha$ -tocopherol, ascorbic acid and alpha-lipoic acid, was very effective to protect against oxidative imbalance and reverse cognitive impairment of mouse models of DS [222]. However, clinical trials performed on both children and young adults with DS have revealed that standard antioxidant supplementations could not replicate the favorable pre-clinical therapeutic effects in humans [223].

Melatonin, a hormone involved in the regulation of sleep and wake cycles was found to be lowered in the serum of children with DS with respect to controls [224]. Due to its strong antioxidant abilities, melatonin was tested in mouse models of DS and was shown to reduce oxidative damage, improve cognitive performance, and reduce age-related degeneration of basal forebrain cholinergic neurons [225,226]. These results make melatonin an interesting antioxidant supplementation to be tested in DS patients.

## 5.4. microRNA

Evidence that miRNAs are associated with regulation of the respiratory chain and ROS generation suggests the potential role of mitomiRs as novel therapeutic targets in DS [227]. While the therapeutic potential of miRNAs is promising, it is necessary to first to know

the transient or long-lasting regulatory effects of mitomiRs, and delivery mechanisms of mitomiRs to mitochondria.

## 6. Concluding remarks

In this review we have analyzed the molecular determinants for mitochondrial dysfunctions and the resulting cellular ROS accumulation in DS (Fig. 1). Many mitochondrial alterations in DS are shared with those found in other neurodegenerative diseases, aging, heart defects, and other disorders associated with DS clinical phenotype (see Fig. 2). We collected evidences demonstrating that mitochondrial alterations principally affect the brain, which is highly vulnerable to energy deficit and susceptible to oxidative stress [19,228]. Therefore, we strongly propose that mitochondrial dysfunction may be a major etiological mechanism in intellectual disability and cognitive decline, which represent key hallmarks of DS.

Therapeutic intervention aimed at improving mitochondrial function and reducing oxidative stress could be more effective during early childhood to prevent neurobehavioral outcomes [229]. We also believe that long-time treatment should be performed in DS, since short-time intervention in neonatal life failed to remain effective in adulthood [230,231]. Therefore, natural bioactive compounds, such as polyphenols extracts, due to their long-term safety profile and efficacy could be strongly recommended as therapeutic interventions in DS [194,211]. We also suggest that combinations of therapeutic agents may better improve outcomes in this complex disease.

In conclusion, we are fully aware that further work should be done prior to confirming the efficacy of the proposed therapeutics in the DS population. However, available evidence suggests that improving mitochondrial function whilst a challenge, may ameliorate cognitive impairment and many other clinical outcomes associated with DS, leading to a better general health and improved quality of life of DS individuals.

## Acknowledgments

This study was partially supported by grants from Fondation Jerome Lejeune, Paris (VACCA/615-VRI2008A and VACCA/1093-VR2012B).

## References

- [1] S.E. Antonarakis, Down syndrome and the complexity of genome dosage imbalance, *Nat. Rev. Genet.* 18 (2017) 147–163, <http://dx.doi.org/10.1038/nrg.2016.154>.
- [2] M. Kazemi, M. Salehi, M. Kheirollahi, Down syndrome: current status, challenges and future perspectives, *Int. J. Mol. Cell. Med.* 5 (2016) 125–133.
- [3] S.N. Van Cleve, W.I. Cohen, Part I: clinical practice guidelines for children with Down syndrome from birth to 12 years, *J. Pediatr. Health Care Off. Publ. Natl. Assoc. Pediatr. Nurse Assoc. Pract.* 20 (2006) 47–54, <http://dx.doi.org/10.1016/j.pedhc.2005.10.004>.
- [4] S.N. Van Cleve, S. Cannon, W.I. Cohen, Part II: clinical practice guidelines for adolescents and young adults with Down syndrome: 12–21 years, *J. Pediatr. Health Care Off. Publ. Natl. Assoc. Pediatr. Nurse Assoc. Pract.* 20 (2006) 198–205, <http://dx.doi.org/10.1016/j.pedhc.2006.02.006>.
- [5] E. Head, I.T. Lott, D.M. Wilcock, C.A. Lemere, Aging in Down syndrome and the development of Alzheimer's disease neuropathology, *Curr. Alzheimer Res.* 13 (2016) 18–29.
- [6] M. Rachidi, C. Lopes, Mental retardation in Down syndrome: from gene dosage imbalance to molecular and cellular mechanisms, *Neurosci. Res.* 59 (2007) 349–369, <http://dx.doi.org/10.1016/j.neures.2007.08.007>.
- [7] J. Park, W.-J. Song, K.C. Chung, Function and regulation of Dyrk1A: towards understanding Down syndrome, *Cell. Mol. Life Sci. CMLS* 66 (2009) 3235–3240, <http://dx.doi.org/10.1007/s00018-009-0123-2>.
- [8] N. Méndez-Barbero, V. Esteban, S. Villahoz, A. Escolano, K. Urso, A. Alfranca, C. Rodríguez, S.A. Sánchez, T. Osawa, V. Andrés, J. Martínez-González, T. Minami, J.M. Redondo, M.R. Campanero, A major role for RCAN1 in atherosclerosis progression, *EMBO Mol. Med.* 5 (2013) 1901–1917, <http://dx.doi.org/10.1002/emmm.201302842>.
- [9] V. Iacobazzi, V. Infantino, A. Castegna, G. Andria, Hyperhomocysteinemia: related genetic diseases and congenital defects, abnormal DNA methylation and newborn screening issues, *Mol. Genet. Metab.* 113 (2014) 27–33, <http://dx.doi.org/10.1016/j.ymgme.2014.07.016>.
- [10] M. Valko, K. Jomova, C.J. Rhodes, K. Kuča, K. Musílek, Redox- and non-redox-metal-induced formation of free radicals and their role in human disease, *Arch. Toxicol.* 90 (2016) 1–37, <http://dx.doi.org/10.1007/s00204-015-1579-5>.
- [11] R. Mashima, Physiological roles of miR-155, *Immunology* 145 (2015) 323–333, <http://dx.doi.org/10.1111/imm.12468>.
- [12] X. Bofil-De Ros, M. Santos, M. Vila-Casadesús, E. Villanueva, N. Andreu, M. Dierssen, C. Fillat, Genome-wide miR-155 and miR-802 target gene identification in the hippocampus of Ts65Dn Down syndrome mouse model by miRNA sponges, *BMC Genom.* 16 (2015) 907, <http://dx.doi.org/10.1186/s12864-015-2160-6>.
- [13] A. Quiñones-Lombraña, J.G. Blanco, Chromosome 21-derived hsa-miR-155-5p regulates mitochondrial biogenesis by targeting Mitochondrial Transcription Factor A (TFAM), *Biochim. Biophys. Acta* 1852 (2015) 1420–1427, <http://dx.doi.org/10.1016/j.bbadis.2015.04.004>.
- [14] Z. Cetin, S. Yakut, E. Mihci, A.E. Manguoglu, S. Berker, I. Keser, G. Luleci, A patient with Down syndrome with a de novo derivative chromosome 21, *Gene* 507 (2012) 159–164, <http://dx.doi.org/10.1016/j.gene.2012.07.018>.
- [15] M.C. Pelleri, E. Cicchini, C. Locatelli, L. Vitale, M. Caracausi, A. Piovesan, A. Rocca, G. Poletti, M. Seri, P. Strippoli, G. Cocchi, Systematic reanalysis of partial trisomy 21 cases with or without Down syndrome suggests a small region on 21q22.13 as critical to the phenotype, *Hum. Mol. Genet.* 25 (2016) 2525–2538, <http://dx.doi.org/10.1093/hmg/ddw116>.
- [16] D. Valenti, L. de Bari, B. De Filippis, A. Henrion-Caude, R.A. Vacca, Mitochondrial dysfunction as a central actor in intellectual disability-related diseases: an overview of Down syndrome, autism, Fragile X and Rett syndrome, *Neurosci. Biobehav. Rev.* 46 (Pt 2) (2014) 202–217, <http://dx.doi.org/10.1016/j.neubiorev.2014.01.012>.
- [17] B. De Filippis, D. Valenti, L. de Bari, D. De Rasmio, M. Musto, A. Fabbri, L. Ricceri, C. Fiorentini, G. Laviola, R.A. Vacca, Mitochondrial free radical overproduction due to respiratory chain impairment in the brain of a mouse model of Rett syndrome: protective effect of CNF1, *Free Radic. Biol. Med.* 83 (2015) 167–177, <http://dx.doi.org/10.1016/j.freeradbiomed.2015.02.014>.
- [18] M. Golpich, E. Amini, Z. Mohamed, R.A. Ali, N.M. Ibrahim, A. Ahmadiani, Mitochondrial dysfunction and biogenesis in neurodegenerative diseases: pathogenesis and treatment, *CNS Neurosci. Ther.* 23 (2017) 5–22, <http://dx.doi.org/10.1111/cns.12655>.
- [19] O. Kann, R. Kovács, Mitochondria and neuronal activity, *Am. J. Physiol. Cell Physiol.* 292 (2007) C641–C657, <http://dx.doi.org/10.1152/ajpcell.00222.2006>.
- [20] J.M. Xavier, C.M.P. Rodrigues, S. Solá, Mitochondria: major regulators of neural development, *Neurosci. Rev. J. Bring. Neurobiol. Neurol. Psychiatry* 22 (2016) 346–358, <http://dx.doi.org/10.1177/1073858415585472>.
- [21] A. Mehrotra, A. Kanwal, S.K. Banerjee, R. Sandhir, Mitochondrial modulators in experimental Huntington's disease: reversal of mitochondrial dysfunctions and cognitive deficits, *Neurobiol. Aging* 36 (2015) 2186–2200, <http://dx.doi.org/10.1016/j.neurobiolaging.2015.02.004>.
- [22] N.R. Das, S.S. Sharma, Cognitive impairment associated with Parkinson's disease: role of mitochondria, *Curr. Neuropharmacol.* 14 (2016) 584–592.
- [23] M.T. Ryan, N.J. Hoogenraad, Mitochondrial-nuclear communications, *Annu. Rev. Biochem.* 76 (2007) 701–722, <http://dx.doi.org/10.1146/annurev.biochem.76.052305.091720>.
- [24] G. Benard, N. Bellance, C. Jose, S. Melsler, K. Nouette-Gaulain, R. Rossignol, Multi-site control and regulation of mitochondrial energy production, *Biochim. Biophys. Acta* 1797 (2010) 698–709, <http://dx.doi.org/10.1016/j.bbabi.2010.02.030>.
- [25] D.F. Rolfe, G.C. Brown, Cellular energy utilization and molecular origin of standard metabolic rate in mammals, *Physiol. Rev.* 77 (1997) 731–758.
- [26] J.-P. Mazat, S. Ransac, M. Heiske, A. Devin, M. Rigoulet, Mitochondrial energetic metabolism—some general principles, *IUBMB Life* 65 (2013) 171–179, <http://dx.doi.org/10.1002/iub.1138>.
- [27] F. Palmieri, F. Bisaccia, L. Capobianco, V. Dolce, G. Fiermonte, V. Iacobazzi, C. Indiveri, L. Palmieri, Mitochondrial metabolite transporters, *Biochim. Biophys. Acta* 1275 (1996) 127–132.
- [28] D.G. Hardie, AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy, *Nat. Rev. Mol. Cell Biol.* 8 (2007) 774–785, <http://dx.doi.org/10.1038/nrm2249>.
- [29] C. Cantó, J. Auwerx, PGC-1 $\alpha$ , SIRT1 and AMPK, an energy sensing network that controls energy expenditure, *Curr. Opin. Lipidol.* 20 (2009) 98–105, <http://dx.doi.org/10.1097/MOL.0b013e328328d0a4>.
- [30] C. Cantó, Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, AMPK regulates energy expenditure by modulating NAD<sup>+</sup> metabolism and SIRT1 activity, *Nature* 458 (2009) 1056–1060, <http://dx.doi.org/10.1038/nature07813>.
- [31] J.E. Dominy, P. Puigserver, Mitochondrial biogenesis through activation of nuclear signaling proteins, *Cold Spring Harb. Perspect. Biol.* 5 (2013), <http://dx.doi.org/10.1101/cshperspect.a015008>.
- [32] M.J. Evans, R.C. Scarpulla, Interaction of nuclear factors with multiple sites in the somatic cytochrome c promoter. characterization of upstream NRF-1, ATF, and intron Sp1 recognition sequences, *J. Biol. Chem.* 264 (1989) 14361–14368.
- [33] R.C. Scarpulla, Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network, *Biochim. Biophys. Acta* 1813 (2011) 1269–1278, <http://dx.doi.org/10.1016/j.bbamer.2010.09.019>.
- [34] N. Gleyzer, K. Vercauteren, R.C. Scarpulla, Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators, *Mol. Cell Biol.* 25 (2005) 1354–1366, <http://dx.doi.org/10.1128/MCB.25.4.1354-1366.2005>.
- [35] M. Falkenberg, M. Gaspari, A. Rantanen, A. Trifunovic, N.-G. Larsson, C.M. Gustafsson, Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA, *Nat. Genet.* 31 (2002) 289–294, <http://dx.doi.org/10.1038/ng909>.

- [36] T. Mello, M. Materozzi, A. Galli, PPARs and mitochondrial metabolism: from NAFLD to HCC, *PPAR Res.* 2016 (2016) 7403230, <http://dx.doi.org/10.1155/2016/7403230>.
- [37] L.J. Eichner, V. Giguère, Estrogen related receptors (ERRs): a new dawn in transcriptional control of mitochondrial gene networks, *Mitochondrion* 11 (2011) 544–552, <http://dx.doi.org/10.1016/j.mito.2011.03.121>.
- [38] L. Gopalakrishnan, R.C. Scarpulla, Differential regulation of respiratory chain subunits by a CREB-dependent signal transduction pathway. role of cyclic amp in cytochrome c and COXIV gene expression, *J. Biol. Chem.* 269 (1994) 105–113.
- [39] B. Wan, R.W. Moreadith, Structural characterization and regulatory element analysis of the heart isoform of cytochrome c oxidase VIa, *J. Biol. Chem.* 270 (1995) 26433–26440.
- [40] J.T. Cunningham, J.T. Rodgers, D.H. Arlow, F. Vazquez, V.K. Mootha, P. Puigserver, mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex, *Nature* 450 (2007) 736–740, <http://dx.doi.org/10.1038/nature06322>.
- [41] H. Xi, Y. Yu, Y. Fu, J. Foley, A. Halees, Z. Weng, Analysis of overrepresented motifs in human core promoters reveals dual regulatory roles of YY1, *Genome Res.* 17 (2007) 798–806, <http://dx.doi.org/10.1101/gr.5754707>.
- [42] F. Morrish, D. Hockenbery, MYC and mitochondrial biogenesis, *Cold Spring Harb. Perspect. Med.* 4 (2014), <http://dx.doi.org/10.1101/cshperspect.a014225>.
- [43] C. Handschin, B.M. Spiegelman, Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism, *Endocr. Rev.* 27 (2006) 728–735, <http://dx.doi.org/10.1210/er.2006-0037>.
- [44] B.N. Finck, D.P. Kelly, PGC-1 coactivators: inducible regulators of energy metabolism in health and disease, *J. Clin. Invest.* 116 (2006) 615–622, <http://dx.doi.org/10.1172/JCI27794>.
- [45] J. Lin, C. Handschin, B.M. Spiegelman, Metabolic control through the PGC-1 family of transcription coactivators, *Cell Metab.* 1 (2005) 361–370, <http://dx.doi.org/10.1016/j.cmet.2005.05.004>.
- [46] M. Uldry, W. Yang, J. St-Pierre, J. Lin, P. Seale, B.M. Spiegelman, Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation, *Cell Metab.* 3 (2006) 333–341, <http://dx.doi.org/10.1016/j.cmet.2006.04.002>.
- [47] Z. Arany, N. Lebrasseur, C. Morris, E. Smith, W. Yang, Y. Ma, S. Chin, B.M. Spiegelman, The transcriptional coactivator PGC-1beta drives the formation of oxidative type IIX fibers in skeletal muscle, *Cell Metab.* 5 (2007) 35–46, <http://dx.doi.org/10.1016/j.cmet.2006.12.003>.
- [48] R. Ventura-Clapier, A. Garnier, V. Veksler, Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha, *Cardiovasc. Res.* 79 (2008) 208–217, <http://dx.doi.org/10.1093/cvr/cvn098>.
- [49] C. Handschin, J. Rhee, J. Lin, P.T. Tarr, B.M. Spiegelman, An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle, *Proc. Natl. Acad. Sci. USA* 100 (2003) 7111–7116, <http://dx.doi.org/10.1073/pnas.1232352100>.
- [50] C. Cantó, J. Auwerx, PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure, *Curr. Opin. Lipidol.* 20 (2009) 98–105, <http://dx.doi.org/10.1097/MOL.0b013e318328d0a4>.
- [51] H.-M. Ni, J.A. Williams, W.-X. Ding, Mitochondrial dynamics and mitochondrial quality control, *Redox Biol.* 4 (2014) 6–13, <http://dx.doi.org/10.1016/j.redox.2014.11.006>.
- [52] R.M. Levitskiy, E.M. Germany, O. Khalimonchuk, Mitochondrial quality control proteases in neuronal welfare, *J. NeuroImmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol.* 11 (2016) 629–644, <http://dx.doi.org/10.1007/s11481-016-9683-8>.
- [53] O. Shirihai, M. Song, G.W. Dorn, How mitochondrial dynamism orchestrates mitophagy, *Circ. Res.* 116 (2015) 1835–1849, <http://dx.doi.org/10.1161/CIRCRESAHA.116.306374>.
- [54] G. Twig, A. Elorza, A.J.A. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, O.S. Shirihai, Fission and selective fusion govern mitochondrial segregation and elimination by autophagy, *EMBO J.* 27 (2008) 433–446, <http://dx.doi.org/10.1038/sj.emboj.7601963>.
- [55] K. Zhang, H. Li, Z. Song, Membrane depolarization activates the mitochondrial protease OMA1 by stimulating self-cleavage, *EMBO Rep.* 15 (2014) 576–585, <http://dx.doi.org/10.1002/embr.201338240>.
- [56] J.R. Friedman, J. Nunnari, Mitochondrial form and function, *Nature* 505 (2014) 335–343, <http://dx.doi.org/10.1038/nature12985>.
- [57] D.C. Chan, Fusion and fission: interlinked processes critical for mitochondrial health, *Annu. Rev. Genet.* 46 (2012) 265–287, <http://dx.doi.org/10.1146/annurev-genet-110410-132529>.
- [58] E. Schrepfer, L. Scorrano, Mitofusins, from mitochondria to metabolism, *Mol. Cell* 61 (2016) 683–694, <http://dx.doi.org/10.1016/j.molcel.2016.02.022>.
- [59] T. MacVicar, T. Langer, OPA1 processing in cell death and disease – the long and short of it, *J. Cell Sci.* 129 (2016) 2297–2306, <http://dx.doi.org/10.1242/jcs.159186>.
- [60] S. Cogliati, C. Frezza, M.E. Soriano, T. Varanita, R. Quintana-Cabrera, M. Corrado, S. Cipolat, V. Costa, A. Casarin, L.C. Gomes, E. Perales-Clemente, L. Salviati, P. Fernandez-Silva, J.A. Enriquez, L. Scorrano, Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency, *Cell* 155 (2013) 160–171, <http://dx.doi.org/10.1016/j.cell.2013.08.032>.
- [61] C. Frezza, S. Cipolat, O. Martins de Brito, M. Micaroni, G.V. Bezoussenko, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, L. Scorrano, OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion, *Cell* 126 (2006) 177–189, <http://dx.doi.org/10.1016/j.cell.2006.06.025>.
- [62] E. Smirnova, L. Griparic, D.L. Shurland, A.M. van der Bliek, Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells, *Mol. Biol. Cell* 12 (2001) 2245–2256.
- [63] H. Otera, C. Wang, M.M. Cleland, K. Setoguchi, S. Yokota, R.J. Youle, K. Mihara, Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells, *J. Cell Biol.* 191 (2010) 1141–1158, <http://dx.doi.org/10.1083/jcb.201007152>.
- [64] C.S. Palmer, L.D. Osellame, D. Laine, O.S. Koutsopoulos, A.E. Frazier, M.T. Ryan, Mid49 and Mid51, new components of the mitochondrial fission machinery, *EMBO Rep.* 12 (2011) 565–573, <http://dx.doi.org/10.1038/embor.2011.54>.
- [65] O.C. Losón, Z. Song, H. Chen, D.C. Chan, Fis1, Mff, Mid49, and Mid51 mediate Drp1 recruitment in mitochondrial fission, *Mol. Biol. Cell* 24 (2013) 659–667, <http://dx.doi.org/10.1091/mbc.E12-10-0721>.
- [66] K. Elgass, J. Pakay, M.T. Ryan, C.S. Palmer, Recent advances into the understanding of mitochondrial fission, *Biochim. Biophys. Acta* 1833 (2013) 150–161, <http://dx.doi.org/10.1016/j.bbamer.2012.05.002>.
- [67] S. Campello, L. Scorrano, Mitochondrial shape changes: orchestrating cell pathophysiology, *EMBO Rep.* 11 (2010) 678–684, <http://dx.doi.org/10.1038/embor.2010.115>.
- [68] A.B. Knott, G. Perkins, R. Schwarzenbacher, E. Bossy-Wetzel, Mitochondrial fragmentation in neurodegeneration, *Nat. Rev. Neurosci.* 9 (2008) 505–518, <http://dx.doi.org/10.1038/nrn2417>.
- [69] B. Oettinghaus, J.M. Schulz, L.M. Restelli, M. Licci, C. Savoia, A. Schmidt, K. Schmitt, A. Grimm, L. Moré, J. Hench, M. Tolnay, A. Eckert, P. D'Adamo, P. Franken, N. Ishihara, K. Mihara, J. Bischofberger, L. Scorrano, S. Frank, Synaptic dysfunction, memory deficits and hippocampal atrophy due to ablation of mitochondrial fission in adult forebrain neurons, *Cell Death Differ.* 23 (2016) 18–28, <http://dx.doi.org/10.1038/cdd.2015.39>.
- [70] D.E. Kuhn, G.J. Nuovo, M.M. Martin, G.E. Malana, A.P. Pleister, J. Jiang, T.D. Schmittgen, A.V. Terry, K. Gardiner, E. Head, D.S. Feldman, T.S. Elton, Human chromosome 21-derived miRNAs are overexpressed in down syndrome brains and hearts, *Biochem. Biophys. Res. Commun.* 370 (2008) 473–477, <http://dx.doi.org/10.1016/j.bbrc.2008.03.120>.
- [71] T.S. Elton, S.E. Sansom, M.M. Martin, Trisomy-21 gene dosage over-expression of miRNAs results in the haploinsufficiency of specific target proteins, *RNA Biol.* 7 (2010) 540–547.
- [72] J.A. Saugstad, MicroRNAs as effectors of brain function with roles in ischemia and injury, neuroprotection, and neurodegeneration, *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* 30 (2010) 1564–1576, <http://dx.doi.org/10.1038/jcbfm.2010.101>.
- [73] S. Bandiera, F. Cartault, A.-S. Jannot, E. Hatem, M. Girard, L. Rifai, C. Loiseau, A. Munnich, S. Lyonnet, A. Henrion-Caude, Genetic variations creating microRNA target sites in the FXN 3'-UTR affect frataxin expression in Friedreich ataxia, *PLoS One* 8 (2013) e54791, <http://dx.doi.org/10.1371/journal.pone.0054791>.
- [74] W.A. Baseler, D. Thapa, R. Jagannathan, E.R. Dabkowski, T.L. Croston, J.M. Hollander, miR-141 as a regulator of the mitochondrial phosphate carrier (Slc25a3) in the type 1 diabetic heart, *Am. J. Physiol. – Cell Physiol.* 303 (2012) C1244–C1251, <http://dx.doi.org/10.1152/ajpcell.00137.2012>.
- [75] A. Aschrafi, A.D. Schwechter, M.G. Mameza, O. Natera-Naranjo, A.E. Gioio, B.B. Kaplan, MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons, *J. Neurosci. Off. J. Soc. Neurosci.* 28 (2008) 12581–12590, <http://dx.doi.org/10.1523/JNEUROSCI.3338-08.2008>.
- [76] A. Aschrafi, A.N. Kar, O. Natera-Naranjo, M.A. MacGibeny, A.E. Gioio, B.B. Kaplan, MicroRNA-338 regulates the axonal expression of multiple nuclear-encoded mitochondrial mRNAs encoding subunits of the oxidative phosphorylation machinery, *Cell. Mol. Life Sci. CMLS* 69 (2012) 4017–4027, <http://dx.doi.org/10.1007/s00018-012-1064-8>.
- [77] S. Zheng, Y. Li, Y. Zhang, X. Li, H. Tang, MiR-101 regulates HSV-1 replication by targeting ATP5B, *Antivir. Res.* 89 (2011) 219–226, <http://dx.doi.org/10.1016/j.antiviral.2011.01.008>.
- [78] I.M. Willers, I. Martínez-Reyes, M. Martínez-Díez, J.M. Cuezva, miR-127-5p targets the 3'UTR of human  $\beta$ -F1-ATPase mRNA and inhibits its translation, *Biochim. Biophys. Acta* 1817 (2012) 838–848, <http://dx.doi.org/10.1016/j.bbabi.2012.03.005>.
- [79] M. Ivan, X. Huang, miR-210: fine-tuning the hypoxic response, *Adv. Exp. Med. Biol.* 772 (2014) 205–227, [http://dx.doi.org/10.1007/978-1-4614-5915-6\\_10](http://dx.doi.org/10.1007/978-1-4614-5915-6_10).
- [80] S. Bandiera, S. Rüberg, M. Girard, N. Cagnard, S. Hanein, D. Chrétien, A. Munnich, S. Lyonnet, A. Henrion-Caude, Nuclear outsourcing of RNA interference components to human mitochondria, *PLoS One* 6 (2011) e20746, <http://dx.doi.org/10.1371/journal.pone.0020746>.
- [81] S. Das, M. Ferlito, O.A. Kent, K.F. Talbot, R. Wang, D. Liu, N. Raghavachari, Y. Yang, S.J. Wheelan, E. Murphy, C. Steenberg, Nuclear miRNA regulates the mitochondrial genome in the heart, *Circ. Res.* (2012), <http://dx.doi.org/10.1161/CIRCRESAHA.112.267732> (CIRCRESAHA.112.267732).
- [82] S. Bandiera, S. Hanein, S. Lyonnet, A. Henrion-Caude, Mitochondria as novel players of the cellular RNA interference, *J. Biol. Chem.* 286 (2011) 1e19, <http://dx.doi.org/10.1074/jbc.L111.240259>.
- [83] B.T. Kren, P.Y.-P. Wong, A. Sarver, X. Zhang, Y. Zeng, C.J. Steer, MicroRNAs identified in highly purified liver-derived mitochondria may play a role in apoptosis, *RNA Biol.* 6 (2009) 65–72.
- [84] H. Yamamoto, K. Morino, Y. Nishio, S. Ugi, T. Yoshizaki, A. Kashiwagi, H. Maegawa, MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead boxj3, *Am. J. Physiol. Endocrinol. Metab.* 303 (2012) E1419–1427, <http://dx.doi.org/10.1152/ajpendo.00097.2012>.
- [85] A. Contestabile, B. Greco, D. Ghezzi, V. Tucci, F. Benfenati, L. Gasparini, Lithium

- rescues synaptic plasticity and memory in Down syndrome mice, *J. Clin. Invest.* 123 (2013) 348–361, <http://dx.doi.org/10.1172/JCI64650>.
- [86] M. Perluigi, D.A. Butterfield, Oxidative stress and Down syndrome: a route toward Alzheimer-like dementia, *Curr. Gerontol. Geriatr. Res.* 2012 (2012) 724904, <http://dx.doi.org/10.1155/2012/724904>.
- [87] R.A. Vacca, D. Valenti, Green tea EGCG plus fish oil omega-3 dietary supplements rescue mitochondrial dysfunctions and are safe in a Down's syndrome child, *Clin. Nutr. Edinb. Scotl.* 34 (2015) 783–784, <http://dx.doi.org/10.1016/j.clnu.2015.04.012>.
- [88] C. Piccoli, A. Izzo, R. Scrima, F. Bonfiglio, R. Manco, R. Negri, G. Quarato, O. Cela, M. Ripoli, M. Prisco, F. Gentile, G. Cali, P. Pinton, A. Conti, L. Nitsch, N. Capitano, Chronic pro-oxidative state and mitochondrial dysfunctions are more pronounced in fibroblasts from Down syndrome foeti with congenital heart defects, *Hum. Mol. Genet.* 22 (2013) 1218–1232, <http://dx.doi.org/10.1093/hmg/ddt529>.
- [89] D. Valenti, A. Tullio, M.F. Caratuzolo, R.S. Merafina, P. Scartezzini, E. Marra, R.A. Vacca, Impairment of F1F0-ATPase, adenine nucleotide translocator and adenylate kinase causes mitochondrial energy deficit in human skin fibroblasts with chromosome 21 trisomy, *Biochem. J.* 431 (2010) 299–310, <http://dx.doi.org/10.1042/BJ20100581>.
- [90] D. Valenti, G.A. Manente, L. Moro, E. Marra, R.A. Vacca, Deficit of complex I activity in human skin fibroblasts with chromosome 21 trisomy and over-production of reactive oxygen species by mitochondria: involvement of the cAMP/PKA signalling pathway, *Biochem. J.* 435 (2011) 679–688, <http://dx.doi.org/10.1042/BJ20101908>.
- [91] D. Valenti, D. De Rasmio, A. Signorile, L. Rossi, L. de Bari, I. Scala, B. Granese, S. Papa, R.A. Vacca, Epigallocatechin-3-gallate prevents oxidative phosphorylation deficit and promotes mitochondrial biogenesis in human cells from subjects with Down's syndrome, *Biochim. Biophys. Acta* 1832 (2013) 542–552, <http://dx.doi.org/10.1016/j.bbadis.2012.12.011>.
- [92] P. Helguera, J. Seiglie, J. Rodriguez, M. Hanna, G. Helguera, J. Busciglio, Adaptive downregulation of mitochondrial function in Down syndrome, *Cell Metab.* 17 (2013) 132–140, <http://dx.doi.org/10.1016/j.cmet.2012.12.005>.
- [93] S.H. Kim, R. Vlkolinsky, N. Cairns, M. Fountoulakis, G. Lubec, The reduction of NADH ubiquinone oxidoreductase 24- and 75-kDa subunits in brains of patients with Down syndrome and Alzheimer's disease, *Life Sci.* 68 (2001) 2741–2750.
- [94] A. Conti, F. Fabbrini, P. D'Agostino, R. Negri, D. Greco, R. Genesio, M. D'Armiento, C. Olla, D. Paladini, M. Zannini, L. Nitsch, Altered expression of mitochondrial and extracellular matrix genes in the heart of human fetuses with chromosome 21 trisomy, *BMC Genom.* 8 (2007) 268, <http://dx.doi.org/10.1186/1471-2164-8-268>.
- [95] D. Valenti, L. de Bari, D. de Rasmio, A. Signorile, A. Henrion-Caude, A. Contestabile, R.A. Vacca, The polyphenols resveratrol and epigallocatechin-3-gallate restore the severe impairment of mitochondria in hippocampal progenitor cells from a Down syndrome mouse model, *Biochim. Biophys. Acta* 1862 (2016) 1093–1104, <http://dx.doi.org/10.1016/j.bbadis.2016.03.003>.
- [96] M. Dierssen, I.F. Vallina, C. Baamonde, S. García-Calatayud, M.A. Lumbraeras, J. Flórez, Alterations of central noradrenergic transmission in Ts65Dn mouse, a model for Down syndrome, *Brain Res.* 749 (1997) 238–244.
- [97] J.E. Telford, S.M. Kilbride, G.P. Davey, Complex I is rate-limiting for oxygen consumption in the nerve terminal, *J. Biol. Chem.* 284 (2009) 9109–9114, <http://dx.doi.org/10.1074/jbc.M809101200>.
- [98] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, *Nat. Cell Biol.* 13 (2011) 1016–1023, <http://dx.doi.org/10.1038/ncb2329>.
- [99] B. Granese, I. Scala, C. Spatuzza, A. Valentino, M. Coletta, R.A. Vacca, P. De Luca, G. Andria, Validation of microarray data in human lymphoblasts shows a role of the ubiquitin-proteasome system and NF- $\kappa$ B in the pathogenesis of Down syndrome, *BMC Med. Genom.* 6 (2013) 24, <http://dx.doi.org/10.1186/1755-8794-6-24>.
- [100] M.J. Kipanyula, W.H. Kimaro, P.F. Seke Etet, The emerging roles of the calcineurin-nuclear factor of activated T-lymphocytes pathway in nervous system functions and diseases, *J. Aging Res.* 2016 (2016) 5081021, <http://dx.doi.org/10.1155/2016/5081021>.
- [101] E.J. Yang, Y.S. Ahn, K.C. Chung, Protein kinase Dyrk1 activates cAMP response element-binding protein during neuronal differentiation in hippocampal progenitor cells, *J. Biol. Chem.* 276 (2001) 39819–39824, <http://dx.doi.org/10.1074/jbc.M104091200>.
- [102] A. Izzo, M. Nitti, N. Mollo, S. Paladino, C. Procaccini, D. Faicchia, G. Cali, R. Genesio, F. Bonfiglio, R. Ciacitello, E. Polishchuk, R. Polishchuk, P. Pinton, G. Matarese, A. Conti, L. Nitsch, Metformin restores the mitochondrial network and reverses mitochondrial dysfunction in Down syndrome cells, *Hum. Mol. Genet.* 26 (2017) 1056–1069, <http://dx.doi.org/10.1093/hmg/ddx016>.
- [103] M. Rachidi, C. Lopes, Mental retardation and associated neurological dysfunctions in Down syndrome: a consequence of dysregulation in critical chromosome 21 genes and associated molecular pathways, *Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc.* 12 (2008) 168–182, <http://dx.doi.org/10.1016/j.ejpn.2007.08.010>.
- [104] S. Jäger, C. Handschin, J. St-Pierre, B.M. Spiegelman, AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 $\alpha$ , *Proc. Natl. Acad. Sci. USA* 104 (2007) 12017–12022, <http://dx.doi.org/10.1073/pnas.0705070104>.
- [105] T. Wenz, PGC-1 $\alpha$  activation as a therapeutic approach in mitochondrial disease, *IUBMB Life* 61 (2009) 1051–1062, <http://dx.doi.org/10.1002/iub.261>.
- [106] E.T. Bersu, F.J. Ahmad, M.J. Schwei, P.W. Baas, Cytoplasmic abnormalities in cultured cerebellar neurons from the trisomy 16 mouse, *Brain Res. Dev. Brain Res.* 109 (1998) 115–120.
- [107] K.H. Flippo, S. Strack, Mitochondrial dynamics in neuronal injury, development and plasticity, *J. Cell Sci.* 130 (2017) 671–681, <http://dx.doi.org/10.1242/jcs.171017>.
- [108] D.A. Butterfield, F. Di Domenico, A.M. Swomley, E. Head, M. Perluigi, Redox proteomics analysis to decipher the neurobiology of Alzheimer-like neurodegeneration: overlaps in Down syndrome and Alzheimer disease brain, *Biochem. J.* 463 (2014) 177–189, <http://dx.doi.org/10.1042/BJ20140772>.
- [109] A. Tramutola, F. Di Domenico, E. Barone, A. Arena, A. Giorgi, L. di Francesco, M.E. Schinina, R. Coccia, E. Head, D.A. Butterfield, M. Perluigi, Polyubiquitinylation profile in Down syndrome brain before and after the development of Alzheimer neuropathology, *Antioxid. Redox Signal.* 26 (2017) 280–298, <http://dx.doi.org/10.1089/ars.2016.6686>.
- [110] E. Barone, A. Arena, E. Head, D.A. Butterfield, M. Perluigi, Disturbance of redox homeostasis in Down syndrome: role of iron dysmetabolism, *Free Radic. Biol. Med.* (2017), <http://dx.doi.org/10.1016/j.freeradbiomed.2017.07.009>.
- [111] K. Ishihara, K. Amano, E. Takaki, A.S. Ebrahim, A. Shimohata, N. Shibasaki, I. Inoue, M. Takaki, Y. Ueda, H. Sago, C.J. Epstein, K. Yamakawa, Increased lipid peroxidation in Down's syndrome mouse models, *J. Neurochem.* 110 (2009) 1965–1976, <http://dx.doi.org/10.1111/j.1471-4159.2009.06294.x>.
- [112] G. Pagano, G. Castello, Oxidative stress and mitochondrial dysfunction in Down syndrome, *Adv. Exp. Med. Biol.* 724 (2012) 291–299, [http://dx.doi.org/10.1007/978-1-4614-0653-2\\_22](http://dx.doi.org/10.1007/978-1-4614-0653-2_22).
- [113] B. Uttara, A.V. Singh, P. Zamboni, R.T. Mahajan, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, *Curr. Neuropharmacol.* 7 (2009) 65–74, <http://dx.doi.org/10.2174/157015909787602823>.
- [114] A. Gimeno, J.L. García-Giménez, L. Audí, N. Toran, P. Andaluz, F. Dasi, J. Viña, F.V. Pallardó, Decreased cell proliferation and higher oxidative stress in fibroblasts from Down syndrome fetuses. Preliminary study, *Biochim. Biophys. Acta* 1842 (2014) 116–125, <http://dx.doi.org/10.1016/j.bbadis.2013.10.014>.
- [115] J. Sastre, F.V. Pallardó, J. Viña, The role of mitochondrial oxidative stress in aging, *Free Radic. Biol. Med.* 35 (2003) 1–8.
- [116] J. Busciglio, B.A. Yankner, Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro, *Nature* 378 (1995) 776–779, <http://dx.doi.org/10.1038/378776a0>.
- [117] M. Perluigi, F. di Domenico, A. Fiorini, A. Coccio, A. Giorgi, C. Foppoli, D.A. Butterfield, M. Giorlandino, C. Giorlandino, M.E. Schinina, R. Coccia, Oxidative stress occurs early in Down syndrome pregnancy: a redox proteomics analysis of amniotic fluid, *Proteom. Clin. Appl.* 5 (2011) 167–178, <http://dx.doi.org/10.1002/prca.201000121>.
- [118] S. Schuchmann, U. Heinemann, Increased mitochondrial superoxide generation in neurons from trisomy 16 mice: a model of Down's syndrome, *Free Radic. Biol. Med.* 28 (2000) 235–250.
- [119] G. Capone, P. Kim, S. Jovanovich, L. Payne, L. Freund, K. Welch, E. Miller, M. Trush, Evidence for increased mitochondrial superoxide production in Down syndrome, *Life Sci.* 70 (2002) 2885–2895, [http://dx.doi.org/10.1016/S0024-3205\(02\)01538-2](http://dx.doi.org/10.1016/S0024-3205(02)01538-2).
- [120] H. Brentani, C.S. de Paula, D. Bordini, D. Rolim, F. Sato, J. Portolese, M.C. Pacifico, J.T. McCracken, Autism spectrum disorders: an overview on diagnosis and treatment, *Rev. Bras. Psiquiatr. Sao Paulo Braz.* 1999 (Suppl. 1) (2013) S62–S72, <http://dx.doi.org/10.1590/1516-4446-2013-S104> (35).
- [121] R. Canitano, Epilepsy in autism spectrum disorders, *Eur. Child Adolesc. Psychiatry* 16 (2007) 61–66, <http://dx.doi.org/10.1007/s00787-006-0563-2>.
- [122] P. Krakowiak, B. Goodlin-Jones, I. Hertz-Picciotto, L.A. Croen, R.L. Hansen, Sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study, *J. Sleep Res.* 17 (2008) 197–206, <http://dx.doi.org/10.1111/j.1365-2869.2008.00650.x>.
- [123] T. Buie, D.B. Campbell, G.J. Fuchs, G.T. Furuta, J. Levy, J. Vandewater, A.H. Whitaker, D. Atkins, M.L. Bauman, A.L. Beaudet, E.G. Carr, M.D. Gershon, S.L. Hyman, P. Jirapinyo, H. Jyonouchi, K. Kooros, R. Kushak, P. Levitt, S.E. Levy, J.D. Lewis, K.F. Murray, M.R. Natowitz, A. Sabra, B.K. Wershil, S.C. Weston, L. Zeltzer, H. Winter, Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report, *Pediatrics* 125 (Suppl. 1) (2010) S1–S18, <http://dx.doi.org/10.1542/peds.2009-1878C>.
- [124] D.A. Rossignol, R.E. Frye, Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis, *Mol. Psychiatry* 17 (2012) 290–314, <http://dx.doi.org/10.1038/mp.2010.136>.
- [125] M.-C. Lai, M.V. Lombardo, S. Baron-Cohen, Autism, *Lancet* 383 (2014) 896–910, [http://dx.doi.org/10.1016/S0140-6736\(13\)61539-1](http://dx.doi.org/10.1016/S0140-6736(13)61539-1).
- [126] A.M. Persico, T. Bourgeron, Searching for ways out of the autism maze: genetic, epigenetic and environmental clues, *Trends Neurosci.* 29 (2006) 349–358, <http://dx.doi.org/10.1016/j.tins.2006.05.010>.
- [127] K.E. Latham, C. Sapienza, N. Engel, The epigenetic lorax: gene–environment interactions in human health, *Epigenomics* 4 (2012) 383–402, <http://dx.doi.org/10.2217/epi.12.31>.
- [128] R.E. Frye, S. Melnyk, D.F. MacFabe, Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder, *Transl. Psychiatry* 3 (2013) e220, <http://dx.doi.org/10.1038/tp.2012.143>.
- [129] E. Napoli, S. Wong, I. Hertz-Picciotto, C. Giulivi, Deficits in bioenergetics and impaired immune response in granulocytes from children with autism, *Pediatrics* 133 (2014) e1405–e1410, <http://dx.doi.org/10.1542/peds.2013-1545>.
- [130] H. Kuwabara, H. Yamasue, S. Koike, H. Inoue, Y. Kawakubo, M. Kuroda, Y. Takano, N. Iwashiro, T. Natsubori, Y. Aoki, Y. Kano, K. Kasai, Altered metabolites in the plasma of autism spectrum disorder: a capillary electrophoresis time-of-flight mass spectrometry study, *PLoS One* 8 (2013) e73814, <http://dx.doi.org/10.1371/journal.pone.0073814>.

- [131] Y. Aoki, T. Watanabe, O. Abe, H. Kuwabara, N. Yahata, Y. Takano, N. Iwashiro, T. Natsubori, H. Takao, Y. Kawakubo, K. Kasai, H. Yamasue, Oxytocin's neurochemical effects in the medial prefrontal cortex underlie recovery of task-specific brain activity in autism: a randomized controlled trial, *Mol. Psychiatry* 20 (2015) 447–453, <http://dx.doi.org/10.1038/mp.2014.74>.
- [132] S. Goh, Z. Dong, Y. Zhang, S. DiMauro, B.S. Peterson, Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: evidence from brain imaging, *JAMA Psychiatry* 71 (2014) 665–671, <http://dx.doi.org/10.1001/jamapsychiatry.2014.179>.
- [133] L. Palmieri, V. Papaleo, V. Porcelli, P. Scarcia, L. Gaita, R. Sacco, J. Hager, F. Rousseau, P. Curatolo, B. Manzi, R. Militerni, C. Bravaccio, S. Trillo, C. Schneider, R. Melmed, M. Elia, C. Lenti, M. Saccani, T. Pascucci, S. Puglisi-Allegra, K.-L. Reichelt, A.M. Persico, Altered calcium homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1, *Mol. Psychiatry* 15 (2010) 38–52, <http://dx.doi.org/10.1038/mp.2008.63>.
- [134] G. Tang, P. Gutierrez Rios, S.-H. Kuo, H.O. Akman, G. Rosoklija, K. Tanji, A. Dwork, E.A. Schon, S. DiMauro, J. Goldman, D. Sulzer, Mitochondrial abnormalities in temporal lobe of autistic brain, *Neurobiol. Dis.* 54 (2013) 349–361, <http://dx.doi.org/10.1016/j.nbd.2013.01.006>.
- [135] A. Signorile, A. Santeramo, G. Tamma, T. Pellegrino, S. D'Oria, P. Lattanzio, D. De Rasmio, Mitochondrial cAMP prevents apoptosis modulating Sirt3 protein level and OPA1 processing in cardiac myoblast cells, *Biochim. Biophys. Acta* 1864 (2017) 355–366, <http://dx.doi.org/10.1016/j.bbamer.2016.11.022>.
- [136] F. Gu, V. Chauhan, K. Kaur, W.T. Brown, G. LaFauci, J. Wegiel, A. Chauhan, Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjects with autism, *Transl. Psychiatry* 3 (2013) e299, <http://dx.doi.org/10.1038/tp.2013.68>.
- [137] S. Rose, S. Melynk, O. Pavliv, S. Bai, T.G. Nick, R.E. Frye, S.J. James, Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain, *Transl. Psychiatry* 2 (2012) e134, <http://dx.doi.org/10.1038/tp.2012.61>.
- [138] S. Rose, S.C. Bennuri, R. Wynne, S. Melynk, S.J. James, R.E. Frye, Mitochondrial and redox abnormalities in autism lymphoblastoid cells: a sibling control study, *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 31 (2017) 904–909, <http://dx.doi.org/10.1096/fj.201601004R>.
- [139] W.D. Graf, J. Marin-Garcia, H.G. Gao, S. Pizzo, R.K. Naviaux, D. Markusic, B.A. Barshop, E. Courchesne, R.H. Haas, Autism associated with the mitochondrial DNA G8363A transfer RNALys mutation, *J. Child Neurol.* 15 (2000) 357–361, <http://dx.doi.org/10.1177/088307380001500601>.
- [140] A. Patowary, R. Nesbitt, M. Archer, R. Bernier, Z. Brkanac, Next generation sequencing mitochondrial DNA analysis in autism spectrum disorder, *Autism Res.* (2017), <http://dx.doi.org/10.1002/aur.1792> (n/a-n/a).
- [141] D. De Rasmio, G. Gattoni, F. Papa, A. Santeramo, C. Pacelli, T. Cocco, L. Micelli, N. Sardo, M. Larizza, M. Scivetti, S. Milano, A. Signorile, The  $\beta$ -adrenoceptor agonist isoproterenol promotes the activity of respiratory chain complex I and lowers cellular reactive oxygen species in fibroblasts and heart myoblasts, *Eur. J. Pharmacol.* 652 (2011) 15–22, <http://dx.doi.org/10.1016/j.ejphar.2010.11.016>.
- [142] D. De Rasmio, A. Signorile, A. Santeramo, M. Larizza, P. Lattanzio, G. Capitanio, S. Papa, Intramitochondrial adenyllyl cyclase controls the turnover of nuclear-encoded subunits and activity of mammalian complex I of the respiratory chain, *Biochim. Biophys. Acta BBA – Mol. Cell Res.* 1853 (2015) 183–191, <http://dx.doi.org/10.1016/j.bbamer.2014.10.016>.
- [143] M. Neri, I. Riezzo, C. Pomara, S. Schiavone, E. Turillazzi, Oxidative-nitrosative stress and myocardial dysfunctions in Sepsis: evidence from the literature and postmortem observations, *Mediat. Inflamm.* 2016 (2016) 3423450, <http://dx.doi.org/10.1155/2016/3423450>.
- [144] Y.W. Chung, S.-M. Kang, An experimental approach to study the function of mitochondria in cardiomyopathy, *BMB Rep.* 48 (2015) 541–548.
- [145] S.-B. Ong, D.J. Hausenloy, Mitochondrial morphology and cardiovascular disease, *Cardiovasc. Res.* 88 (2010) 16–29, <http://dx.doi.org/10.1093/cvr/cvq237>.
- [146] S.-B. Ong, A.R. Hall, D.J. Hausenloy, Mitochondrial dynamics in cardiovascular health and disease, *Antioxid. Redox Signal.* 19 (2013) 400–414, <http://dx.doi.org/10.1089/ars.2012.4777>.
- [147] L. Chen, Q. Gong, J.P. Stice, A.A. Knowlton, Mitochondrial OPA1, apoptosis, and heart failure, *Cardiovasc. Res.* 84 (2009) 91–99, <http://dx.doi.org/10.1093/cvr/cvp181>.
- [148] R. Anand, T. Wai, M.J. Baker, N. Kladt, A.C. Schauss, E. Rugarli, T. Langer, The *i*-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission, *J. Cell Biol.* 204 (2014) 919–929, <http://dx.doi.org/10.1083/jcb.201308006>.
- [149] T. Wai, J. García-Prieto, M.J. Baker, C. Merkwirth, P. Benit, P. Rustin, F.J. Rupérez, C. Barbas, B. Ibañez, T. Langer, Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice, *Science* 350 (2015) aad0116, <http://dx.doi.org/10.1126/science.aad0116>.
- [150] E. Marzetti, A. Csizsar, D. Dutta, G. Balagopal, R. Calvani, C. Leeuwenburgh, Role of mitochondrial dysfunction and altered autophagy in cardiovascular aging and disease: from mechanisms to therapeutics, *Am. J. Physiol. Heart Circ. Physiol.* 305 (2013) H459–H476, <http://dx.doi.org/10.1152/ajpheart.00936.2012>.
- [151] Y. Hirota, D. Kang, T. Kanki, The physiological role of mitophagy: new insights into phosphorylation events, *Int. J. Cell Biol.* 2012 (2012) 354914, <http://dx.doi.org/10.1155/2012/354914>.
- [152] N.A. Shirwany, M.-H. Zou, AMPK in cardiovascular health and disease, *Acta Pharmacol. Sin.* 31 (2010) 1075–1084, <http://dx.doi.org/10.1038/aps.2010.139>.
- [153] G.W. Dorn, R.B. Vega, D.P. Kelly, Mitochondrial biogenesis and dynamics in the developing and diseased heart, *Genes Dev.* 29 (2015) 1981–1991, <http://dx.doi.org/10.1101/gad.269894.115>.
- [154] N.B. Madungwe, N.F. Zilberstein, Y. Feng, J.C. Bopassa, Critical role of mitochondrial ROS is dependent on their site of production on the electron transport chain in ischemic heart, *Am. J. Cardiovasc. Dis.* 6 (2016) 93–108.
- [155] D. Maggiorani, N. Manzella, D.E. Edmondson, A. Mattevi, A. Parini, C. Binda, J. Miale-Perez, Monoamine oxidases, oxidative stress, and altered mitochondrial dynamics in cardiac ageing, *Oxid. Med. Cell. Longev.* 2017 (2017) 3017947, <http://dx.doi.org/10.1155/2017/3017947>.
- [156] L. Zhang, J. Wu, X. Duan, X. Tian, H. Shen, Q. Sun, G. Chen, NADPH Oxidase, A potential target for treatment of stroke, *Oxid. Med. Cell. Longev.* 2016 (2016) 5026984, <http://dx.doi.org/10.1155/2016/5026984>.
- [157] A.M. Robert, L. Robert, Xanthine oxidoreductase, free radicals and cardiovascular disease. A critical review, *Pathol. Oncol. Res.* 20 (2014) 1–10, <http://dx.doi.org/10.1007/s12253-013-9698-x>.
- [158] F.M. Yakes, B. Van Houten, Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress, *Proc. Natl. Acad. Sci. USA* 94 (1997) 514–519.
- [159] N. Abaci, M. Arkan, T. Tansel, N. Sahin, A. Cakiris, F. Pacal, S. Sirma Ekmecci, E. Gök, D. Üstek, Mitochondrial mutations in patients with congenital heart defects by next generation sequencing technology, *Cardiol. Young.* 25 (2015) 705–711, <http://dx.doi.org/10.1017/S1047951114000754>.
- [160] J.W. Walters, D. Amos, K. Ray, N. Santanam, Mitochondrial redox status as a target for cardiovascular disease, *Curr. Opin. Pharmacol.* 27 (2016) 50–55, <http://dx.doi.org/10.1016/j.coph.2016.01.006>.
- [161] D. Dutta, R. Calvani, R. Bernabei, C. Leeuwenburgh, E. Marzetti, Contribution of impaired mitochondrial autophagy to cardiac aging: mechanisms and therapeutic opportunities, *Circ. Res.* 110 (2012) 1125–1138, <http://dx.doi.org/10.1161/CIRCRESAHA.111.246108>.
- [162] J.H. Lee, A.J. Lee, L.-H. Dang, D. Pang, S. Kisselev, S.J. Krinsky-McHale, W.B. Zigman, J.A. Luchsinger, W. Silverman, B. Tycko, L.N. Clark, N. Schupf, Candidate gene analysis for Alzheimer's disease in adults with Down syndrome, *Neurobiol. Aging* 56 (2017) 150–158, <http://dx.doi.org/10.1016/j.neurobiolaging.2017.04.018>.
- [163] P.I. Moreira, C. Carvalho, X. Zhu, M.A. Smith, G. Perry, Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology, *Biochim. Biophys. Acta BBA – Mol. Basis Dis.* 1802 (2010) 2–10, <http://dx.doi.org/10.1016/j.bbadis.2009.10.006>.
- [164] H. Zheng, E.H. Koo, The amyloid precursor protein: beyond amyloid, *Mol. Neurodegener.* 1 (2006) 5, <http://dx.doi.org/10.1186/1750-1326-1-5>.
- [165] D.M. Walsh, D.J. Selkoe, A $\beta$  Oligomers – a decade of discovery, *J. Neurochem.* 101 (2007) 1172–1184, <http://dx.doi.org/10.1111/j.1471-4159.2006.04426.x>.
- [166] R.H. Swerdlow, J.M. Burns, S.M. Khan, The Alzheimer's disease mitochondrial cascade hypothesis, *J. Alzheimers Dis. Jad.* 20 (Suppl. 2) (2010) S265–S279, <http://dx.doi.org/10.3233/JAD-2010-100339>.
- [167] M.-Y. Cha, S.-H. Han, S.M. Son, H.-S. Hong, Y.-J. Choi, J. Byun, I. Mook-Jung, Mitochondria-specific accumulation of amyloid  $\beta$  induces mitochondrial dysfunction leading to apoptotic cell death, *PLoS One* 7 (2012) e34929, <http://dx.doi.org/10.1371/journal.pone.0034929>.
- [168] J.X. Chen, S.D. Yan, Amyloid- $\beta$ -induced mitochondrial dysfunction, *J. Alzheimers Dis. Jad.* 12 (2007) 177–184.
- [169] C. Caspersen, N. Wang, J. Yao, A. Sosunov, X. Chen, J.W. Lustbader, H.W. Xu, D. Stern, G. McKhann, S.D. Yan, Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease, *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 19 (2005) 2040–2041, <http://dx.doi.org/10.1096/fj.05-3735fje>.
- [170] J. Yao, R.W. Irwin, L. Zhao, J. Nilsen, R.T. Hamilton, R.D. Brinton, Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease, *Proc. Natl. Acad. Sci. USA* 106 (2009) 14670–14675, <http://dx.doi.org/10.1073/pnas.0903563106>.
- [171] H. Zhang, Y. Zhang, Y. Chen, X. Huang, F. Zhou, W. Wang, B. Xian, X. Zhang, E. Masliah, Q. Chen, J.-D.J. Han, G. Bu, J.C. Reed, F.-F. Liao, Y.-G. Chen, H. Xu, Apoptosin is a novel pro-apoptotic protein and mediates cell death in neurodegeneration, *J. Neurosci.* 32 (2012) 15565–15576, <http://dx.doi.org/10.1523/JNEUROSCI.3668-12.2012>.
- [172] B. Sheng, X. Wang, B. Su, H. Lee, G. Casadesus, G. Perry, X. Zhu, Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease, *J. Neurochem.* 120 (2012) 419–429, <http://dx.doi.org/10.1111/j.1471-4159.2011.07581.x>.
- [173] M. Manczak, P.H. Reddy, Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimer's disease neurons: implications for mitochondrial dysfunction and neuronal damage, *Hum. Mol. Genet.* 21 (2012) 2538–2547, <http://dx.doi.org/10.1093/hmg/dds072>.
- [174] A. Smilansky, L. Dangoor, I. Nakdimon, D. Ben-Hail, D. Mizrahi, V. Shoshan-Barmatz, The voltage-dependent anion channel 1 mediates amyloid  $\beta$  toxicity and represents a potential target for Alzheimer disease therapy, *J. Biol. Chem.* 290 (2015) 30670–30683, <http://dx.doi.org/10.1074/jbc.M115.691493>.
- [175] V.K. Rao, E.A. Carlson, S.S. Yan, Mitochondrial permeability transition pore is a potential drug target for neurodegeneration, *Biochim. Biophys. Acta* 1842 (2014) 1267–1272, <http://dx.doi.org/10.1016/j.bbadis.2013.09.003>.
- [176] P. Mecocci, U. MacGarvey, M.F. Beal, Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease, *Ann. Neurol.* 36 (1994) 747–751, <http://dx.doi.org/10.1002/ana.410360510>.
- [177] N.R. Phillips, J.W. Simpkins, R.K. Roby, Mitochondrial DNA deletions in Alzheimer's brains: a review, *Alzheimers Dement. J. Alzheimers Assoc.* 10 (2014) 393–400, <http://dx.doi.org/10.1016/j.jalz.2013.04.508>.

- [178] D. Harman, Aging: a theory based on free radical and radiation chemistry, *J. Gerontol.* 11 (1956) 298–300.
- [179] P. Jezek, L. Hlavatá, Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism, *Int. J. Biochem. Cell Biol.* 37 (2005) 2478–2503, <http://dx.doi.org/10.1016/j.biocel.2005.05.013>.
- [180] C. Venkateshappa, G. Harish, A. Mahadevan, M.M. Srinivas Bharath, S.K. Shankar, Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease, *Neurochem. Res.* 37 (2012) 1601–1614, <http://dx.doi.org/10.1007/s11064-012-0755-8>.
- [181] P.K. Mandal, M. Tripathi, S. Sugunan, Brain oxidative stress: detection and mapping of anti-oxidant marker “Glutathione” in different brain regions of healthy male/female, MCI and Alzheimer patients using non-invasive magnetic resonance spectroscopy, *Biochem. Biophys. Res. Commun.* 417 (2012) 43–48, <http://dx.doi.org/10.1016/j.bbrc.2011.11.047>.
- [182] N. Braidý, A. Poljak, R. Grant, T. Jayasena, H. Mansour, T. Chan-Ling, G.J. Guillemin, G. Smythe, P. Sachdev, Mapping NAD(+) metabolism in the brain of ageing Wistar rats: potential targets for influencing brain senescence, *Biogerontology* 15 (2014) 177–198, <http://dx.doi.org/10.1007/s10522-013-9489-5>.
- [183] J.D. Pandya, R. Grondin, H.M. Yonutas, H. Haghazadeh, D.M. Gash, Z. Zhang, P.G. Sullivan, Decreased mitochondrial bioenergetics and calcium buffering capacity in the basal ganglia correlates with motor deficits in a nonhuman primate model of aging, *Neurobiol. Aging* 36 (2015) 1903–1913, <http://dx.doi.org/10.1016/j.neurobiolaging.2015.01.018>.
- [184] J. Sastre, A. Millán, J. García de la Asunción, R. Plá, G. Juan, F.V. Pallardó, E. O'Connor, J.A. Martín, M.T. Droy-Lefaix, J. Viña, A Ginkgo biloba extract (EG761) prevents mitochondrial aging by protecting against oxidative stress, *Free Radic. Biol. Med.* 24 (1998) 298–304.
- [185] O. Krestinina, T. Azarashvili, Y. Baburina, A. Galvita, D. Grachev, R. Stricker, G. Reiser, In aging, the vulnerability of rat brain mitochondria is enhanced due to reduced level of 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNP) and subsequently increased permeability transition in brain mitochondria in old animals, *Neurochem. Int.* 80 (2015) 41–50, <http://dx.doi.org/10.1016/j.neuint.2014.09.008>.
- [186] T. Kurokawa, S. Asada, S. Nishitani, O. Hazeki, Age-related changes in manganese superoxide dismutase activity in the cerebral cortex of senescence-accelerated prone and resistant mouse, *Neurosci. Lett.* 298 (2001) 135–138, [http://dx.doi.org/10.1016/S0304-3940\(00\)01755-9](http://dx.doi.org/10.1016/S0304-3940(00)01755-9).
- [187] H. Nakahara, T. Kanno, Y. Inai, K. Utsumi, M. Hiramatsu, A. Mori, L. Packer, Mitochondrial dysfunction in the senescence accelerated mouse (SAM), *Free Radic. Biol. Med.* 24 (1998) 85–92.
- [188] G. Amadoro, V. Corsetti, F. Florenzano, A. Atlante, A. Bobba, V. Nicolin, S.L. Nori, P. Calissano, Morphological and bioenergetic demands underlying the mitophagy in post-mitotic neurons: the Pink–Parkin pathway, *Front. Aging Neurosci.* 6 (2014), <http://dx.doi.org/10.3389/fnagi.2014.00018>.
- [189] V. Todorova, A. Blokland, Mitochondria and synaptic plasticity in the mature and aging nervous system, *Curr. Neuropharmacol.* 15 (2017) 166–173.
- [190] E. McDonnell, B.S. Peterson, H.M. Bonzme, M.D. Hirschey, SIRT3 regulates progression and development of diseases of aging, *Trends Endocrinol. Metab. Tem.* 26 (2015) 486–492, <http://dx.doi.org/10.1016/j.tem.2015.06.001>.
- [191] L. Guarente, H. Franklin, Epstein lecture: sirtuins, aging, and medicine, *N. Engl. J. Med.* 364 (2011) 2235–2244, <http://dx.doi.org/10.1056/NEJMr1100831>.
- [192] J. Wang, H. Lei, J. Hou, J. Liu, Involvement of oxidative stress in SAMP10 mice with age-related neurodegeneration, *Neurol. Sci.* 36 (2015) 743–750, <http://dx.doi.org/10.1007/s10072-014-2029-5>.
- [193] A. Olszewska, A. Szewczyk, Mitochondria as a pharmacological target: magnum overview, *IUBMB Life* 65 (2013) 273–281, <http://dx.doi.org/10.1002/iub.1147>.
- [194] R.A. Vacca, D. Valenti, S. Caccamese, M. Daglia, N. Braidý, S.M. Nabavi, Plant polyphenols as natural drugs for the management of Down syndrome and related disorders, *Neurosci. Biobehav. Rev.* 71 (2016) 865–877, <http://dx.doi.org/10.1016/j.neubiorev.2016.10.023>.
- [195] R. Tsao, Chemistry and biochemistry of dietary polyphenols, *Nutrients* 2 (2010) 1231–1246, <http://dx.doi.org/10.3390/nu2121231>.
- [196] M. Ajami, H. Pazoki-Toroudi, H. Amani, S.F. Nabavi, N. Braidý, R.A. Vacca, A.G. Atanasov, A. Mocan, S.M. Nabavi, Therapeutic role of sirtuins in neurodegenerative disease and their modulation by polyphenols, *Neurosci. Biobehav. Rev.* 73 (2017) 39–47, <http://dx.doi.org/10.1016/j.neubiorev.2016.11.022>.
- [197] J. Pérez-Hernández, V.J. Zaldívar-Machorro, D. Villanueva-Porras, E. Vega-Ávila, A. Chavarría, A potential alternative against neurodegenerative diseases: phytochemicals, *Oxid. Med. Cell. Longev.* 2016 (2016) 8378613, <http://dx.doi.org/10.1155/2016/8378613>.
- [198] S. Banerjee, J. Chatterjee, Efficient extraction strategies of tea (*Camellia sinensis*) biomolecules, *J. Food Sci. Technol.* 52 (2015) 3158–3168, <http://dx.doi.org/10.1007/s13197-014-1487-3>.
- [199] N.A. Singh, A.K.A. Mandal, Z.A. Khan, Potential neuroprotective properties of epigallocatechin-3-gallate (EGCG), *Nutr. J.* 15 (2016), <http://dx.doi.org/10.1186/s12937-016-0179-4>.
- [200] M.M. Abd El Mohsen, G. Kuhnle, A.R. Rechner, H. Schroeter, S. Rose, P. Jenner, C.A. Rice-Evans, Uptake and metabolism of epicatechin and its access to the brain after oral ingestion, *Free Radic. Biol. Med.* 33 (2002) 1693–1702.
- [201] B. Zhang, D. Rusciano, N.N. Osborne, Orally administered epigallocatechin gallate attenuates retinal neuronal death in vivo and light-induced apoptosis in vitro, *Brain Res.* 1198 (2008) 141–152, <http://dx.doi.org/10.1016/j.brainres.2007.12.015>.
- [202] M.H. Yan, X. Wang, X. Zhu, Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease, *Free Radic. Biol. Med.* 62 (2013) 90–101, <http://dx.doi.org/10.1016/j.freeradbiomed.2012.11.014>.
- [203] E.K. Schroeder, N.A. Kelsey, J. Doyle, E. Breed, R.J. Bouchard, F.A. Loucks, R.A. Harbison, D.A. Linseman, Green tea epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in neurons, *Antioxid. Redox Signal.* 11 (2009) 469–480, <http://dx.doi.org/10.1089/ars.2008.2215>.
- [204] J. Yu, Y. Jia, Y. Guo, G. Chang, W. Duan, M. Sun, B. Li, C. Li, Epigallocatechin-3-gallate protects motor neurons and regulates glutamate level, *FEBS Lett.* 584 (2010) 2921–2925, <http://dx.doi.org/10.1016/j.febslet.2010.05.011>.
- [205] B.A. Sutherland, O.M. Shaw, A.N. Clarkson, D.N. Jackson, I.A. Sammut, I. Appleton, Neuroprotective effects of (-)-epigallocatechin gallate following hypoxia-ischemia-induced brain damage: novel mechanisms of action, *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 19 (2005) 258–260, <http://dx.doi.org/10.1096/fj.04-2806fje>.
- [206] C.H. Mazucanti, J.V. Cabral-Costa, A.R. Vasconcelos, D.Z. Andreotti, C. Scavone, E.M. Kawamoto, Longevity pathways (mTOR, SIRT, Insulin/IGF-1) as key modulatory targets on aging and neurodegeneration, *Curr. Top. Med. Chem.* 15 (2015) 2116–2138.
- [207] B.L. Tang, Sirt1 and the Mitochondria, *Mol. Cells* 39 (2016) 87–95, <http://dx.doi.org/10.14348/molcells.2016.2318>.
- [208] W.-J. Ok, H.-J. Cho, H.-H. Kim, D.-H. Lee, H.-Y. Kang, H.-W. Kwon, M.H. Rhee, M. Kim, H.-J. Park, Epigallocatechin-3-gallate has an anti-platelet effect in a cyclic AMP-dependent manner, *J. Atheroscler. Thromb.* 19 (2012) 337–348.
- [209] X. Yin, N. Jin, J. Shi, Y. Zhang, Y. Wu, C.-X. Gong, K. Iqbal, F. Liu, Dyrk1A overexpression leads to increase of 3R-tau expression and cognitive deficits in Ts65Dn Down syndrome mice, *Sci. Rep.* 7 (2017) 619, <http://dx.doi.org/10.1038/s41598-017-00682-y>.
- [210] I.C. Burckhardt, D. Gozal, E. Dayyat, Y. Cheng, R.C. Li, A.D. Goldbart, B.W. Row, Green tea catechin polyphenols attenuate behavioral and oxidative responses to intermittent hypoxia, *Am. J. Respir. Crit. Care Med.* 177 (2008) 1135–1141, <http://dx.doi.org/10.1164/rccm.200701-1100C>.
- [211] R. de la Torre, S. de Sola, G. Hernandez, M. Farré, J. Pujol, J. Rodriguez, J.M. Espadaler, K. Langohr, A. Cuenca-Royo, A. Principe, L. Xicota, N. Janel, S. Caturana-Solarz, G. Sanchez-Benavides, H. Bléhaut, I. Dueñas-Espín, L. Del Hoyo, B. Benejam, L. Blanco-Hinojo, S. Videla, M. Fitó, J.M. Delabar, M. Dierssen, TESAD study group, safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial, *Lancet Neurol.* 15 (2016) 801–810, [http://dx.doi.org/10.1016/S1474-4422\(16\)30034-5](http://dx.doi.org/10.1016/S1474-4422(16)30034-5).
- [212] N. Latruffe, A. Lançon, R. Frazzi, V. Aires, D. Delmas, J.-J. Michaille, F. Djouadi, J. Bastin, M. Cherkaoui-Malki, Exploring new ways of regulation by resveratrol involving miRNAs, with emphasis on inflammation, *Ann. N.Y. Acad. Sci.* 1348 (2015) 97–106, <http://dx.doi.org/10.1111/nyas.12819>.
- [213] M. Stringer, I. Abeysekera, J. Thomas, J. LaCombe, K. Stancombe, R.J. Stewart, K.J. Dria, J.M. Wallace, C.R. Goodlett, R.J. Roper, Epigallocatechin-3-gallate (EGCG) consumption in the Ts65Dn model of Down syndrome fails to improve behavioral deficits and is detrimental to skeletal phenotypes, *Physiol. Behav.* 177 (2017) 230–241, <http://dx.doi.org/10.1016/j.physbeh.2017.05.003>.
- [214] P. Picone, S. Vilasi, F. Librizzi, M. Contardi, D. Nuzzo, L. Caruana, S. Baldassano, A. Amato, F. Mulè, P.L. San Biagio, D. Giacomazza, M. Di Carlo, Biological and biophysical aspects of metformin-induced effects: cortex mitochondrial dysfunction and promotion of toxic amyloid pre-fibrillar aggregates, *Aging* 8 (2016) 1718–1734, <http://dx.doi.org/10.18632/aging.101004>.
- [215] E. Barini, O. Antico, Y. Zhao, F. Asta, V. Tucci, T. Catalani, R. Marotta, H. Xu, L. Gasparini, Metformin promotes tau aggregation and exacerbates abnormal behavior in a mouse model of tauopathy, *Mol. Neurodegener.* 11 (2016) 16, <http://dx.doi.org/10.1186/s13024-016-0082-7>.
- [216] L. Tian, J. Busciglio, Mitochondrial dysfunction and Down's syndrome: is there a role for coenzyme Q(10)? *BioFactors* 37 (2011) 386–392, <http://dx.doi.org/10.1002/biof.184>.
- [217] J. Muchová, I. Žitňanová, Z. Ďuračková, Oxidative stress and Down syndrome. Do antioxidants play a role in therapy? *Physiol. Res.* 63 (2014) 535–542.
- [218] M.J. Acosta, L. Vazquez Fonseca, M.A. Desbats, C. Cerqua, R. Zordan, E. Trevisson, L. Salvati, Coenzyme Q biosynthesis in health and disease, *Biochim. Biophys. Acta* 1857 (2016) 1079–1085, <http://dx.doi.org/10.1016/j.bbabi.2016.03.036>.
- [219] X. Yang, Y. Zhang, H. Xu, X. Luo, J. Yu, J. Liu, R.C.-C. Chang, Neuroprotection of coenzyme Q10 in neurodegenerative diseases, *Curr. Top. Med. Chem.* 16 (2016) 858–866.
- [220] M.E. Zaki, H.T. El-Bassyouni, A.M.S. Tosson, E. Youness, J. Hussein, Coenzyme Q10 and pro-inflammatory markers in children with Down syndrome: clinical and biochemical aspects, *J. Pediatr.* 93 (2017) 100–104, <http://dx.doi.org/10.1016/j.jpeds.2016.04.012>.
- [221] L. Tian, L. Padella, L. Santoro, P. Carnevali, F. Principi, F. Brugé, O. Gabrielli, G.P. Littarru, Prolonged coenzyme Q10 treatment in Down syndrome patients: effect on DNA oxidation, *Neurobiol. Aging* 33 (2012) e1–e8, <http://dx.doi.org/10.1016/j.neurobiolaging.2011.03.025> (626).
- [222] M. Shichiri, Y. Yoshida, N. Ishida, Y. Hagihara, H. Iwahashi, H. Tamai, E. Niki,  $\alpha$ -Tocopherol suppresses lipid peroxidation and behavioral and cognitive impairments in the Ts65Dn mouse model of Down syndrome, *Free Radic. Biol. Med.* 50 (2011) 1801–1811, <http://dx.doi.org/10.1016/j.freeradbiomed.2011.03.023>.
- [223] I.T. Lott, Antioxidants in Down syndrome, *Biochim. Biophys. Acta* 1822 (2012) 657–663, <http://dx.doi.org/10.1016/j.bbdis.2011.12.010>.
- [224] J. Uberos, J. Romero, A. Molina-Carballo, A. Muñoz-Hoyos, Melatonin and elimination of kynurenines in children with Down's syndrome, *J. Pediatr. Endocrinol. Metab. JPEM* 23 (2010) 277–282.

- [225] E.B. Parisotto, V. Vidal, S. García-Cerro, S. Lantigua, D. Wilhelm Filho, E.J. Sanchez-Barceló, C. Martínez-Cué, N. Rueda, Chronic melatonin administration reduced oxidative damage and cellular senescence in the hippocampus of a mouse model of Down syndrome, *Neurochem. Res.* 41 (2016) 2904–2913, <http://dx.doi.org/10.1007/s11064-016-2008-8>.
- [226] A. Corrales, P. Martínez, S. García, V. Vidal, E. García, J. Flórez, E.J. Sanchez-Barceló, C. Martínez-Cué, N. Rueda, Long-term oral administration of melatonin improves spatial learning and memory and protects against cholinergic degeneration in middle-aged Ts65Dn mice, a model of Down syndrome, *J. Pineal Res.* 54 (2013) 346–358, <http://dx.doi.org/10.1111/jpi.12037>.
- [227] V. Simion, W.D. Nadim, H. Benedetti, C. Pichon, S. Morisset-Lopez, P. Baril, Pharmacomodulation of microRNA expression in neurocognitive diseases: obstacles and future opportunities, *Curr. Neuropharmacol.* 15 (2017) 276–290.
- [228] B.H. Natelson, Brain dysfunction as one cause of CFS symptoms including difficulty with attention and concentration, *Front. Physiol.* 4 (2013), <http://dx.doi.org/10.3389/fphys.2013.00109>.
- [229] F. Stagni, A. Giacomini, S. Guidi, E. Ciani, R. Bartesaghi, Timing of therapies for Down syndrome: the sooner, the better, *Front. Behav. Neurosci.* 9 (2015) 265, <http://dx.doi.org/10.3389/fnbeh.2015.00265>.
- [230] F. Stagni, A. Giacomini, M. Emili, S. Trazzi, S. Guidi, M. Sassi, E. Ciani, R. Rimondini, R. Bartesaghi, Short- and long-term effects of neonatal pharmacotherapy with epigallocatechin-3-gallate on hippocampal development in the Ts65Dn mouse model of Down syndrome, *Neuroscience* 333 (2016) 277–301, <http://dx.doi.org/10.1016/j.neuroscience.2016.07.031>.
- [231] G. Deidda, M. Parrini, S. Naskar, I.F. Bozarth, A. Contestabile, L. Cancedda, Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome, *Nat. Med.* 21 (2015) 318–326, <http://dx.doi.org/10.1038/nm.3827>.