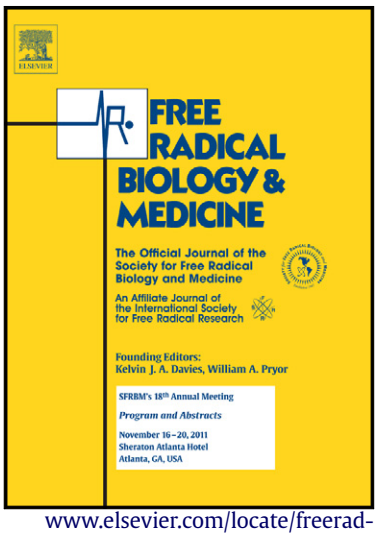

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Exploring the possible link between MeCP2 and oxidative stress in Rett Syndrome

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Abstract

Rett syndrome (RTT, MIM 312750), is a rare and orphan progressive neurodevelopmental disorder affecting almost exclusively the female gender with a frequency of 1:15,000 live births. The disease is characterized by a period of 6 to 18 months of apparently normal neurodevelopment, followed by an early neurological regression, with a progressive loss of acquired cognitive, social, and motor skills. RTT is known to be caused in the 95% of the cases by sporadic de novo loss-of-function mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene encoding methyl-CpG binding protein 2 (MeCP2), a nuclear protein able to regulates gene expression. Despite almost two decades of research into the functions and role of MeCP2, little is known about the mechanisms leading from *MECP2* mutation to the disease.

Oxidative stress (OS) is involved in the pathogenic mechanisms of several neurodevelopmental and neurodegenerative disorders although in many cases it is not clear whether OS is a cause of a consequence of the pathology. Fairly recently it has been demonstrated the presence of a systemic OS also in RTT patients with the strong correlation with the patients clinical status. At today it is not clear the link between *MECP2* mutation and the redox imbalance found in RTT. Animal studies have anyway suggested a possible direct correlation between Mecp2 mutation and increased OS levels. In addition, the restoration of Mecp2 function in astrocytes significantly improves the developmental outcome of *Mecp2*-null mice and re-expression of *Mecp2* gene in the brain of null mice rescued oxidative damage, suggesting that Mecp2 loss-of-function can be involved in oxidative brain damage.

Starting from the evidences that oxidative damage in the brain of *Mecp2*-null mice precedes the onset of symptoms, we evaluated whether, based on the current literature, the dysfunctions described in RTT could be a consequence or, in contrast, are caused by OS. We also analyzed if the therapies, that at least partially rescued some RTT symptoms, can have a role in the defense against OS. At this stage we can propose that OS could be one of the main causes of the dysfunctions observed in RTT. In addition, it should be mentioned that the major part of the therapies

recommended to alleviate RTT symptoms have been shown to interfere with oxidative homeostasis, suggesting that MeCP2 could be somehow involved in the protection of the brain from OS.

Keywords: Rett syndrome; neurodevelopmental disorder; methyl-CpG-binding protein 2; oxidative stress; brain damage; brain derived neurotrophic factor; 4-hydroxy-2-nonenal; mitochondria.

Rett Syndrome and Redox imbalance

Rett syndrome (RTT) is a progressive neurodevelopmenal disorder that affects girls almost exclusively with an incidence of 1 in 15,000 [1,2]. First recognized by the Viennese pediatrician Andreas Rett in 1966 [3], the disorder is characterized by apparently normal development for the first 6–18 months of age followed by a stagnation and then rapid regression of acquired language and motor abilities [1,4]. Typical or classical RTT progresses through four stages and its distinctive features are the loss of purposeful hand movements, replaced by repetitive and stereotypic hand movements, microcephaly, seizures, ataxia, autistic-like traits, cardiac abnormalities, breathing irregularities such as hyperventilation and apnoeas [4].

Pathogenic mutations in X-linked "methyl-CpG-binding protein 2" gene (*MECP2*) account for up to 95% of typical and approximately 75% of atypical RTT cases [5-8]. MeCP2 is a multifunctional protein that is involved in transcriptional regulation as well as modulating chromatin structure and RNA splicing [9-11].

MeCP2 is widely expressed in many tissues, but with higher expression in the brain, where it is specifically detected in neurons [12]. More recently, MeCP2 expression has also been proven in astrocytes, oligodendrocytes and microglia [13-15]. At present, more than 700 *MECP2* mutations have been identified [16, 17], among which the eight most common lead to a wide phenotypic variability and different degrees of disease severity [18].

Effectively, the spectrum of RTT phenotypes is quite broad and, in addition to *MECP2*-related classical form, includes also other variant or "atypical" RTT, which have been found to cluster in some distinct clinical groupings, such as preserved speech variant, early seizure variant, and congenital variant [19, 20]. These forms may be either milder or more severe than classical RTT. The preserved speech variant, the so-called Zappella variant, is a benign form of RTT characterized by the ability of patients to retain some speech and the ability to walk [21]. While mutations responsible for most severe forms have been identified in cyclin-dependent kinase-like 5 (*CDKL5*) and forkhead box G1 (*FOXG1*) genes and associated with an early-onset seizure variant (Hanefeld

variant) and congenital form (Rolando variant), respectively [22, 23]. Despite the characterization of the underlying genetic mutations and continuing progress into the knowledge of MeCP2 functions, the molecular mechanisms leading from defective protein to the disease expression remain still not fully understood.

New insights could arise from the growing evidence of a potential relationships between OS and RTT [24-27, 32-38].

OS is a well-known factor implicated in pathogenesis and progression of several human diseases, such as cardiovascular diseases, cancer, diabetes mellitus, asthma, neurological diseases and ageing [39, 40]. By definition, OS occurs when the antioxidant response is insufficient to balance the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to a disruption of redox signalling and control and/or molecular damage and developing or worsening of several pathologies [41].

Within a given limit, the low or moderate production of oxidants is a physiological process, since they are formed in normal aerobic metabolism and act with beneficial effects on cellular redox signaling and immune function [42]. Usually, exposure of cells to free radicals induces the activation of cytoprotective signaling pathways in order to promote cell survival and to maintain the optimal redox balance. A variety of antioxidant enzymes functions as scavengers of ROS and these include, among others, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin (TRX), and the peroxiredoxin (PRDX). In addition, there is also a non-enzymatic system represented by low-molecular-weight compounds, including reduced glutathione (GSH), vitamins C and E, β-carotene, lipoid acid, melatonin, uric acid, bilirubin and metal-chelating proteins [43].

Over time, if not properly managed by these cellular detoxification mechanisms, the continual exposure of cells to ROS/RNS results to be cytotoxic. In fact, at high levels reactive species disrupt cell structures and functions, causing membrane lipid peroxidation, protein oxidation, DNA damage [43].

The brain is particularly prone to ROS and RNS production and highly susceptible towards redox stress, because of its high lipid and metal ions content coupled with its high metabolic rate and relatively low concentrations of antioxidants. Several lines of evidence have established that OS is a causative or at least pivotal factor for a large number of brain illnesses, including Parkinson's, Huntington's, Alzheimer's, prions, Down's syndrome, ataxia, multiple sclerosis, amyotrophic lateral sclerosis, schizophrenia, depression and epilepsy [39].

Since the end of 80's, a potential relationship between OS and RTT has been explored, first in humans and more recently in animal models, and generally found to be related to either increased OS and/or altered antioxidant defenses.

The first report indicating an impaired antioxidant defense in RTT dates back to 1987. In a post-mortem study where Sofić et al. reported a severe reduction of vitamin C and GSH in the brain of a RTT patients [24]. Serum vitamin E deficiency and decreased erythrocyte SOD activity also indicate low antioxidant protection and higher vulnerability to oxidative damage in RTT [25, 26]. Moreover, the decline in GSH levels is detected in plasma, erythrocytes and skin fibroblasts from RTT patients [30, 34, 35]. More recently, similar findings have been shown also from our group in a microarray study [44]. To cope the redox imbalance, a probable feedback mechanism in RTT implies the compensatory up-regulation of a subset of gene related to antioxidant cellular defense systems, including SOD, CAT, PRDX1, glutathione transferases (GST) and enzymes involved in the detoxification of lipid peroxidation products [44]. Likewise, our previous PBMC gene expression analysis confirmed the overexpression of transcripts for CAT and GST found in human RTT brain [45].

In line with the changes in antioxidant defense systems, an increase in OS biomarkers has also been observed in RTT. Blood samples from RTT patients reveal increased levels of plasma and intraerythrocyte non-protein-bound iron (NPBI), plasma protein carbonyls, plasma and membrane erythrocyte 4-hydroxynonenal protein adducts (4HNE-PA), plasma malondialdehyde, and Isoprostanes [26-29, 31-33, 35], indicating the strong evidence of systemic oxidative damage to

lipids and proteins. Of note, levels of above cited OS markers change as a function of the time and of phenotype severity, namely clinical stage and different clinical form of disorder [28, 31-33].

The critical role of OS in RTT is also supported by the evidence that exogenous administration of ω-3 polyunsaturated fatty acids significantly decreases the levels of NPBI, Isoprostanes and 4HNE-PA, partially rescues the altered erythrocyte morphology observed in RTT and reduces at the same time the clinical severity of the disorder [29, 31, 33, 35, 46]. Therefore, it is conceivable that chronic OS in RTT might underlie at least some of the typical symptoms and contributes to disease progression.

The cellular redox imbalance observed in RTT patients [34, 47], can also negatively affects not only cell functions but also systemic metabolic pathways. For instance, oxidative post-translational modifications of Scavenger Receptor B1 (SRB1) by 4HNE, a known lipid peroxidation byproduct, are accompanied by a dramatic reduction of the receptor levels [47]. Given SRB1 crucial role in the uptake of HDL-derived cholesterol and cholesteryl ester in the liver and other tissue [48], SRB1 oxidative damage seems to contribute to altered cholesterol metabolism [49] and plasma lipid profile of RTT patients with an imbalance in both high density lipoprotein (HDL) and low density lipoprotein (LDL) levels [47, 50].

Rett syndrome as a possible mitochondriopathy

Beyond the known function in ATP production, mitochondria represent the major source of free radicals in the cell environment, thereby the harmful structural and/or functional changes in these organelles are usually correlated to redox imbalance in a variety of pathological conditions [51]. Indeed, there are now several lines of evidence indicate that mitochondria are involved in RTT. In fact, it has been reported that RTT shares many common clinical features with mitochondrial diseases, including hypotonia, delayed development or regression of previously acquired skills, and seizures. In addition, a metabolic profile showing abnormal cerebrospinal fluid lactate and pyruvate

levels led to hypothesize a mitochondrial impairment in RTT [52]. The possible role of mitochondria in RTT has been already suggested in works from 30 years ago, where several morphological alterations of mitochondria have been repeatedly reported both in humans and mouse models [37, 53-58]. In this regard, electron microscopy studies revealed abnormally swollen and dumb-bell shaped mitochondria with vacuolization, granular inclusions and membranous changes in muscle and frontal lobe biopsies of RTT patients [53-56]. In other papers, ultrastructural changes were confirmed and resulted also associated with abnormalities in mitochondrial respiratory chain enzymes and therefore to energy metabolism impairment [59, 60]. Recently, we have reported the overexpression of genes related to mitochondrial biology in peripheral blood mononuclear cells isolated from RTT patients, that can be indicative of anomalous cellular energy requirement [44]. Besides in humans, mitochondrial abnormalities have been also confirmed in brain and muscle mitochondria from *Mecp2*-mutant mice [36, 37, 58, 61].

In 2006, Kriaucionis et al. reported that *Uqcrc1* gene, which encodes a subunit of respiratory complex III, is a target of MecP2 [62]. In the case of Mecp2 KO animal, there is an increased expression of *Uqcrc1* gene that leads to elevated mitochondria respiration rates, associated with the respiratory complex III, and an overall reduction in coupling. Therefore, there is an increased O_2 consumption and ROS production in brain mitochondria of *Mecp2*-null mice [62]. Subsequently, a magnetic resonance study confirmed the impairment of mitochondrial respiration and the decrease of ATP levels in brain of *MeCP2*-deficient mice [63]. As consequence of mitochondrial dysfunctions, Groβer et al. demonstrated an increased oxidative burden and a more vulnerable brain cells to oxidative damage in in *Mecp2*$^{-/y}$ mouse hippocampus [36].

Evidence of the defective mitochondria and decrease in GSH levels has been also reported in the skeletal muscle of *Mecp2*^{tm1Tam} mouse model of RTT [37]. In this study the authors suggested that the detected mitochondrial alterations could be a contributing factor to the progressive deterioration in RTT mobility through the accumulation of free radicals.

In line with previous reports, an aberrant function of complex II of the mitochondrial respiratory chain coupled to hydrogen peroxide overproduction has been also observed in brain of MeCP2-308 heterozygous female mice [38]. Indeed, as a proof of concept, the recovery of complex II activity prevented ROS overproduction [38]. Due to the absence of neurodegenerative aspects in RTT, it is likely that such mitochondrial defects are not sufficient to induce cell death, however they can affect and potentially worsen the neuronal functions also through an oxidative imbalance, thus contributing to the clinical manifestations and progression of RTT.

Due to the main role of mitochondria in energy production, any structural and/or functional alteration of these organelles in RTT could lead to the energy metabolism defects. Effectively, this appears consistent with data showing a reduction of ATP levels in brain of several RTT animal models [38, 63].

It is well known that impairment in mitochondrial respiration elicits an enhanced production of free radical species in several human pathological conditions [64, 65]. Thus, the role of mitochondrial dysfunctions seems to be well linked to RTT and it is possible that a chronic mitochondrial deregulation may contribute to the systemic OS fund in RTT. It is then possible to speculate that, since mitochondrial modifications are already present at presymptomatic stage of the disease, the altered mitochondria functions play a direct role in the development of the pathology [36].

RTT animal models as useful tool to study the disease

To better understand the possible link that might associate MecP2 mutations with increased OS levels in RTT, several researchers have focused on RTT mouse models. Indeed, over the last few decades, a number of mouse models have been developed with the attempt to better understand the molecular mechanisms involved in RTT pathogenesis as well as to develop new therapeutic strategies.

Despite the fact that murine models cannot display all aspects of the human RTT, they are anyway good experimental tool to understand the cellular pathways involved in RTT, thanks also to the fact that RTT animal models are able to recapitulate many of the clinical key features observed in human patients [66].

Several different strategies have been able to modify or abolish the Mecp2 expression and/or function, generating a wide range of mouse models of the disease: *Mecp2* knockout mice; *Mecp2* mutant mice expressing a truncated protein; cell-type specific *Mecp2* deletions or mutations; mice expressing reduced levels of *Mecp2*; mice overexpressing wildtype full-length MeCP2; knock-in mice carrying RTT-associated *MECP2* mutations [67-69]. Up to now, the multiple RTT mouse models have permitted to identified and characterized some of the molecular pathways that appear altered in this disorder, leading also to test some therapeutic strategies in mice.

Furthermore, the availability of these models has also given the opportunity to explore the hypothesis of the OS involvement in the pathophysiology of disorder, and to evaluate a possible causative role of OS in RTT etiopathogenesis.

In line with our previous findings, which showed high circulating levels of OS markers in RTT patients [27-29, 31-33, 35] suggesting a possible role of the redox imbalance in RTT pathology, a recent study from our lab provided clear evidence that Mecp2 deficiency is associated with oxidative brain damage in several *Mecp2* mutant mouse models [30]. In fact, the levels of different OS markers, including NPBI, Isoprostanes (F_2-isoprostanes, F_4-neuroprostanes, F_2-dihomo-isoprostanes) and 4HNE-PA, have been found increased in whole brains of two different mouse models of RTT, *Mecp2*-null and *Mecp2*-308 animals. In particular, in both experimental models the brain oxidative damage precedes the clinical manifestations of the disorder; this imply the existence of a temporal window in which an early OS-modulating therapy could reduce/limit phenotype severity. Interestingly, we observed a full rescue of the brain redox homeostasis following brain specific *Mecp2* gene reactivation in *Mecp2* stop/y NestinCre animals [30]. This finding means that the OS imbalance can be a reversible phenomenon by the restoration of a correct Mecp2 function,

thereby suggesting that Mecp2 might to be involved in the protection of the brain from OS [30]. In addition the above mentioned study support the idea that OS is also involved in the clinical feature of the pathology since there is a clear correlation between increased OS markers and the development of RTT symptoms.

The availability of a wide spectrum of RTT experimental models, either cells or animals, have been instrumental not only for the study of the disorder and MeCP2 functions, but also has stimulated the research aimed to develop effective treatments for RTT.

In this respect it appears of crucial importance the encouraging findings obtained in experimental models, indicating that the condition is not irreversible and the rescue of the RTT-like phenotypes is possible.

In fact, in last decade independent research groups have shown that some RTT-like symptoms are reversible in experimental models following reactivation of silent Mecp2 alleles [70-73] or transgene-mediated Mecp2 replacement [74-80], even at late stages of disease progression.

Link between Mecp2 and OS

To date, the exact molecular mechanisms linking *MECP2* mutations to the subsequent OS derangement are still unidentified although there are several reports that have suggested a possible connection between altered MeCP2 functions and cellular redox imbalance. In a recent exome sequencing study has been identified variants in genes related to OS in classical RTT patients within two sisters with the same *MECP2* mutation, but different phenotype (classical versus Zappella variant) [81]. This finding suggests that multiple variants associated with a specific *MECP2* mutation can have different phenotypic effect in this disorder. In particular, the different susceptibility to OS may modulate some clinical manifestations and explain the expressed phenotype variability found among patients [81]. In addition, it is well known that RTT patients suffer from highly irregular breathing coupled to abnormal pulmonary gas exchanges and reduced

arterial oxygen levels, all symptoms related to *MECP2* mutation [27, 82-84]. Therefore, the chronic intermittent hypoxia associated to these irregularities might be able to explain, at least partly, the redox imbalance observed in RTT [27, 85].

In support of this theory, a number of convincing evidences have highlighted a redox homeostasis dysregulation in RTT mice, mainly linked to mitochondrial abnormalities [36-38, 58, 61].

In addition it has been reported that MeCP2 is capable of controlling the expression of several genes that seem play a role in redox balance, such as brain derived neurotrophic factor (BDNF) and proline dehydrogenase (Prodh) [86, 87], although MeCP2-dependent regulation of BDNF gene transcription is very complex and context-dependent [88], making difficult to clearly extrapolate the direct effect of MeCP2 on BDNF transcription. Reports using either RTT mouse models or RTT patients convey that BDNF dysfunction is a hallmark of disease pathology and, interestingly, the enhancement of BDNF signaling in animal models reverses and improves the pathological manifestations of RTT, including respiratory dysfunctions [89-91]. In this regard, new evidences indicate that BDNF is able to protect circulating angiogenic cells by increasing expression of manganese superoxide dismutase (MnSOD), thereby enhancing cellular redox defensive system [92]. Moreover, the relationship between BDNF levels and OS parameters is also confirmed in other mental disorders [93].

In addition, a recent paper demonstrated a functional interplay between MeCP2 and SIRT1 in the regulation of BDNF expression [94]. SIRT1 is a crucial cellular survival protein that acts as NAD-dependent histone deacetylase and is also involved in defending cells from OS, eliciting also neuroprotective effects through its anti-oxidative and anti-inflammatory functions [95, 96].

An other target gene of MeCP2 is *Prodh*, whose expression has been found to be up-regulated in the brain of a *Mecp2*-null (KO) mouse model [86]. *Prodh* encodes for the proline dehydrogenase (ProDH), a mitochondrial inner-membrane flavoenzyme that catalyzes the oxidation of proline, leading to the release of electrons, that can be transferred to either electron transfer systems or to molecular oxygen [97]. ProDH is not only essential for proline catabolism but also plays key roles

in providing energy, shuttling redox potential between cellular compartments and ROS production. In addition, this amino acid has a physiologically protective role against OS. Thereby, the oxidation by ProDH not only contributes to lower the amount of proline in the cell, hence abolishing its protective function, but can also generate ROS [97].

In a recent work, by the use of transcription factor protein arrays, Leoh et al. identified MeCP2 as a interacting partner of LEDGF/p75 (lens epithelium-derived growth factor p75) and its short splice variant LEDGF/p52 [98]. LEDGF/p75 is a transcription coactivator and a stress survival protein that protects against OS-induced cellular damage and death [99]. In particular, LEDGF/p75 promote cell survival under stress by transcriptionally activating genes encoding protective proteins such as heat shock protein 27 (Hsp27), αB-crystallin, peroxiredoxin 6 (PRDX6), and vascular endothelial growth factor c (VEGF-c) [100-102]. Presumably, LEDGF/p75 transactivates these stress genes by binding to heat shock elements (HSE) and stress elements (STRE) in their promoter regions [100-102]. These results suggest a key role of MeCP2 in cellular redox homeostasis by being able to regulate the expression of stress survival genes.

There is a growing interest in the involvement of OS in the epigenetic regulation of gene expression and specifically in controlling DNA methylation status [103]. ROS production is associated with increased DNA damage and alterations of both hypermethylation and hypomethylation of the DNA [104], also changing the DNA methyltransferase expression [105]. MeCP2 binds to both methylated and unmethylated DNA, but it associates preferentially with methylated regions [106], therefore chronic accumulation of ROS might play an important role in the disrupting the MeCP2 regulation of epigenetic gene expression. In this regards, the first evidence of these mechanisms dates back in 2004, when Valinluck et al. reported that the MeCP2-binding to methylated CpG sites is inhibited by OS. In particular, the selective modification of guanine to 8-hydroxyguanine at CpG sites reduce the binding affinity of MeCP2 to its target sequences by at least an order of magnitude [107]. Because the initial binding of the MeCP2 to methylated CpGs is a critical event in the chromatin remodeling and thus in epigenetic regulation of gene activity, the oxidative damage to DNA can

interfere with subsequent steps in the chromatin condensation cascade, resulting in potentially heritable epigenetic alterations and disregulation of transcriptional activity [107]. This report demonstrates that MeCP2 can be both, the starting point and one of the several end targets of free radical mediated processes.

All these observations corroborate the concept of a possible direct relationship between MeCP2 deficit and cellular redox imbalance [Figure 1].

Finally, it is worth it mentioning that the previously cited study of exome on RTT revealed the occurrence of multiple gene variations associated with the main *MECP2* mutation, some of which affect genes related to OS [81]. Therefore, it is possible that redox imbalance in RTT might not be exclusively dependent on MeCP2 dysfunction, but rather by a combination of numerous factors.

MeCP2 dysfunction and therapies under the oxidative stress light

Several therapeutic approaches have been tested in RTT with the main propose to either modify the effect of *MECP2* mutation or to ameliorate the oxidative damage present in RTT. Herein there is an overview of the possible targets that have shown to alleviate RTT clinical features.

BDNF

Brain-derived neurotrophic factor (BDNF) has been shown to have an essential role in neuronal survival and synaptic plasticity. In RTT patients, analyzing autopsy brain samples low levels of BDNF expression were detected [90]. In Mecp2 mutant mice a low overall levels of BDNF protein was also reported [89, 108, 109]. Moreover, also BDNF intracellular trafficking may been disrupted indeed, in RTT patients carrying BDNF Val66Met polymorphism known to impede BDNF maturation process, severity and course of neuropsychiatric symptoms were found correlated [110].

The interplay between OS and BDNF has been described not only in different pathologies but also in processes like aging and in different life styles.

The antioxidant effect of calorie restriction has been reported to protect against cognitive decline via up regulation of BDNF. Indeed, high fat diet increases OS and decreases BDNF therefore modulating synaptic plasticity and cognitive functions [111].

The effect of BDNF on synaptic plasticity and cognition, caused by high in saturated fat diet, can be prevented by supplementation with an antioxidant [112, 113]

In aged rats, antioxidant treatment attenuates age-induced cognitive impairment modulating the BDNF levels [114]. Moreover, neuronal death caused by stroke is believed to involve OS. Calorie restriction has been described not only to prevent but also to reduce damages induced by stroke. Indeed, fasting after a moderate stroke injury results in a lower OS and induction of neurotrophic factors such as BDNF [115].

A growing amount of data have been published indicating that decreased level of BDNF and increased OS have a role also in the pathophysiology of different neurological and neurodegenerative disorders. Moreover, several evidences suggested that antioxidant increase the levels of BDNF triggering a neuroprotective effect.

Dendritic spine

In RTT it has been described a dendritic spine dysgenesis, more particular, a lower spine density and an atypical morphology resulting in a decreased proportion of mushroom-type spines have been observed in the cortex and hippocampus [116-118]. Mouse models of RTT recapitulate the abnormalities observed in human and in particular show impaired dendritic complexity [119, 120], lower dendritic spine density [121-124] and lack of mushroom-type spines both in cortical and hippocampal neurons [125, 126].

It seems important to mention that ROS produce spine pathology. Indeed, spine loss was observed in rat hippocampal CA1 pyramidal neurons after induction of OS induced by exposure to ozone [127]. Similar in rat prefrontal cortex, striatum, and olfactory bulb, ozone induced spine loss with vacuolation of dendrites and spines [128, 129].

Omega 3

Starting from the evidence of enhanced OS and lipid peroxidation in RTT patients, the effect of tω-3 polyunsaturated fatty acids (ω-3 PUFAs) supplementation in RTT has been studied. Indeed, ω-3 PUFAs have been shown to have multiple beneficial effects, able also to indirectly ameliorate the cellular redox unbalance in several pathologies..

The ω-3 PUFAs oil supplemented RTT patients show a significant reduction in the clinical severity of the symptoms (in particular, motor-related signs, nonverbal communication deficits, and breathing abnormalities) and significant decrease in all the examined OS markers.

These findings suggest that a dietary intervention at an early stage in RTT could lead to a partial clinical and biochemical rescue.

Statins

Cholesterol homeostasis has a central role in numerous pathologies including neurodegenerative diseases, indeed cholestrol is involved in pathways related to neuron function and survival.

Homeostasis of cholesterol is perturbed in Mecp2 null mice; indeed, in the brain and body system, it has been detected an elevated synthesis of cholesterol. Statin drugs , chemically mimicking the genetic effect of downregulating the cholesterol pathway improved systemic perturbations of lipid metabolism, alleviate motor symptoms and confer increased longevity in Mecp2 mutant mice [49, 130].

Recent studies suggests that statins, HMG-CoA reductase inhibitors, have neuroprotective/antioxidant properties [131]. Indeed, statins, independently of their effects on biosynthesis of cholesterol, can act as free-radical scavengers [132-134]. *In vivo* studies in dogs it has been demonstrated that high-dose atorvastatin, increasing the ratio of GSH to reduced GSH in the brain, reduced markers of oxidative and nitrosative stress (i.e., protein carbonyls, 4-hydroxy-2-nonenal, 3-nitrotyrosine). These data suggested that this drug act as neuroprotectant and CNS antioxidant [135]. Moreover, it has been shown that atorvastatin ameliorates cerebral vasospasm, in

an *in vivo* model of subarachnoid hemorrhage in rat, attenuating neuronal apoptosis through inibition of brain caspase-3 activity and DNA fragmentation [136, 137]. Furthermore, in rat cerebrocortical neuronal cultures, rosuvastatin protected neurons from stress induced by oxygen-glucose deprivation and the mechanism of neuroprotection involved the decreasing of ROS levels [138]. Similarly, simvastatin decreased neuronal death induced by oxygen-glucose deprivation and subsequent reoxygenation by inhibiting production of 4-hydroxy-2E-nonenal (4HNE), a cytotoxic product of lipid peroxidation, and at least in part by maintaining the activity of NF-kappaB [139]. In addition clinical investigations indicated that statin treatment reduced cerebral expression of OS markers (i.e., nitrotyrosine and F_2-isoprostanes) [140, 141].

These data suggest that statins can act in RTT not only stabilizing the homeostasis of cholesterol but also acting as neuroprotective against OS.

Insulin-like growth factor-1

Insulin-like growth factor-1 (IGF-1) molecule is FDA approved for the treatment of growth failure in children. Currently IGF-1 is studied in an early-stage clinical trial to determine the side-effect profile and disease improvements in RTT (ClinicalTrials.gov identifier: NCT01253317).

IGF-1 penetrate the blood–brain barrier and is able to stimulate proliferation of neural progenitors, neuronal survival, neurite outgrowth and synapse formation [142].

The rational of this trial is based on preclinical trials, done in Mecp2 mutant mice, using a tripeptide fragment of IGF-1 which improved motor function, dendritic spine density and motility, and breathing rhythm [121, 123].

Moreover, *in vitro* IGF-1 also increased synapse number in neurons differentiated from induced pleuripotential stem cells reprogrammed from skin fibroblasts of RTT patients [143].

It has been proposed that IGF-1 mediate neuroprotection challenging with OS. Indeed, IGF-1-mediated neuroprotection, in neuronal cells, acts through induction of NF-kB that promotes activation of phosphoinositol (PI) 3-kinase.

Valproic acid

Occurrence of seizure disorder have been reported in approximately 85% of RTT individuals during their lifetimes. Valproic acid (VPA) is an old generation anti-epileptic drug that has been used as monoterapy to treat epilepsy in RTT patients. Although, one study found a significant effect in controlling seizures [144], another study did not demonstrate any beneficial effect [145].

Recently, an analysis in mouse model of RTT suggested that Valproic acid could alleviate RTT neurological symptoms [146].

It has been suggested that oxidative damage may play a role both in initiation and progression of epilepsy. Therefore ameliorating tissue damage, reducing OS, could favorably alter the clinical course of the disease [147].

Valproic acid treatment has been associated with increases formation of ROS. Although, the majority of OS measurements in patients with epilepsy treated with VPA have been performed on peripheral tissues, such as plasma, serum or red blood cells [145, 148-154]. However, not necessarily OS originated from various sources in the body reflects the OS in the CNS acid. Indeed, VPA was shown to be protective against OS in neuronal cells in both *in vitro* and *in vivo* models of epilepsy. It was suggested that VPA can produce neuroprotective effects inhibiting lipid peroxidation and protein oxidation [155] and increasing the level of GSH [156].

Lamotrigine

Lamotrigine, a phenyltriazine derivative, is second generation antiepileptic drug that blocks voltage-sensitive sodium channels inhibiting the release of excitatory amino acids [157].

An open pilot study to evaluate the effect of Lamotrigine in RTT patients, demonstrated that girls respond well in terms of seizure frequency and behavioral issues.

Lamotrigine has neuroprotective proprieties especially in neurodegenerative diseases. Recently, it has been reported that Lamotrigine treatment may cause an increase in levels of the antioxidants

GSH, GSH-R, SOD and CAT. Suggesting that Lamotregine may produce beneficial neuroprotective effects reducing oxidative– nitrosative stress and enhancing protective antioxidants [158].

Trolox

In RTT patients several alterations of mitochondrial function including decreased levels of succinate cytochrome c reductase and cytochrome c oxidase, a proton leak across the inner mitochondrial membrane, and a reduced respiratory capacity have been observed [60, 62, 159, 160]. Moreover, lowered blood serum levels of vitamin E and reduced activity of the ROS-detoxifying enzyme SOD are also evident [26].

Lower serum levels of vitamin E were detected in patients affected by of RTT syndrome suggesting that the oxidative free radical metabolism may be impaired in this disorder [25].

The deficiencies in cellular ROS-scavenging capabilities combined with impaired mitochondrial function could easily explain OS damages observed in RTT patients and in RTT mouse models [26, 27, 30].

Starting from the hypothesis that these observed changes may contribute to the manifestation of symptoms and disease progression, it was analyzed whether free radical scavengers are capable of improving neuronal and mitochondrial function in hippocampal slices of adult MeCP2-/Y mice. In particular, the effect of vitamin E derivative Trolox was evaluated. Accordingly with the hypothesis, it was observed that Trolox dampens neuronal hyperexcitability, reinstates synaptic plasticity, and improves the hypoxia tolerance in a mouse model of RTT [61].

Curcumin

RTT patients present circulatory problems that become increasingly severe in adulthood.

Studying the relation between peripheral microcirculation and the loss of MeCP2, in MeCP2 knockout mouse model Panighini et al. (2013) highlighted that, in these mice, vessels showed a reduced endothelium-dependent relaxation, due to a reduced Nitric Oxide (NO) availability

secondary to an increased ROS generation. Moreover, they observed an intravascular increase in superoxide anion production, and a decreased vascular eNOS expression. It has also been shown that cucumin administration restored endothelial NO availability, decreased intravascular ROS production and normalized vascular eNOS gene expression. Moreover, pathological stereotyped movements that are one of the characteristics of the pathological animals, were partially reversed by curcumin administration [161].

Conclusion

Despite MeCP2 was identified more that twenty years ago, and more that 700 mutations that link MeCP2 to RTT have been identified, the molecular mechanisms leading from defective protein to the disease expression remain still not fully understood. OS is considered a relevant process associated to neurological disorders and the relation between OS and RTT have been described in both humans and in mouse models. Recently it has been re-evaluated the possible role that mitochondria might have in the development of the pathology thanks to the fact that it is one the primary endogenous source of ROS and also to the abnormalities observed in the mitochondria of blood mononuclear cells, brain and muscle of both RTT patients and mouse models.

In brain of Mecp2-null mice OS damages precede the onset of symptoms. Therefore, dysfunctions observed in RTT patients and mouse model, like low levels of BDNF, dendritic spine dysgenesis or lipid peroxidation that have been described in different systems to be caused by OS, can be consequence of the OS produced in RTT by mitochondrial abnormalities.

Several key features of RTT suggest that the disorder could be at least partially treatable in humans. First, as above mentioned, even if RTT is a severe brain pathology, no obvious signs of neuronal and glial degeneration appear in patients and nor in animal models [162-164].

Second, typically the RTT clinical symptoms manifest months after birth, therefore exist a time window in which it is possible to intervene therapeutically.

Although there is not an official cure for RTT patients, several treatments that have been used aimed to ameliorate, et least partially, the clinical symptoms of the disease. As reported above, many of them were able in reducing at least some of the multiple symptoms related to RTT, and unexpectedly, all these treatments have also been described to act as "antioxidant" or to be neuroprotective towards oxidative damage.

Our analysis suggests that MeCP2 having a crucial role in chromatin dynamics, can act as modulator of genes essential in antioxidant and radical scavengers pathways. Moreover, epigenetic drugs that have also antioxidant properties can be promising compounds for the treatment of neuronal dysfunction in Rett syndrome.

Of note it is that any cure aimed to contrast MecP2 dysfunction should be applied in the first month after birth or even earlier to avoid the evolution of the clinical symptoms present in RTT (Scheme 1).

Taken together, these aspects leave the hope to achieve through an appropriate pharmacological therapy or genetic intervention a significant improvement of the RTT conditions if not to reverse the disease in humans.

References

[1] Chahrour, M. and Zoghbi, H.Y. The story of Rett syndrome: from clinic to neurobiology. *Neuron*. **56**:422-437; 2007.

[2] Laurvick, C.L.; de Klerk, N.; Bower, C.; Christodoulou, J.; Ravine, D.; Ellaway, C.; Williamson, S. and Leonard, H. Rett syndrome in Australia: a review of the epidemiology. *J Pediatr*. **148**:347-352; 2006.

[3] Rett, A. [On a unusual brain atrophy syndrome in hyperammonemia in childhood]. *Wien Med Wochenschr*. **116**:723-726; 1966.

[4] Hagberg, B. Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev*. **8**:61-65; 2002.

[5] Amir, R.E.; Van den Veyver, I.B.; Wan, M.; Tran, C.Q.; Francke, U. and Zoghbi, H.Y. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet*. **23**:185-188; 1999.

[6] Bienvenu, T.; Carrie, A.; de Roux, N.; Vinet, M.C.; Jonveaux, P.; Couvert, P.; Villard, L.; Arzimanoglou, A.; Beldjord, C.; Fontes, M.; Tardieu, M. and Chelly, J. MECP2 mutations account for most cases of typical forms of Rett syndrome. *Hum Mol Genet*. **9**:1377-1384; 2000.

[7] Percy, A.K.; Lane, J.B.; Childers, J.; Skinner, S.; Annese, F.; Barrish, J.; Caeg, E.; Glaze, D.G. and MacLeod, P. Rett syndrome: North American database. *J Child Neurol*. **22**:1338-1341; 2007.

[8] Neul, J.L.; Fang, P.; Barrish, J.; Lane, J.; Caeg, E.B.; Smith, E.O.; Zoghbi, H.; Percy, A. and Glaze, D.G. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. *Neurology*. **70**:1313-1321; 2008.

[9] Georgel, P.T.; Horowitz-Scherer, R.A.; Adkins, N.; Woodcock, C.L.; Wade, P.A. and Hansen, J.C. Chromatin compaction by human MeCP2. Assembly of novel secondary

chromatin structures in the absence of DNA methylation. *J Biol Chem*. **278**:32181-32188; 2003.

[10] Young, J.I.; Hong, E.P.; Castle, J.C.; Crespo-Barreto, J.; Bowman, A.B.; Rose, M.F.; Kang, D.; Richman, R.; Johnson, J.M.; Berget, S. and Zoghbi, H.Y. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *Proc Natl Acad Sci U S A*. **102**:17551-17558; 2005.

[11] Chahrour, M.; Jung, S.Y.; Shaw, C.; Zhou, X.; Wong, S.T.; Qin, J. and Zoghbi, H.Y. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science*. **320**:1224-1229; 2008.

[12] Skene, P.J.; Illingworth, R.S.; Webb, S.; Kerr, A.R.; James, K.D.; Turner, D.J.; Andrews, R. and Bird, A.P. Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. *Mol Cell*. **37**:457-468; 2010.

[13] Ballas, N.; Lioy, D.T.; Grunseich, C. and Mandel, G. Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. *Nat Neurosci*. **12**:311-317; 2009.

[14] Maezawa, I. and Jin, L.W. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J Neurosci*. **30**:5346-5356; 2010.

[15] Zachariah, R.M.; Olson, C.O.; Ezeonwuka, C. and Rastegar, M. Novel MeCP2 isoform-specific antibody reveals the endogenous MeCP2E1 expression in murine brain, primary neurons and astrocytes. *PLoS One*. **7**:e49763; 2012.

[16] Christodoulou, J.; Grimm, A.; Maher, T. and Bennetts, B. RettBASE: The IRSA MECP2 variation database-a new mutation database in evolution. *Hum Mutat*. **21**:466-472; 2003.

[17] http://mecp2.chw.edu.au/

[18] Amir, R.E. and Zoghbi, H.Y. Rett syndrome: methyl-CpG-binding protein 2 mutations and phenotype-genotype correlations. *Am J Med Genet*. **97**:147-152; 2000.

[19] Hagberg, B.A. and Skjeldal, O.H. Rett variants: a suggested model for inclusion criteria. *Pediatr Neurol*. **11**:5-11; 1994.

[20] Neul, J.L.; Kaufmann, W.E.; Glaze, D.G.; Christodoulou, J.; Clarke, A.J.; Bahi-Buisson, N.; Leonard, H.; Bailey, M.E.; Schanen, N.C.; Zappella, M.; Renieri, A.; Huppke, P. and Percy, A.K. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol*. **68**:944-950; 2010.

[21] Zappella, M.; Meloni, I.; Longo, I.; Hayek, G. and Renieri, A. Preserved speech variants of the Rett syndrome: molecular and clinical analysis. *Am J Med Genet*. **104**:14-22; 2001.

[22] Ariani, F.; Hayek, G.; Rondinella, D.; Artuso, R.; Mencarelli, M.A.; Spanhol-Rosseto, A.; Pollazzon, M.; Buoni, S.; Spiga, O.; Ricciardi, S.; Meloni, I.; Longo, I.; Mari, F.; Broccoli, V.; Zappella, M. and Renieri, A. FOXG1 is responsible for the congenital variant of Rett syndrome. *Am J Hum Genet*. **83**:89-93; 2008.

[23] Scala, E.; Ariani, F.; Mari, F.; Caselli, R.; Pescucci, C.; Longo, I.; Meloni, I.; Giachino, D.; Bruttini, M.; Hayek, G.; Zappella, M. and Renieri, A. CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. *J Med Genet*. **42**:103-107; 2005.

[24] Sofić, E.; Riederer, P.; Killian, W. and Rett, A. Reduced concentrations of ascorbic acid and glutathione in a single case of Rett syndrome: a postmortem brain study. *Brain Dev*. **9**:529-531; 1987.

[25] Formichi, P.; Battisti, C.; Dotti, M.T.; Hayek, G.; Zappella, M. and Federico, A. Vitamin E serum levels in Rett syndrome. *J Neurol Sci*. **156**:227-230; 1998.

[26] Sierra, C.; Vilaseca, M.A.; Brandi, N.; Artuch, R.; Mira, A.; Nieto, M. and Pineda, M. Oxidative stress in Rett syndrome. *Brain Dev*. **23 Suppl 1**:S236-239; 2001.

[27] De Felice, C.; Ciccoli, L.; Leoncini, S.; Signorini, C.; Rossi, M.; Vannuccini, L.; Guazzi, G.; Latini, G.; Comporti, M.; Valacchi, G. and Hayek, J. Systemic oxidative stress in classic Rett syndrome. *Free Radic Biol Med*. **47**:440-448; 2009.

[28] De Felice, C.; Signorini, C.; Durand, T.; Oger, C.; Guy, A.; Bultel-Ponce, V.; Galano, J.M.; Ciccoli, L.; Leoncini, S.; D'Esposito, M.; Filosa, S.; Pecorelli, A.; Valacchi, G. and Hayek, J.

F2-dihomo-isoprostanes as potential early biomarkers of lipid oxidative damage in Rett syndrome. *J Lipid Res*. **52**:2287-2297; 2011.

[29] De Felice, C.; Signorini, C.; Durand, T.; Ciccoli, L.; Leoncini, S.; D'Esposito, M.; Filosa, S.; Oger, C.; Guy, A.; Bultel-Ponce, V.; Galano, J.M.; Pecorelli, A.; De Felice, L.; Valacchi, G. and Hayek, J. Partial rescue of Rett syndrome by omega-3 polyunsaturated fatty acids (PUFAs) oil. *Genes Nutr*. **7**:447-458; 2012.

[30] De Felice, C.; Della Ragione, F.; Signorini, C.; Leoncini, S.; Pecorelli, A.; Ciccoli, L.; Scalabri, F.; Marracino, F.; Madonna, M.; Belmonte, G.; Ricceri, L.; De Filippis, B.; Laviola, G.; Valacchi, G.; Durand, T.; Galano, J.M.; Oger, C.; Guy, A.; Bultel-Ponce, V.; Guy, J.; Filosa, S.; Hayek, J. and D'Esposito, M. Oxidative brain damage in Mecp2-mutant murine models of Rett syndrome. *Neurobiol Dis*. **68**:66-77; 2014.

[31] Leoncini, S.; De Felice, C.; Signorini, C.; Pecorelli, A.; Durand, T.; Valacchi, G.; Ciccoli, L. and Hayek, J. Oxidative stress in Rett syndrome: natural history, genotype, and variants. *Redox Rep*. **16**:145-153; 2011.

[32] Pecorelli, A.; Ciccoli, L.; Signorini, C.; Leoncini, S.; Giardini, A.; D'Esposito, M.; Filosa, S.; Hayek, J.; De Felice, C. and Valacchi, G. Increased levels of 4HNE-protein plasma adducts in Rett syndrome. *Clin Biochem*. **44**:368-371; 2011.

[33] Signorini, C.; De Felice, C.; Leoncini, S.; Giardini, A.; D'Esposito, M.; Filosa, S.; Della Ragione, F.; Rossi, M.; Pecorelli, A.; Valacchi, G.; Ciccoli, L. and Hayek, J. F(4)-neuroprostanes mediate neurological severity in Rett syndrome. *Clin Chim Acta*. **412**:1399-1406; 2011.

[34] Signorini, C.; Leoncini, S.; De Felice, C.; Pecorelli, A.; Meloni, I.; Ariani, F.; Mari, F.; Amabile, S.; Paccagnini, E.; Gentile, M.; Belmonte, G.; Zollo, G.; Valacchi, G.; Durand, T.; Galano, J.M.; Ciccoli, L.; Renieri, A. and Hayek, J. Redox imbalance and morphological changes in skin fibroblasts in typical Rett syndrome. *Oxid Med Cell Longev*. **2014**:195935; 2014.

[35] Ciccoli, L.; De Felice, C.; Paccagnini, E.; Leoncini, S.; Pecorelli, A.; Signorini, C.; Belmonte, G.; Valacchi, G.; Rossi, M. and Hayek, J. Morphological changes and oxidative damage in Rett Syndrome erythrocytes. *Biochim Biophys Acta*. **1820**:511-520; 2012.

[36] Groβer, E.; Hirt, U.; Janc, O.A.; Menzfeld, C.; Fischer, M.; Kempkes, B.; Vogelgesang, S.; Manzke, T.U.; Opitz, L.; Salinas-Riester, G. and Muller, M. Oxidative burden and mitochondrial dysfunction in a mouse model of Rett syndrome. *Neurobiol Dis*. **48**:102-114; 2012.

[37] Gold, W.A.; Williamson, S.L.; Kaur, S.; Hargreaves, I.P.; Land, J.M.; Pelka, G.J.; Tam, P.P. and Christodoulou, J. Mitochondrial dysfunction in the skeletal muscle of a mouse model of Rett syndrome (RTT): implications for the disease phenotype. *Mitochondrion*. **15**:10-17; 2014.

[38] De Filippis, B.; Valenti, D.; de Bari, L.; De Rasmo, D.; Musto, M.; Fabbri, A.; Ricceri, L.; Fiorentini, C.; Laviola, G. and Vacca, R.A. Mitochondrial free radicals overproduction due to respiratory chain impairment in brain of a mouse model of Rett syndrome. Protective effect of CNF1. *Free Radic Biol Med* 2015; doi:10.1016/j.freeradbiomed.2015.02.014

[39] Thanan, R.; Oikawa, S.; Hiraku, Y.; Ohnishi, S.; Ma, N.; Pinlaor, S.; Yongvanit, P.; Kawanishi, S. and Murata, M. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *Int J Mol Sci*. **16**:193-217; 2014.

[40] Heistad, D.D.; Wakisaka, Y.; Miller, J.; Chu, Y. and Pena-Silva, R. Novel aspects of oxidative stress in cardiovascular diseases. *Circ J*. **73**:201-207; 2009.

[41] Sies, H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* **4C**:180-183; 2015.

[42] Finkel, T. Reactive oxygen species and signal transduction. *IUBMB Life*. **52**:3-6; 2001.

[43] Halliwell, B. and Gutteridge, J.M.C.: *Free radicals in biology and medicine,* 4th eds. New York: Oxford University Press Inc.; 2007.

[44] Pecorelli, A.; Leoni, G.; Cervellati, F.; Canali, R.; Signorini, C.; Leoncini, S.; Cortelazzo, A.; De Felice, C.; Ciccoli, L.; Hayek, J. and Valacchi, G. Genes related to mitochondrial functions, protein degradation, and chromatin folding are differentially expressed in lymphomonocytes of Rett syndrome patients. *Mediators Inflamm*. **2013**:137629; 2013.

[45] Colantuoni, C.; Jeon, O.H.; Hyder, K.; Chenchik, A.; Khimani, A.H.; Narayanan, V.; Hoffman, E.P.; Kaufmann, W.E.; Naidu, S. and Pevsner, J. Gene expression profiling in postmortem Rett Syndrome brain: differential gene expression and patient classification. *Neurobiol Dis*. **8**:847-865; 2001.

[46] Maffei, S.; De Felice, C.; Cannarile, P.; Leoncini, S.; Signorini, C.; Pecorelli, A.; Montomoli, B.; Lunghetti, S.; Ciccoli, L.; Durand, T.; Favilli, R. and Hayek, J. Effects of omega-3 PUFAs supplementation on myocardial function and oxidative stress markers in typical Rett syndrome. *Mediators Inflamm*. **2014**:983178; 2014.

[47] Sticozzi, C.; Belmonte, G.; Pecorelli, A.; Cervellati, F.; Leoncini, S.; Signorini, C.; Ciccoli, L.; De Felice, C.; Hayek, J. and Valacchi, G. Scavenger receptor B1 post-translational modifications in Rett syndrome. *FEBS Lett*. **587**:2199-2204; 2013.

[48] Valacchi, G.; Sticozzi, C.; Lim, Y. and Pecorelli, A. Scavenger receptor class B type I: a multifunctional receptor. *Ann N Y Acad Sci*. **1229**:E1-7; 2011.

[49] Buchovecky, C.M.; Turley, S.D.; Brown, H.M.; Kyle, S.M.; McDonald, J.G.; Liu, B.; Pieper, A.A.; Huang, W.; Katz, D.M.; Russell, D.W.; Shendure, J. and Justice, M.J. A suppressor screen in Mecp2 mutant mice implicates cholesterol metabolism in Rett syndrome. *Nat Genet*. **45**:1013-1020; 2013.

[50] Segatto, M.; Trapani, L.; Di Tunno, I.; Sticozzi, C.; Valacchi, G.; Hayek, J. and Pallottini, V. Cholesterol metabolism is altered in Rett syndrome: a study on plasma and primary cultured fibroblasts derived from patients. *PLoS One*. **9**:e104834; 2014.

[51] Albers, D.S. and Beal, M.F. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease. *J Neural Transm Suppl*. **59**:133-154; 2000.

[52] Matsuishi, T.; Urabe, F.; Percy, A.K.; Komori, H.; Yamashita, Y.; Schultz, R.S.; Ohtani, Y.; Kuriya, N. and Kato, H. Abnormal carbohydrate metabolism in cerebrospinal fluid in Rett syndrome. *J Child Neurol*. **9**:26-30; 1994.

[53] Eeg-Olofsson, O.; al-Zuhair, A.G.; Teebi, A.S. and al-Essa, M.M. Abnormal mitochondria in the Rett syndrome. *Brain Dev*. **10**:260-262; 1988.

[54] Eeg-Olofsson, O.; al-Zuhair, A.G.; Teebi, A.S.; Daoud, A.S.; Zaki, M.; Besisso, M.S. and Al-Essa, M.M. Rett syndrome: a mitochondrial disease? *J Child Neurol*. **5**:210-214; 1990.

[55] Ruch, A.; Kurczynski, T.W. and Velasco, M.E. Mitochondrial alterations in Rett syndrome. *Pediatr Neurol*. **5**:320-323; 1989.

[56] Wakai, S.; Kameda, K.; Ishikawa, Y.; Miyamoto, S.; Nagaoka, M.; Okabe, M.; Minami, R. and Tachi, N. Rett syndrome: findings suggesting axonopathy and mitochondrial abnormalities. *Pediatr Neurol*. **6**:339-343; 1990.

[57] Cardaioli, E.; Dotti, M.T.; Hayek, G.; Zappella, M. and Federico, A. Studies on mitochondrial pathogenesis of Rett syndrome: ultrastructural data from skin and muscle biopsies and mutational analysis at mtDNA nucleotides 10463 and 2835. *J Submicrosc Cytol Pathol*. **31**:301-304; 1999.

[58] Belichenko, P.V.; Wright, E.E.; Belichenko, N.P.; Masliah, E.; Li, H.H.; Mobley, W.C. and Francke, U. Widespread changes in dendritic and axonal morphology in Mecp2-mutant mouse models of Rett syndrome: evidence for disruption of neuronal networks. *J Comp Neurol*. **514**:240-258; 2009.

[59] Dotti, M.T.; Manneschi, L.; Malandrini, A.; De Stefano, N.; Caznerale, F. and Federico, A. Mitochondrial dysfunction in Rett syndrome. An ultrastructural and biochemical study. *Brain Dev*. **15**:103-106; 1993.

[60] Coker, S.B. and Melnyk, A.R. Rett syndrome and mitochondrial enzyme deficiencies. *J Child Neurol*. **6**:164-166; 1991.

[61] Janc, O.A. and Muller, M. The free radical scavenger Trolox dampens neuronal hyperexcitability, reinstates synaptic plasticity, and improves hypoxia tolerance in a mouse model of Rett syndrome. *Front Cell Neurosci*. **8**:56; 2014.

[62] Kriaucionis, S.; Paterson, A.; Curtis, J.; Guy, J.; Macleod, N. and Bird, A. Gene expression analysis exposes mitochondrial abnormalities in a mouse model of Rett syndrome. *Mol Cell Biol*. **26**:5033-5042; 2006.

[63] Saywell, V.; Viola, A.; Confort-Gouny, S.; Le Fur, Y.; Villard, L. and Cozzone, P.J. Brain magnetic resonance study of Mecp2 deletion effects on anatomy and metabolism. *Biochem Biophys Res Commun*. **340**:776-783; 2006.

[64] Raha, S. and Robinson, B.H. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci*. **25**:502-508; 2000.

[65] Valenti, D.; de Bari, L. ; De Filippis, B.; Henrion-Caude, A.; Vacca, R.A. 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**:202-217; 2014.

[66] Ricceri, L.; De Filippis, B. and Laviola, G. Mouse models of Rett syndrome: from behavioural phenotyping to preclinical evaluation of new therapeutic approaches. *Behav Pharmacol*. **19**:501-517; 2008.

[67] Katz, D.M.; Berger-Sweeney, J.E.; Eubanks, J.H.; Justice, M.J.; Neul, J.L.; Pozzo-Miller, L.; Blue, M.E.; Christian, D.; Crawley, J.N.; Giustetto, M.; Guy, J.; Howell, C.J.; Kron, M.; Nelson, S.B.; Samaco, R.C.; Schaevitz, L.R.; St Hillaire-Clarke, C.; Young, J.L.; Zoghbi, H.Y. and Mamounas, L.A. Preclinical research in Rett syndrome: setting the foundation for translational success. *Dis Model Mech*. **5**:733-745; 2012.

[68] Calfa, G.; Percy, A.K. and Pozzo-Miller, L. Experimental models of Rett syndrome based on Mecp2 dysfunction. *Exp Biol Med (Maywood)*. **236**:3-19; 2011.

[69] Ezeonwuka, C.D. and Rastegar, M. MeCP2-Related Diseases and Animal Models. *Diseases*. **2**:45-70; 2014.

[70] Guy, J.; Gan, J.; Selfridge, J.; Cobb, S. and Bird, A. Reversal of neurological defects in a mouse model of Rett syndrome. *Science*. **315**:1143-1147; 2007.

[71] Lioy, D.T.; Wu, W.W. and Bissonnette, J.M. Autonomic dysfunction with mutations in the gene that encodes methyl-CpG-binding protein 2: insights into Rett syndrome. *Auton Neurosci*. **161**:55-62; 2011.

[72] Robinson, L.; Guy, J.; McKay, L.; Brockett, E.; Spike, R.C.; Selfridge, J.; De Sousa, D.; Merusi, C.; Riedel, G.; Bird, A. and Cobb, S.R. Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. *Brain*. **135**:2699-2710; 2012.

[73] Lang, M.; Wither, R.G.; Colic, S.; Wu, C.; Monnier, P.P.; Bardakjian, B.L.; Zhang, L. and Eubanks, J.H. Rescue of behavioral and EEG deficits in male and female Mecp2-deficient mice by delayed Mecp2 gene reactivation. *Hum Mol Genet*. **23**:303-318; 2014.

[74] Alvarez-Saavedra, M.; Saez, M.A.; Kang, D.; Zoghbi, H.Y. and Young, J.I. Cell-specific expression of wild-type MeCP2 in mouse models of Rett syndrome yields insight about pathogenesis. *Hum Mol Genet*. **16**:2315-2325; 2007.

[75] Collins, A.L.; Levenson, J.M.; Vilaythong, A.P.; Richman, R.; Armstrong, D.L.; Noebels, J.L.; David Sweatt, J. and Zoghbi, H.Y. Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. *Hum Mol Genet*. **13**:2679-2689; 2004.

[76] Giacometti, E.; Luikenhuis, S.; Beard, C. and Jaenisch, R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *Proc Natl Acad Sci U S A*. **104**:1931-1936; 2007.

[77] Jugloff, D.G.; Vandamme, K.; Logan, R.; Visanji, N.P.; Brotchie, J.M. and Eubanks, J.H. Targeted delivery of an Mecp2 transgene to forebrain neurons improves the behavior of female Mecp2-deficient mice. *Hum Mol Genet*. **17**:1386-1396; 2008.

[78] Luikenhuis, S.; Giacometti, E.; Beard, C.F. and Jaenisch, R. Expression of MeCP2 in postmitotic neurons rescues Rett syndrome in mice. *Proc Natl Acad Sci U S A*. **101**:6033-6038; 2004.

[79] Pitcher, M.R.; Herrera, J.A.; Buffington, S.A.; Kochukov, M.Y.; Merritt, J.K.; Fisher, A.R.; Schanen, N.C.; Costa-Mattioli, M. and Neul, J.L. Rett syndrome like phenotypes in the R255X Mecp2 mutant mouse are rescued by MECP2 transgene. *Hum Mol Genet.* **24**:2662-2672; 2015.

[80] Garg, S.K.; Lioy, D.T.; Cheval, H.; McGann, J.C.; Bissonnette, J.M.; Murtha, M.J.; Foust, K.D.; Kaspar, B.K.; Bird, A. and Mandel, G. Systemic delivery of MeCP2 rescues behavioral and cellular deficits in female mouse models of Rett syndrome. *J Neurosci*. **33**:13612-13620; 2013.

[81] Grillo, E.; Lo Rizzo, C.; Bianciardi, L.; Bizzarri, V.; Baldassarri, M.; Spiga, O.; Furini, S.; De Felice, C.; Signorini, C.; Leoncini, S.; Pecorelli, A.; Ciccoli, L.; Mencarelli, M.A.; Hayek, J.; Meloni, I.; Ariani, F.; Mari, F. and Renieri, A. Revealing the complexity of a monogenic disease: rett syndrome exome sequencing. *PLoS One*. **8**:e56599; 2013.

[82] Julu, P.O.; Kerr, A.M.; Apartopoulos, F.; Al-Rawas, S.; Engerstrom, I.W.; Engerstrom, L.; Jamal, G.A. and Hansen, S. Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. *Arch Dis Child*. **85**:29-37; 2001.

[83] Stettner, G.M.; Huppke, P.; Gartner, J.; Richter, D.W. and Dutschmann, M. Disturbances of breathing in Rett syndrome: results from patients and animal models. *Adv Exp Med Biol*. **605**:503-507; 2008.

[84] Katz, D.M.; Dutschmann, M.; Ramirez, J.M. and Hilaire, G. Breathing disorders in Rett syndrome: progressive neurochemical dysfunction in the respiratory network after birth. *Respir Physiol Neurobiol*. **168**:101-108; 2009.

[85] Kulkarni, A.C.; Kuppusamy, P. and Parinandi, N. Oxygen, the lead actor in the pathophysiologic drama: enactment of the trinity of normoxia, hypoxia, and hyperoxia in disease and therapy. *Antioxid Redox Signal*. **9**:1717-1730; 2007.

[86] Urdinguio, R.G.; Lopez-Serra, L.; Lopez-Nieva, P.; Alaminos, M.; Diaz-Uriarte, R.; Fernandez, A.F. and Esteller, M. Mecp2-null mice provide new neuronal targets for Rett syndrome. *PLoS One*. **3**:e3669; 2008.

[87] Katz, D.M. Brain-derived neurotrophic factor and Rett syndrome. *Handb Exp Pharmacol*. **220**:481-495; 2014.

[88] KhorshidAhmad, T.; Acosta, C.; Cortes, C.; Lakowski, T.M.; Gangadaran, S. and Namaka, M. Transcriptional Regulation of Brain-Derived Neurotrophic Factor (BDNF) by Methyl CpG Binding Protein 2 (MeCP2): a Novel Mechanism for Re-Myelination and/or Myelin Repair Involved in the Treatment of Multiple Sclerosis (MS). *Mol Neurobiol.* 2015; DOI 10.1007/s12035-014-9074-1

[89] Chang, Q.; Khare, G.; Dani, V.; Nelson, S. and Jaenisch, R. The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. *Neuron*. **49**:341-348; 2006.

[90] Deng, V.; Matagne, V.; Banine, F.; Frerking, M.; Ohliger, P.; Budden, S.; Pevsner, J.; Dissen, G.A.; Sherman, L.S. and Ojeda, S.R. FXYD1 is an MeCP2 target gene overexpressed in the brains of Rett syndrome patients and Mecp2-null mice. *Hum Mol Genet*. **16**:640-650; 2007.

[91] Kline, D.D.; Ogier, M.; Kunze, D.L. and Katz, D.M. Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice. *J Neurosci*. **30**:5303-5310; 2010.

[92] He, T. and Katusic, Z.S. Brain-derived neurotrophic factor increases expression of MnSOD in human circulating angiogenic cells. *Microvasc Res*. **83**:366-371; 2012.

[93] Zhang, X.Y.; Chen, D.C.; Tan, Y.L.; Tan, S.P.; Wang, Z.R.; Yang, F.D.; Okusaga, O.O.; Zunta-Soares, G.B. and Soares, J.C. The interplay between BDNF and oxidative stress in chronic schizophrenia. *Psychoneuroendocrinology*. **51**:201-208; 2015.

[94] Zocchi, L. and Sassone-Corsi, P. SIRT1-mediated deacetylation of MeCP2 contributes to BDNF expression. *Epigenetics*. **7**:695-700; 2012.

[95] Salminen, A.; Kaarniranta, K. and Kauppinen, A. Crosstalk between Oxidative Stress and SIRT1: Impact on the Aging Process. *Int J Mol Sci*. **14**:3834-3859; 2013.

[96] Cheng, Y.; Takeuchi, H.; Sonobe, Y.; Jin, S.; Wang, Y.; Horiuchi, H.; Parajuli, B.; Kawanokuchi, J.; Mizuno, T. and Suzumura, A. Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. *J Neuroimmunol*. **269**:38-43; 2014.

[97] Servet, C.; Ghelis, T.; Richard, L.; Zilberstein, A. and Savoure, A. Proline dehydrogenase: a key enzyme in controlling cellular homeostasis. *Front Biosci (Landmark Ed)*. **17**:607-620; 2012.

[98] Leoh, L.S.; van Heertum, B.; De Rijck, J.; Filippova, M.; Rios-Colon, L.; Basu, A.; Martinez, S.R.; Tungteakkhun, S.S.; Filippov, V.; Christ, F.; De Leon, M.; Debyser, Z. and Casiano, C.A. The stress oncoprotein LEDGF/p75 interacts with the methyl CpG binding protein MeCP2 and influences its transcriptional activity. *Mol Cancer Res*. **10**:378-391; 2012.

[99] Singh, D.P.; Ohguro, N.; Chylack, L.T., Jr. and Shinohara, T. Lens epithelium-derived growth factor: increased resistance to thermal and oxidative stresses. *Invest Ophthalmol Vis Sci*. **40**:1444-1451; 1999.

[100] Singh, D.P.; Fatma, N.; Kimura, A.; Chylack, L.T., Jr. and Shinohara, T. LEDGF binds to heat shock and stress-related element to activate the expression of stress-related genes. *Biochem Biophys Res Commun*. **283**:943-955; 2001.

[101] Fatma, N.; Singh, D.P.; Shinohara, T. and Chylack, L.T., Jr. Transcriptional regulation of the antioxidant protein 2 gene, a thiol-specific antioxidant, by lens epithelium-derived growth factor to protect cells from oxidative stress. *J Biol Chem*. **276**:48899-48907; 2001.

[102] Cohen, B.; Addadi, Y.; Sapoznik, S.; Meir, G.; Kalchenko, V.; Harmelin, A.; Ben-Dor, S. and Neeman, M. Transcriptional regulation of vascular endothelial growth factor C by

oxidative and thermal stress is mediated by lens epithelium-derived growth factor/p75. *Neoplasia*. **11**:921-933; 2009.

[103] Donkena, K.V.; Young, C.Y. and Tindall, D.J. Oxidative stress and DNA methylation in prostate cancer. *Obstet Gynecol Int*. **2010**:302051; 2010.

[104] Lim, S.O.; Gu, J.M.; Kim, M.S.; Kim, H.S.; Park, Y.N.; Park, C.K.; Cho, J.W.; Park, Y.M. and Jung, G. Epigenetic changes induced by reactive oxygen species in hepatocellular carcinoma: methylation of the E-cadherin promoter. *Gastroenterology*. **135**:2128-2140, 2140 e1-8; 2008.

[105] Campos, A.C.; Molognoni, F.; Melo, F.H.; Galdieri, L.C.; Carneiro, C.R.; D'Almeida, V.; Correa, M. and Jasiulionis, M.G. Oxidative stress modulates DNA methylation during melanocyte anchorage blockade associated with malignant transformation. *Neoplasia*. **9**:1111-1121; 2007.

[106] Hansen, J.C.; Ghosh, R.P. and Woodcock, C.L. Binding of the Rett syndrome protein, MeCP2, to methylated and unmethylated DNA and chromatin. *IUBMB Life*. **62**:732-738; 2010.

[107] Valinluck, V.; Tsai, H.H.; Rogstad, D.K.; Burdzy, A.; Bird, A. and Sowers, L.C. Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). *Nucleic Acids Res*. **32**:4100-4108; 2004.

[108] Wang, H.; Chan, S.A.; Ogier, M.; Hellard, D.; Wang, Q.; Smith, C. and Katz, D.M. Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in Mecp2 null mice. *J Neurosci*. **26**:10911-10915; 2006.

[109] Abuhatzira, L.; Makedonski, K.; Kaufman, Y.; Razin, A. and Shemer, R. MeCP2 deficiency in the brain decreases BDNF levels by REST/CoREST-mediated repression and increases TRKB production. *Epigenetics*. **2**:214-222; 2007.

[110] Zeev, B.B.; Bebbington, A.; Ho, G.; Leonard, H.; de Klerk, N.; Gak, E.; Vecsler, M. and Christodoulou, J. The common BDNF polymorphism may be a modifier of disease severity in Rett syndrome. *Neurology*. **72**:1242-1247; 2009.

[111] Kishi, T.; Hirooka, Y.; Nagayama, T.; Isegawa, K.; Katsuki, M.; Takesue, K. and Sunagawa, K. Calorie Restriction Improves Cognitive Decline via Up-Regulation of Brain-Derived Neurotrophic Factor. *Int Heart J*. **56**:110-115; 2015.

[112] Wu, A.; Ying, Z. and Gomez-Pinilla, F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *Eur J Neurosci*. **19**:1699-1707; 2004.

[113] Liu, Y.; Fu, X.; Lan, N.; Li, S.; Zhang, J.; Wang, S.; Li, C.; Shang, Y.; Huang, T. and Zhang, L. Luteolin protects against high fat diet-induced cognitive deficits in obesity mice. *Behav Brain Res*. **267**:178-188; 2014.

[114] Belviranli, M. and Okudan, N. The effects of Ginkgo biloba extract on cognitive functions in aged female rats: The role of oxidative stress and brain-derived neurotrophic factor. *Behav Brain Res*. **278C**:453-461; 2015.

[115] Manzanero, S.; Gelderblom, M.; Magnus, T. and Arumugam, T.V. Calorie restriction and stroke. *Exp Transl Stroke Med*. **3**:8; 2011.

[116] Armstrong, D.; Dunn, J.K.; Antalffy, B. and Trivedi, R. Selective dendritic alterations in the cortex of Rett syndrome. *J Neuropathol Exp Neurol*. **54**:195-201; 1995.

[117] Belichenko, P.V.; Oldfors, A.; Hagberg, B. and Dahlstrom, A. Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents. *Neuroreport*. **5**:1509-1513; 1994.

[118] Chapleau, C.A.; Calfa, G.D.; Lane, M.C.; Albertson, A.J.; Larimore, J.L.; Kudo, S.; Armstrong, D.L.; Percy, A.K. and Pozzo-Miller, L. Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. *Neurobiol Dis*. **35**:219-233; 2009.

[119] Fukuda, T.; Itoh, M.; Ichikawa, T.; Washiyama, K. and Goto, Y. Delayed maturation of neuronal architecture and synaptogenesis in cerebral cortex of Mecp2-deficient mice. *J Neuropathol Exp Neurol*. **64**:537-544; 2005.

[120] Nguyen, M.V.; Du, F.; Felice, C.A.; Shan, X.; Nigam, A.; Mandel, G.; Robinson, J.K. and Ballas, N. MeCP2 is critical for maintaining mature neuronal networks and global brain anatomy during late stages of postnatal brain development and in the mature adult brain. *J Neurosci*. **32**:10021-10034; 2012.

[121] Tropea, D.; Giacometti, E.; Wilson, N.R.; Beard, C.; McCurry, C.; Fu, D.D.; Flannery, R.; Jaenisch, R. and Sur, M. Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. *Proc Natl Acad Sci U S A*. **106**:2029-2034; 2009.

[122] Chapleau, C.A.; Boggio, E.M.; Calfa, G.; Percy, A.K.; Giustetto, M. and Pozzo-Miller, L. Hippocampal CA1 pyramidal neurons of Mecp2 mutant mice show a dendritic spine phenotype only in the presymptomatic stage. *Neural Plast*. **2012**:976164; 2012.

[123] Landi, S.; Putignano, E.; Boggio, E.M.; Giustetto, M.; Pizzorusso, T. and Ratto, G.M. The short-time structural plasticity of dendritic spines is altered in a model of Rett syndrome. *Sci Rep*. **1**:45; 2011.

[124] Castro, J.; Garcia, R.I.; Kwok, S.; Banerjee, A.; Petravicz, J.; Woodson, J.; Mellios, N.; Tropea, D. and Sur, M. Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett Syndrome. *Proc Natl Acad Sci U S A*. **111**:9941-9946; 2014.

[125] Chao, H.T.; Zoghbi, H.Y. and Rosenmund, C. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. *Neuron*. **56**:58-65; 2007.

[126] Baj, G.; Patrizio, A.; Montalbano, A.; Sciancalepore, M. and Tongiorgi, E. Developmental and maintenance defects in Rett syndrome neurons identified by a new mouse staging system in vitro. *Front Cell Neurosci*. **8**:18; 2014.

[127] Avila-Costa, M.R.; Colin-Barenque, L.; Fortoul, T.I.; Machado-Salas, P.; Espinosa-Villanueva, J.; Rugerio-Vargas, C. and Rivas-Arancibia, S. Memory deterioration in an

oxidative stress model and its correlation with cytological changes on rat hippocampus CA1. *Neurosci Lett*. **270**:107-109; 1999.

[128] Avila-Costa, M.R.; Colin-Barenque, L.; Fortoul, T.I.; Machado-Salas, J.P.; Espinosa-Villanueva, J.; Rugerio-Vargas, C.; Borgonio, G.; Dorado, C. and Rivas-Arancibia, S. Motor impairments in an oxidative stress model and its correlation with cytological changes on rat striatum and prefrontal cortex. *Int J Neurosci*. **108**:193-200; 2001.

[129] Colin-Barenque, L.; Avila-Costa, M.R.; Fortoul, T.; Rugerio-Vargas, C.; Machado-Salas, J.P.; Espinosa-Villanueva, J. and Rivas-Arancibia, S. Morphologic alteration of the olfactory bulb after acute ozone exposure in rats. *Neurosci Lett*. **274**:1-4; 1999.

[130] Justice, M.J.; Buchovecky, C.M.; Kyle, S.M. and Djukic, A. A role for metabolism in Rett syndrome pathogenesis: New clinical findings and potential treatment targets. *Rare Dis*. **1**:e27265; 2013.

[131] Thompson, B.J. and Ronaldson, P.T. Drug delivery to the ischemic brain. *Adv Pharmacol*. **71**:165-202; 2014.

[132] Kassan, M.; Montero, M.J. and Sevilla, M.A. In vitro antioxidant activity of pravastatin provides vascular protection. *Eur J Pharmacol*. **630**:107-111; 2010.

[133] Barone, E.; Cenini, G.; Di Domenico, F.; Martin, S.; Sultana, R.; Mancuso, C.; Murphy, M.P.; Head, E. and Butterfield, D.A. Long-term high-dose atorvastatin decreases brain oxidative and nitrosative stress in a preclinical model of Alzheimer disease: a novel mechanism of action. *Pharmacol Res*. **63**:172-180; 2011.

[134] Butterfield, D.A.; Barone, E.; Di Domenico, F.; Cenini, G.; Sultana, R.; Murphy, M.P.; Mancuso, C. and Head, E. Atorvastatin treatment in a dog preclinical model of Alzheimer's disease leads to up-regulation of haem oxygenase-1 and is associated with reduced oxidative stress in brain. *Int J Neuropsychopharmacol*. **15**:981-987; 2012.

[135] Barone, E.; Mancuso, C.; Di Domenico, F.; Sultana, R.; Murphy, M.P.; Head, E. and Butterfield, D.A. Biliverdin reductase-A: a novel drug target for atorvastatin in a dog pre-clinical model of Alzheimer disease. *J Neurochem*. **120**:135-146; 2012.

[136] Cheng, G.; Wei, L.; Zhi-Dan, S.; Shi-Guang, Z. and Xiang-Zhen, L. Atorvastatin ameliorates cerebral vasospasm and early brain injury after subarachnoid hemorrhage and inhibits caspase-dependent apoptosis pathway. *BMC Neurosci*. **10**:7; 2009.

[137] Pan, H.C.; Yang, D.Y.; Ou, Y.C.; Ho, S.P.; Cheng, F.C. and Chen, C.J. Neuroprotective effect of atorvastatin in an experimental model of nerve crush injury. *Neurosurgery*. **67**:376-388; 2010.

[138] Domoki, F.; Kis, B.; Gaspar, T.; Snipes, J.A.; Parks, J.S.; Bari, F. and Busija, D.W. Rosuvastatin induces delayed preconditioning against oxygen-glucose deprivation in cultured cortical neurons. *Am J Physiol Cell Physiol*. **296**:C97-105; 2009.

[139] Lim, J.H.; Lee, J.C.; Lee, Y.H.; Choi, I.Y.; Oh, Y.K.; Kim, H.S.; Park, J.S. and Kim, W.K. Simvastatin prevents oxygen and glucose deprivation/reoxygenation-induced death of cortical neurons by reducing the production and toxicity of 4-hydroxy-2E-nonenal. *J Neurochem*. **97**:140-150; 2006.

[140] Shishehbor, M.H.; Aviles, R.J.; Brennan, M.L.; Fu, X.; Goormastic, M.; Pearce, G.L.; Gokce, N.; Keaney, J.F., Jr.; Penn, M.S.; Sprecher, D.L.; Vita, J.A. and Hazen, S.L. Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. *Jama*. **289**:1675-1680; 2003.

[141] Davignon, J.; Jacob, R.F. and Mason, R.P. The antioxidant effects of statins. *Coron Artery Dis*. **15**:251-258; 2004.

[142] D'Ercole, A.J.; Ye, P. and O'Kusky, J.R. Mutant mouse models of insulin-like growth factor actions in the central nervous system. *Neuropeptides*. **36**:209-220; 2002.

[143] Marchetto, M.C.; Carromeu, C.; Acab, A.; Yu, D.; Yeo, G.W.; Mu, Y.; Chen, G.; Gage, F.H. and Muotri, A.R. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell*. **143**:527-539; 2010.

[144] Krajnc, N.; Zupancic, N. and Orazem, J. Epilepsy treatment in Rett syndrome. *J Child Neurol*. **26**:1429-1433; 2011.

[145] Huppke, P.; Kohler, K.; Brockmann, K.; Stettner, G.M. and Gartner, J. Treatment of epilepsy in Rett syndrome. *Eur J Paediatr Neurol*. **11**:10-16; 2007.

[146] Guo, W.; Tsujimura, K.; Otsuka, I.M.; Irie, K.; Igarashi, K.; Nakashima, K. and Zhao, X. VPA alleviates neurological deficits and restores gene expression in a mouse model of Rett syndrome. *PLoS One*. **9**:e100215; 2014.

[147] Martinc, B.; Grabnar, I. and Vovk, T. The role of reactive species in epileptogenesis and influence of antiepileptic drug therapy on oxidative stress. *Curr Neuropharmacol*. **10**:328-343; 2012.

[148] Niketic, V.; Ristic, S.; Saicic, Z.S.; Spasic, M.; Buzadzic, B. and Stojkovic, M. Activities of antioxidant enzymes and formation of the glutathione adduct of hemoglobin (Hb ASSG) in epileptic patients with long-term antiepileptic therapy. *Farmaco*. **50**:811-813; 1995.

[149] Graf, W.D.; Oleinik, O.E.; Glauser, T.A.; Maertens, P.; Eder, D.N. and Pippenger, C.E. Altered antioxidant enzyme activities in children with a serious adverse experience related to valproic acid therapy. *Neuropediatrics*. **29**:195-201; 1998.

[150] Yuksel, A.; Cengiz, M.; Seven, M. and Ulutin, T. Erythrocyte glutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children with valproate and carbamazepine monotherapy. *J Basic Clin Physiol Pharmacol*. **11**:73-81; 2000.

[151] Michoulas, A.; Tong, V.; Teng, X.W.; Chang, T.K.; Abbott, F.S. and Farrell, K. Oxidative stress in children receiving valproic acid. *J Pediatr*. **149**:692-696; 2006.

[152] Schulpis, K.H.; Lazaropoulou, C.; Regoutas, S.; Karikas, G.A.; Margeli, A.; Tsakiris, S. and Papassotiriou, I. Valproic acid monotherapy induces DNA oxidative damage. *Toxicology*. **217**:228-232; 2006.

[153] Sobaniec, W.; Solowiej, E.; Kulak, W.; Bockowski, L.; Smigielska-Kuzia, J. and Artemowicz, B. Evaluation of the influence of antiepileptic therapy on antioxidant enzyme activity and lipid peroxidation in erythrocytes of children with epilepsy. *J Child Neurol*. **21**:558-562; 2006.

[154] Varoglu, A.O.; Yildirim, A.; Aygul, R.; Gundogdu, O.L. and Sahin, Y.N. Effects of valproate, carbamazepine, and levetiracetam on the antioxidant and oxidant systems in epileptic patients and their clinical importance. *Clin Neuropharmacol*. **33**:155-157; 2010.

[155] Wang, J.F.; Azzam, J.E. and Young, L.T. Valproate inhibits oxidative damage to lipid and protein in primary cultured rat cerebrocortical cells. *Neuroscience*. **116**:485-489; 2003.

[156] Cui, J.; Shao, L.; Young, L.T. and Wang, J.F. Role of glutathione in neuroprotective effects of mood stabilizing drugs lithium and valproate. *Neuroscience*. **144**:1447-1453; 2007.

[157] Tufan, K.; Oztanir, N.; Ofluoglu, E.; Ozogul, C.; Uzum, N.; Dursun, A.; Pasaoglu, H. and Pasaoglu, A. Ultrastructure protection and attenuation of lipid peroxidation after blockade of presynaptic release of glutamate by lamotrigine in experimental spinal cord injury. *Neurosurg Focus*. **25**:E6; 2008.

[158] Ozkul, A.; Sair, A.; Akyol, A.; Yenisey, C.; Dost, T. and Tataroglu, C. Effects of lithium and lamotrigine on oxidative-nitrosative stress and spatial learning deficit after global cerebral ischemia. *Neurochem Res*. **39**:853-861; 2014.

[159] Gibson, J.H.; Slobedman, B.; K, N.H.; Williamson, S.L.; Minchenko, D.; El-Osta, A.; Stern, J.L. and Christodoulou, J. Downstream targets of methyl CpG binding protein 2 and their abnormal expression in the frontal cortex of the human Rett syndrome brain. *BMC Neurosci*. **11**:53; 2010.

[160] Li, Y.; Wang, H.; Muffat, J.; Cheng, A.W.; Orlando, D.A.; Loven, J.; Kwok, S.M.; Feldman, D.A.; Bateup, H.S.; Gao, Q.; Hockemeyer, D.; Mitalipova, M.; Lewis, C.A.; Vander Heiden, M.G.; Sur, M.; Young, R.A. and Jaenisch, R. Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. *Cell Stem Cell*. **13**:446-458; 2013.

[161] Panighini, A.; Duranti, E.; Santini, F.; Maffei, M.; Pizzorusso, T.; Funel, N.; Taddei, S.; Bernardini, N.; Ippolito, C.; Virdis, A. and Costa, M. Vascular dysfunction in a mouse model of Rett syndrome and effects of curcumin treatment. *PLoS One*. **8**:e64863; 2013.

[162] Jellinger, K.; Armstrong, D.; Zoghbi, H.Y. and Percy, A.K. Neuropathology of Rett syndrome. *Acta Neuropathol*. **76**:142-158; 1988.

[163] Chen, R.Z.; Akbarian, S.; Tudor, M. and Jaenisch, R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat Genet*. **27**:327-331; 2001.

[164] Bauman, M.L.; Kemper, T.L. and Arin, D.M. Microscopic observations of the brain in Rett syndrome. *Neuropediatrics*. **26**:105-108; 1995.

Figure 1. Link between *MECP2* mutations and oxidative imbalance in RTT.

It appears that *MECP2* mutations can affect the expression of genes such as "brain derived neurotrophic factor" (BDNF) and "proline dehydrogenase" (Prodh), that can be also play a role in cellular redox defence. In addition, aberrant mitochondrial functions, in particular mitochondrial respiratory chain dysfunctions, overproduce ROS and contribute to alter the cellular redox homeostasis, increasing oxidative post-translational modifications of proteins and inducing cell dysfunctions. The cellular redox imbalance it also detectable at systemic level with the increase of OS markers, such as Isoprostanes and 4-hydroxynonenal protein adducts (4HNE-PA), and the decrease of antioxidant molecules, including vitamin E.

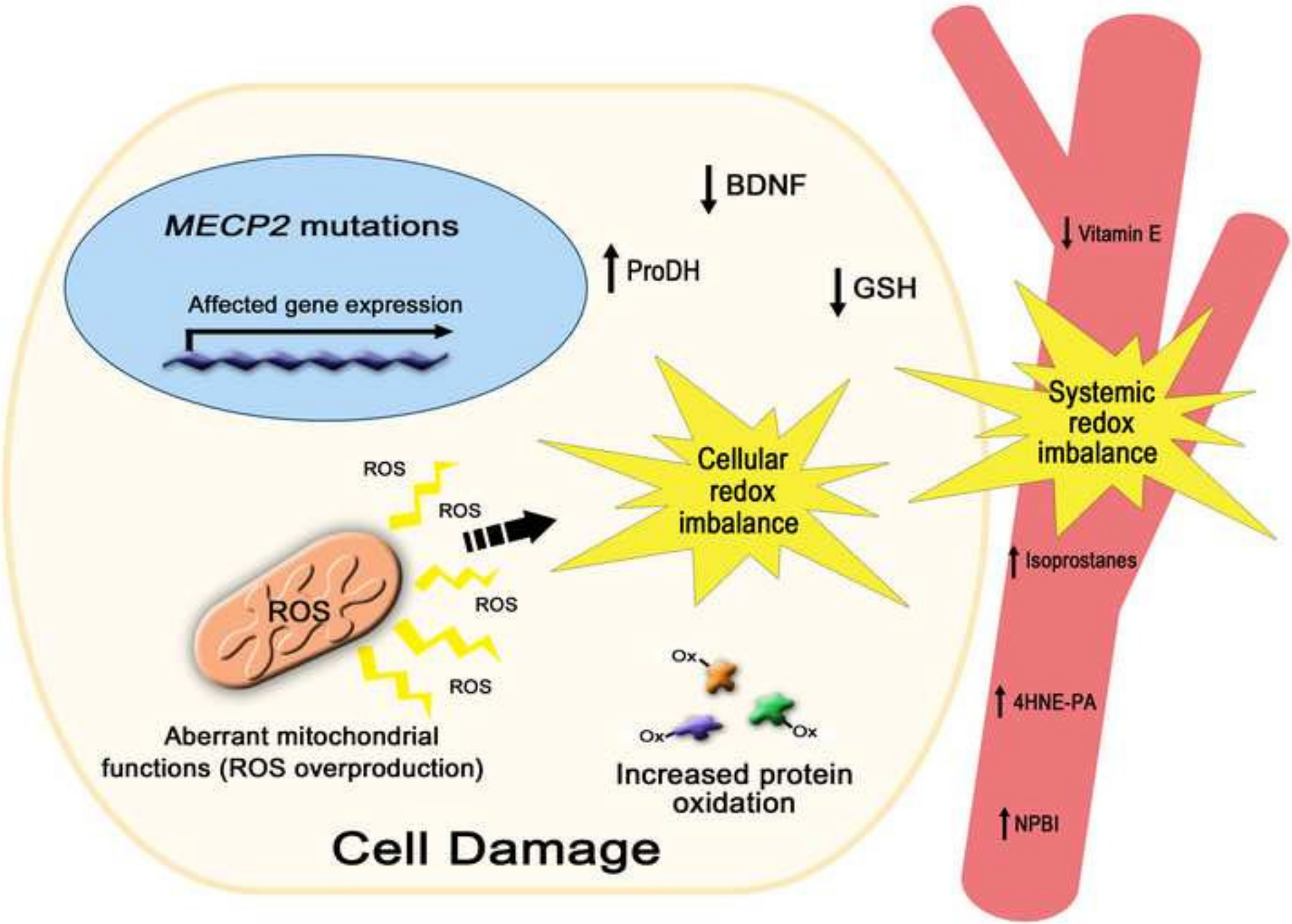
Scheme 1

- Possible mechanisms involved in MeCP2 mutation induced OS in RTT. MecP2 mutations are correlated with mitochondria respiratory chain abnormalities, altered expression of OS related genes (BDNF, Prodh, etc) contributing, therefore, to the redox imbalance present in RTT. Therapies aimed to ameliorate systemic OS as well as, gene therapy, and MecP2 manipulation, could have a beneficial effects on RTT clinical features.

Highlights

- RTT is known to be caused in the 95% of the cases by *MECP2* mutations
- At today, there are no treatments for RTT
- Systemic oxidative stress has been identified in RTT patients
- Animal models have showed a possible direct involvement of *MECP2* mutations in imbalance redox state
- Therapies aimed to ameliorate the redox imbalance can possibly improve the clinical conditions

Figure



Scheme 1