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Invited review

Rett syndrome treatment in mouse models: Searching for effective targets and strategies

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ABSTRACT

Rett syndrome (RTT) is a pervasive developmental disorder, primarily affecting girls with a prevalence of 1 in every 10,000 births; it represents the second most common cause of intellectual disability in females. Mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*) have been identified as clear etiological factors in more than 90% of classical RTT cases. Whereas the mechanisms leading to the severe, progressive and specific neurological dysfunctions when this gene is mutated still remain to be elucidated, a series of different mouse models have been generated, bearing different *Mecp2* mutation. Neurobehavioural analysis in these mouse lines have been carried out and phenotyping analysis can be now utilised to preclinically evaluate the effects of potential RTT treatments. This review summarizes the different results achieved in this research field taking into account different key targets identified to ameliorate RTT phenotype in mouse models, including those not directly downstream of MeCP2 and those limited to the early phases of postnatal development.

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1. Clinical and neurobiological aspects of the syndrome

Rett syndrome (RTT) is a pervasive developmental disorder, primarily affecting girls with a prevalence of 1 in every 10,000 births (Hagberg, 2002); it represents the second most common cause of intellectual disability in females (Hagberg et al., 1983; Rett, 1966). Mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (*MECP2*) have been identified as clear etiological factors in more than 90% of classical RTT cases (Amir et al., 1999; Chahrour and Zoghbi, 2007). A portion of RTT patients with no *MECP2* mutation has been found mutated in related genes such as *CDKL5* (Mari et al., 2005), interacting with MeCP2 and FoxG1 for the congenital form (Ariani et al., 2008). The *MECP2* gene encodes two closely related proteins which selectively bind to methylated CpGs, sequences of the DNA constituted by the repetition of the same dinucleotide (cytosine followed by guanine) and mostly located within gene promoters (Jones et al., 1998). Although initially MeCP2 was thought to act primarily as a transcriptional repressor, subsequent studies have clearly showed that MeCP2 can both activate and repress transcription (Chahrour et al., 2008) and can also globally regulate chromatin remodelling (Cohen et al., 2011). Although several MeCP2-target genes have been proposed [for an overview: (Chadwick and Wade, 2007)], and a preferential role for Mecp2 in maintaining neuronal/glial function has been recently suggested in mouse models (Derecki et al., 2012; Lioy et al., 2011; Maezawa and Jin, 2010; Maezawa et al., 2009), the mechanisms leading to the severe, progressive and specific neuronal dysfunctions when this gene is mutated still remain to be elucidated [see for a review (Guy et al., 2011)].

RTT patients undergo an apparently normal prenatal and perinatal development until about 6–18 months of age, and indeed this remains a "necessary" diagnostic criteria for RTT (Hagberg et al., 2002); however, old data in RTT patients reported subtle disturbances of tone, feeding, cry, and behaviour shortly after birth in some patients (Naidu et al., 1995) and early postnatal alterations in general movements (main patterns of spontaneous neonatal movements) have been detected in videos from family video archives (RTT patients $n = 22$) (Einspieler et al., 2005) whereas no data are available so far on RTT prenatal development. This early phase is followed by a regression period, characterized by a profound loss of acquired developmental skills in the areas of communication and hand use as well as by head growth deceleration, usually leading to microcephaly (Hagberg, 2002). At the end of this period, development reaches a plateau associated with a wide variety of specific symptoms: stereotyped hand movements, major breathing abnormalities, bloating, EEG irregularities, sleep

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problems, gait dispraxia, back deformities, feeding abnormalities as well as autistic-like behaviours (Hagberg, 2002; Mount et al., 2001). During the last part of their life, RTT patients undergo a noteworthy worsening of motor performance whereas lifespan is extremely variable (Chahrour and Zoghbi, 2007).

Despite a relatively homogeneous genetic origin, both the severity of symptoms and the progression of the disease of the patients carrying a mutation in the *MECP2* gene can be extremely variable (Erlandson and Hagberg, 2005). Different causes have been proposed to contribute to the great phenotypic variation among RTT patients. First of all, different mutations have been described to determine different phenotypes: milder phenotypes have been found to be associated with C-terminal deletions, (a type of mutation which accounts for about 10% of RTT cases) whereas early truncating mutations are responsible for more severe symptoms (Calfa et al., 2011). Moreover, X-linked genes are subjected to the X-chromosome inactivation phenomenon, where one of the two X chromosomes is randomly inactivated in every cell of the body. The RTT phenotype has been observed to vary depending on the number of cells expressing the wild type allele versus the mutated one; skewed pattern favouring the wild type allele, would result in a milder phenotype (Hoffbuhr et al., 2002). In an RTT mouse model [*Mecp2* null mice (Guy et al., 2001)] in both motor and somatosensory cortex a pronounced X chromosome inactivation favouring wt Mecp2 expression was observed (>73%) in heterozygous females (Jost et al., 2011). Degree of skewing in human RTT brains still remains a controversial issues because data are available for peripheral (blood) tissues only.

No selective cure exists for treating this devastating disorder: preclinical research, however, has been very active upon this issue in the last years: this review aims to summarize the results achieved in this research field taking into account the different key targets and strategies identified to contrast RTT in mouse models.

2. Mouse models of Rett syndrome

The discovery of a monogenic origin for classical RTT (Amir et al., 1999) initially led to the creation of two mouse lines carrying mutations in the *Mecp2* gene (Chen et al., 2001; Guy et al., 2001). Subsequently, the availability of several transgenic mouse models for RTT allowed in-depth studies on the biological basis of *Mecp2* gene functions, also because their phenotype partially recapitulate the RTT features. The Mecp2-308/y model (Shahbazian et al., 2002), bears a truncating mutation, leading to the expression of a protein truncated at aminoacid 308; this protein includes the methyl binding domain and a portion of the transcriptional repressor domain still intact. The presence of a residual protein function in this model appears associated with a milder phenotype, a delayed onset of symptoms and a prolonged life-span in comparison with knockout mice (Shahbazian et al., 2002). These latter features are in agreement with clinical data from RTT patients carrying C-terminal deletions of the MeCP2 gene (about 10% of RTT cases) who show milder phenotypes (de Leon-Guerrero et al., 2011).

The number of available models has dramatically increased in the recent years also reviewed elsewhere (Calfa et al., 2011; Guy et al., 2011; Ricceri et al., 2008) now including forebrain specific conditional MeCP2 mutant mice (Chen et al., 2001); hypothalamus – specific conditional MeCP2 knockout mice (Fyffe et al., 2008). Selectively knocking out MeCP2 in tyrosine hydroxylase (TH) positive neurons (Samaco et al., 2009) reproduced part of the RTT symptoms; a selective loss of *Mecp2* in adult mice was obtained by infusions of Cre-expressing lentiviruses in basolateral amygdala of floxed Mecp2 mice (Chen et al., 2001), a procedure leading to the knockdown of *Mecp2* to 50% of normal levels in this region and associated with anxiety and emotional changes (not motor coordination ones) (Adachi et al., 2009). More recently, lack of MeCP2 only in GABAergic neurons actually recapitulated most of the features observed in MeCP2 null mice, including altered synaptic plasticity and breathing irregularities (Chao et al., 2010), rendering this model one of the most promising not only to evaluate the role of GABAergic neurons, but more generally the pathogenetic role of disturbances of the excitation/inhibition balance in RTT.

As a whole, these mutant mice are regarded as good models for their high construct validity [i.e. the extent to which a model reproduces aetiology and pathophysiology of a human disorder (McKinney, 1984)]. Moreover, although the behavioural characterization of these mutant mice is far from complete, indications are available also suggesting noticeable face validity [i.e. the degree to which a model resembles the symptoms of the disorder (McKinney, 1984)] for these models, as *Mecp2* mutant mice have been reported to recapitulate many RTT symptoms: an accurate phenotyping of these mutant mice thus started to offer to RTT research preclinical surrogate markers to evaluate treatment efficacy (Crawley, 2007).

3. Major symptoms in RTT mouse models

Major symptoms have so far been reported in different RTT mouse models during the clearly symptomatic phase. Briefly, major symptoms are here reported according to physiological and behavioural domains.

3.1. Respiratory domain: breathing dysfunction

Respiratory impairments have been anecdotally reported for most of the mouse models. By accurate analysis of breathing patterns performed with plethysmographs, a progressive worsening of breathing disturbances has been highlighted in *Mecp2* null mice (Viemari et al., 2005). In particular, alternating periods of fast and slow respiratory frequencies as well as apneas of variable durations were reported. Breathing patterns of heterozygous females from both knockout and CNS conditional mutant models have been investigated (Bissonnette and Knopp, 2006): when studied under hypoxic conditions, both models showed initial response to hypoxia that exceeded wild type (wt); only knockout mice, however, showed signs of respiratory depression. The analysis of the breathing phenotype of *Mecp2* null mice using the perfused working hearth-brainstem preparation (Stettner et al., 2007) identified a dysfunction of the central and vagal post-inspiratory activity in this RTT mouse model.

3.2. General locomotor activity, motor and sensory domains

Severe motor deficits have been reported in *Mecp2* mutant mice. Abnormalities in grip strength, reduced motor coordination assessed by rotarod testing, and severely impaired swim performance were reported in MeCP2 Jaenisch (Stearns et al., 2007). In MeCP2 null mice, a reduced latency to fall off the rotarod (a paradigm mostly used for evaluating motor skills) at 5 weeks of age in both sexes, indicative of a motor coordination deficit has been highlighted (Santos et al., 2007). In MeCP2-308 truncation model, motor abnormalities are definitively more subtle in young animals (8–10 weeks of age) but well evident in dowel test, vertical pole test and in the rotarod test at 3 months of age (De Filippis et al., 2010; Shahbazian et al., 2002). Lower locomotor activity in an open field has also been reported (De Filippis et al., 2010; Shahbazian et al., 2002) as well as abnormalities in circadian locomotor cyclicity (marked decrease in the typical hyperactivity profile generally associated with the dark phase of the L/D cycle) (De Filippis et al., 2010; Moretti et al., 2005). Mutant male mice also