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Review

Breathing challenges in Rett Syndrome: Lessons learned from humans and animal models☆,☆☆

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ABSTRACT

Breathing disturbances are a major challenge in Rett Syndrome (RTT). These disturbances are more pronounced during wakefulness; but irregular breathing occurs also during sleep. During the day patients can exhibit alternating bouts of hypoventilation and irregular hyperventilation. But there is significant individual variability in severity, onset, duration and type of breathing disturbances. Research in mouse models of RTT suggests that different areas in the ventrolateral medulla and pons give rise to different aspects of this breathing disorder. Pre-clinical experiments in mouse models that target different neuromodulatory and neurotransmitter receptors and MeCP2 function within glia cells can partly reverse breathing abnormalities. The success in animal models raises optimism that one day it will be possible to control or potentially cure the devastating symptoms also in human patients with RTT.

1. Introduction

Rett Syndrome (RTT) affects approximately 1 in 10,000 female births (Laurvick et al., 2006; Neul et al., 2010). Children with RTT develop apparently normal until the age of 18 months. At this age, most patients with RTT achieve the normal milestones with regards to motor functions and communication skills. But subsequently girls enter a stagnation phase (Hagberg, 2005) that is followed by a developmental regression (Neul, 2012). This regression is characterized by a loss of hand skills, mobility skills, and speech, and the girls typically show stereotypic hand movements, develop ataxia, gait apraxia and often seizures. Microcephaly, growth deficits, scoliosis are also characteristic features (Weng et al., 2011a). The loss of communication skills is one of the reasons why RTT is categorized as an autism spectrum disorder (Castro et al., 2013; Neul, 2012).

Among the core symptoms of RTT, severe disturbances in breathing are particularly devastating (Glaze, 2005; Katz et al., 2009; Kerr, 1992; Ogier and Katz, 2008; Rohdin et al., 2007; Weese-Mayer et al., 2008, 2006). 65–93% of RTT patients display bouts of hypoventilation that alternate with irregular breathing or hyperventilation (Amir et al., 2000; Julu et al., 2001). But the breathing disturbances are variable and a large catalog of disturbances has been reported (Kerr, 1992). The breathing abnormalities have been categorized by various authors as periods of forced breathing, deep breathing, hyperventilation (rapid shallow breathing), hypoventilation, central and obstructive apneas, apneustic breathing, Valsalva's maneuvers, Biot's breathing, periodic breathing and breath holds (Julu et al., 2001; Weese-Mayer et al., 2008). Moreover, many of these breathing disturbances are associated with a significant dysregulation in cardio-respiratory coupling (Julu et al., 2001; Weese-Mayer et al., 2008). The complexity of the breathing phenotype in Rett Syndrome is in part explained by differences in the genotype/phenotype relationships, specifically the types of mutation and degree of X-chromosome inactivation (Amir et al., 2000).

2. The genetic basis of Rett Syndrome

Rett Syndrome is caused by a mutation in the methyl-CpG binding protein 2 (MECP2) gene (Amir et al., 1999). The genomic locus of *MECP2* in humans is approximately 80 kb and consists of 4 exons from which two different isoforms of MeCP2 may be transcribed, differing in their inclusion of the second exon. The basic structure of the *MECP2* locus and protein is conserved across species. Absence of the second intron allows for translation from the first exon and

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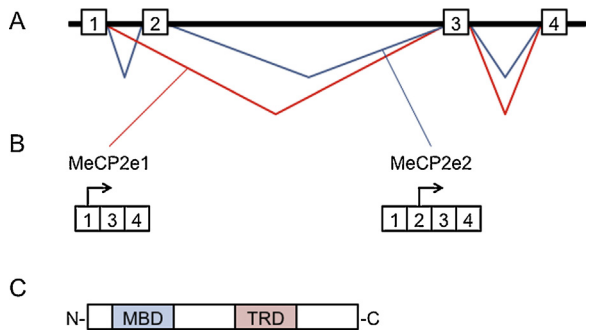

Fig. 1. Structure and organization of *MECP2*. The genomic locus of *MECP2* is spread across 76 kb and is composed of 4 exons (A). Alternative splicing generates two different isofoms of *MECP2* (B). Both isoforms possess the main functional domains of MeCP2 (C). Arrows indicate translation start sites. MBD methyl CpG binding domain. TRD transcription repression domain.

is referred to as the MeCP2e1 isoform, inclusion of the second exon stops translation products originating from the first exon and utilizes its own separate translation start site to generate the MeCP2e2 isoform (Fig. 1) (Adkins and Georgel, 2011). Thus, the two isoforms differ in their N-terminal sequences and in their expression patterns; MeCP2e1 is the predominant isoform expressed in the brain, MeCP2e2 is expressed in peripheral tissues as well as in the brain during early postnatal development before being restricted to subregions such as the dorsal hypothalamus and cortical layer V (Dragich et al., 2007).

At the protein level, MeCP2 contains two distinct functional domains, originally defined by deletion mapping and in vitro methyl-CpG-binding and transcription assays, a methyl-CpG binding domain (MBD), and a transcriptional repression domain (TRD) (Fig. 1) (Nan et al., 1993). The putative nuclear localization sequence (NLS) for MeCP2 was deletion mapped to the region containing amino acids 255–286 (Nan et al., 1996). However, despite the occurrence of these conserved domains within the protein, the structure of MeCP2 is believed to be highly disordered, a feature that may contribute to its functional interactions with chromatin (Adkins and Georgel, 2011). MeCP2 has been shown to bind methylated CpG sites and repress transcription by the recruitment of histone deacetylase complexes; alternatively, MeCP2 also is capable of activating transcription through interactions with CREB (Chahrour et al., 2008). Several genes are misexpressed by loss of MeCP2 including reduction of factors that promote general neuronal survival and plasticity such as BDNF, and genes that define neuronal subpopulations such as GAD1, GAD2, TPH2, or TH (Chahrour et al., 2008; Chao et al., 2010; Samaco et al., 2009). The gene expression changes within neuronal subpopulations often result in biosynthetic deficiencies, reducing the ability to synthesize and release normal quantities of their neurotrasnmitters such as GABA, serotonin, dopamine, or norepinephrine contributing to neurological dysfunction. Additionally, loss of MeCP2 function has been tied to increased expression of FXYD1 a modulator of Na^+/K^+ ATPase activity tied to dendritic morphology (Deng et al., 2007).

3. Breath-holds and apneic events in Rett Syndrome

Breath holds or apneic events are consistently observed in RTT (Figs. 2 and 3) (Weese-Mayer et al., 2008). These events have a periodic nature (Fig. 2A) and they are interspersed by bouts of hyperventilation and irregular breaths (Julu et al., 2001; Southall et al., 1988; Weese-Mayer et al., 2008).

Yet, defining these breathing cessations has been the source of considerable confusion. Indeed, the same type of events may have been described by different authors as “breath-hold”, “apnea” or “Valsalva maneuver” (Julu et al., 2001; Weese-Mayer et al., 2008). The existing confusion may be surprising, because breath holds,

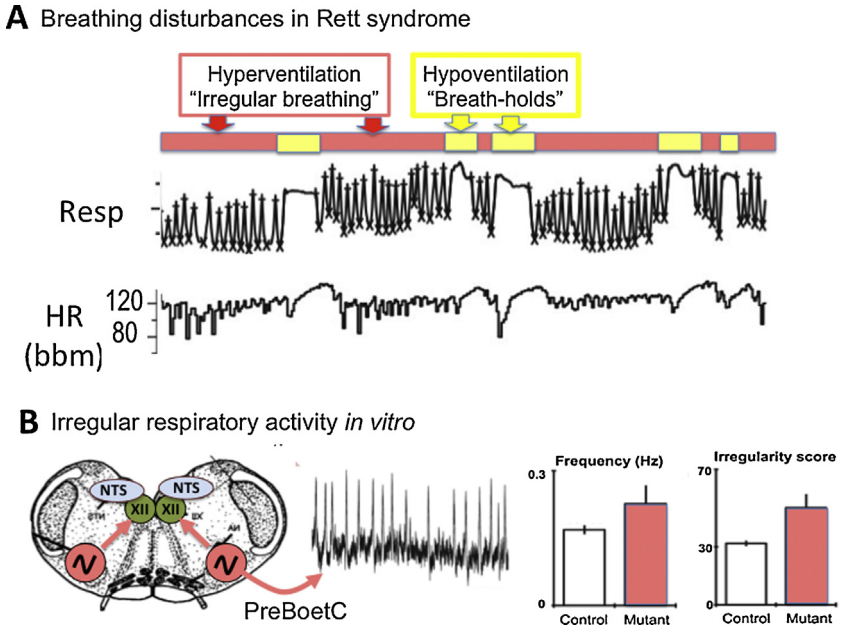

Fig. 2. Respiratory disturbances in Rett Syndrome patient and animal model. (A) Breathing disturbances in Rett Syndrome typically consist of alternating periods of hyperventilation and hypoventilation. During hyperventilation, respiratory activity is typically irregular with regards to frequency and amplitude. Hypoventilation is characterized by long-lasting events that are referred to as breath-holds; for more explanation see text. RESP (upper trace) indicates the respiratory trace – upwards deflections represent inspiration. HR (lower trace) represents the simultaneously recorded heart rate. *Panel A: modified from* Weese-Mayer et al. (2006). (B) The respiratory network isolated in a transverse slice preparation (see schematic) from MECP2 mutant mice exhibits irregular respiratory rhythmic activity that can be recorded as integrated population activity (trace). The slice preparation contains the pre-Boetzinger complex (preBoetC, in this figure marked in red with a curve to symbolize an oscillator), the nucleus tractus solitarius (NTS, marked in blue) and the hypoglossal nucleus (XII, marked in green). The respiratory rhythmic activity in the slice tends to be faster and is significantly more irregular as indicated in the graphs. *Panel B: modified from* Viemari et al. (2005). More details regarding the recording conditions can be found in Viemari et al. (2005).