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Recent insights into genotype–phenotype relationships in patients with Rett syndrome using a fine grain scale

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ABSTRACT

Mutations in *MECP2* gene cause Rett syndrome (RTT), a neurodevelopmental disorder affecting around 1 in 10,000 female births. The clinical picture of RTT appears quite heterogeneous for each single feature. Mutations in *MECP2* gene have been associated with the onset of RTT. The most known gene function consists of transcriptional repression of specific target genes, mainly by the binding of its methyl binding domain (MBD) to methylated CpG nucleotides and recruiting co-repressors and histone deacetylase binding to DNA by its transcription repressor domain (TRD). This study aimed at evaluating a cohort of 114 Rett syndrome (RTT) patients with a detailed scale measuring the different kinds of impairments produced by the syndrome. The sample included relatively large subsets of the most frequent mutations, so that genotype–phenotype correlations could be tested. Results revealed that frequent missense mutations showed a specific profile in different areas of impairment. The R306C mutation, considered as producing mild impairment, was associated to a moderate phenotype in which behavioural characteristics were mainly affected. A notable difference emerged by comparing mutations truncating the protein before and after the nuclear localization signal; such a difference concerned prevalently the motor-functional and autonomy skills of the patients, affecting the management of everyday activities.

1. Introduction

Rett syndrome (RTT; OMIM 312750) is a developmental disorder, almost exclusively affecting females, resulting in severe mental retardation and neurological disability. RTT progresses from the latent onset of symptoms to a more complete expression of the disorder. In late infancy, after a period of superficially normal but subtly flawed development, RTT patients undergo striking developmental regression. RTT is characterized by the loss of pre-existing hand use – such as object reach, grasp, and manipulation, and by the appearance of distinctive hand stereotypies – such as hand wringing, tapping, and

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mouthing (Fabio, Giannatiempo, Antonietti, & Budden, 2009b). Post-regression patients, even though persons with severe intellectual disabilities, often regain social interest (Antonietti, Castelli, Fabio, & Marchetti, 2008; Castelli, Antonietti, Fabio, Lucchini, & Marchetti, 2013; Fabio, Castelli, Marchetti, & Antonietti, 2013) and are relatively stable for an extended period. After this period, progressive motor deterioration occurs in the form of weakness, wasting, and dystonia. During this period, crude self-feeding capabilities may be retained, but voluntary hand use is generally exceedingly limited and hand stereotypies become pervasive (Smeets, Pelc, & Dan, 2011; Weng, Bailey, & Cobb, 2011). Some studies showed that patients often remain visually attentive to objects and people, tracking their movements and even showing preferences by "eye pointing" (Fabio, Antonietti, Marchetti, & Castelli, 2009a). There is a cohort of RTT patients, who does not fit into the above "classical" form and have been grouped in according to an atypical RTT phenotype, which is characterized by the age of the regression onset (frusta forma, late regression onset, neonatal encephalopathy) or by for the presence of verbal speech (Preserved Speech Variant). Renieri et al. (2009) proposed the term "Zappella variant" rather than "preserved speech variant" to described milder forms of RTT, because other aspects, besides speech, are involved.

Considering this range of behavioural patterns, it is important to analyze if specific phenotypic symptoms in RTT are related to specific genotypes. Amir et al. (1999) firstly demonstrated that mutations in the *MECP2* gene, mapping to Xq28 region, are associated with RTT. *MECP2* encodes Methyl-CpG-binding 2 protein, which belongs to a large family of DNA-binding protein characterized by the presence of methyl binding domain (MBD) that selectively binds 5-methylcytosine residues in symmetrically positioned CpG dinucleotides (Fan & Hutnick, 2005; Nikitina et al., 2007). Additionally, a transcriptional repression domain (TRD), which interacts with various co-repressor complexes (Jones et al., 1998; Nan, Campoy, & Bird, 1997; Nan et al., 1998), a bipartite nuclear localization signal (NLS) and a WW domain binding region (WDR) (Buschdorf & Strätling, 2004) have been recognized in the protein structure. As regards the functional role of these domains, it has been showed (Jones et al., 1998) that after binding to methylated CpG nucleotides, *MECP2* can recruit, by its TRD, histone deacetylases to a transcriptional repressor complex, silencing target genes; the WDR seems to be involved in protein–protein interaction (Buschdorf and Strätling, 2004). Two alternatively spliced *MECP2* transcripts, with a different ATG, have been characterized (Mnatzakanian et al., 2004) determining the production of MeCP2E1 and MeCP2E2 proteins isoforms. To date, it has been estimated that 80% of RTT patients carry mutations within *MECP2* gene, up to 95% considering the classical form. Recently CDKL5 and FOXg1 gene mutations have been identified in girls affected by atypical RS with early-onset seizures and by some congenital variant forms (Ariani et al., 2008; Scala et al., 2005).

More than 200 different mutations of *MECP2* gene have been reported in the Rett Base (IRSA MECP2 Variation Database; http://mecp2.chw.edu.au/mecp2/), but eight mutations (Arg106Trp, Arg133Cys, Thr158Met, Arg168X, Arg255X, Arg270X, Arg294X, Arg306Cys) affect around 67% of RTT females. A remaining 10% of RTT cases show a large group of C-terminal frameshift mutations.

Several studies have reported genotype-phenotype correlations, but with conflicting results. Most authors reporting data from different cohorts of RTT patients (Auranen et al., 2001; Chae, Hwang, & Kim, 2002; Weaving et al., 2003) demonstrated that no correlation exists between missense vs. truncating mutations, whereas others (Colvin et al., 2004; Huppke, Held, Hanefeld, Engel, & Laccone, 2002; Monros et al., 2001) reported that the truncating defects are more severe than the missense ones. Studies aimed at comparing mutations affecting the different functional domains share the opinion that defects affecting the C-terminal domain give milder clinical score (Colvin et al., 2004; Huppke et al., 2002). Differences in clustering the mutations, the heterogeneity in size of the analyzed cohorts, the selected clinical parameters, and variation in the age of subjects are likely to explain the conflicting results.

Another important factor modulating phenotype expression is the randomization of X-inactivation; when present, it is expected to influence phenotypical severity. Nevertheless, most studies reported that RTT patients' lymphocytes fail to show a skewed X chromosome inactivation (XCI) (Amir et al., 2000; Hoffbuhr, Moses, Jerdonek, Naidu, & Hoffman, 2002; Van den Veyver & Zoghbi, 2001). Yet, Knudsen (Knudsen et al., 2006) found a significant increment of skewed XCI in RTT patients and their mothers. However, the inclusion of this datum in genotype–phenotype correlation studies remains controversial because of the bias of tissue mosaicism. In fact, the brain cannot respect the same ratio as those found in blood or fibroblasts, which are the investigated tissues. Moreover a role of SNPs and CNVs as modifier elements of phenotype in patients carrying the same *MECP2* mutations should be considered (Artuso et al., 2011).

Given that conflicting findings about genotype–phenotype relationships in RTT are still under discussion (Halbach et al., 2011), it seems worthwhile to further investigate this relationship in order to overcome some methodological flaws (Halbach et al., 2011; Ham, Kumar, Deeter, & Schanen, 2005). In this study, we investigated the effect of *MECP2* mutations on the phenotypic variability within a group of 114 RTT patients, focusing on specific methodological issues. More precisely, our study was performed taking into account what was recommended by Ham et al. (2005) concerning the weak points of the previous studies. Firstly, biases coming from missing data, multiple testing, and age differences were minimized. Secondly, a main new feature was added to this analytic examination of the RTT phenotype, by using the Rett Assessment Rating Scale (R.A.R.S.) (Vignoli et al., 2010), a specific instrument devised to measure the intensity of RTT symptoms and to provide a specific RTT behavioural profile. R.A.R.S. is a standardized scale, which provides sub-scores concerning the specific symptomatic areas and an overall score that expresses the severity of the clinical condition of the RTT patient. Both the sub-scores and the overall score are normalized measures.

The specific aim of this study was to correlate disease-causing genetic mutations to the variety and intensity of the detailed symptomatic parameters assessed by R.A.R.S. and to provide insights into the effect of MECP2 mutations on RTT phenotype.