Correlations Between Linguistic Phenotype and GeneticAlterations in Rett Syndrome

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Abstract

Rett syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in the MECP2 gene affecting around 1 in 10,000 female births. Clinical manifestations include severe linguistic and motor impairments that are the core of phenotype symptoms. Some patients show a moderate level of conservation of linguistic functions while others lose the use of functional verbal communication. This paper aims at correlating residual linguistic capacity, connected to breathing alterations, to specific RTT genotype. In particular, the relation between breathing alterations and the pathological severity caused by locus'mutation is investigated.

Keywords: Rett Syndrome; Language Impairment; Genotype/Phenotype correlation.

Introduction

Rett syndrome (RTT) is a neurodevelopmental disorder, affecting around 1/10.000 female births (Chahrour & Zoghbi2007). Females are primarily affected, even though a few cases of males are reported in the literature (Leonard et al., 2001; Cohen et al., 2002). In 1999 the mutational screening of candidate genes in the region Xq28 allowed the identification of MECP2 gene as a cause of the standard form. Ten years later, different research studies regarding the causes of RTT variations have also been carried out(Amir et al., 1999; Guy et al., 2011).Another study demonstrated that a second gene called CDKL5 localised in the X chromosome, is involved in the variant with early onset convulsions (Weaving et al., 2005).

Recently, the FOXG1 gene, localised in chromosome 14 has been identified as the first autosomal gene associated to RTT, in particular to the congenital variant (Ariani et al., 2008).These results demonstrated that RTT presents genetic and clinical heterogeneity and they provide data for molecular bases to understand pathogenic mechanisms of the disease and to establish targeted therapeutic strategies.

After its nosographical description, RTT was classified among Pervasive Developmental Disorders (APA, 2000), but it has been moved to the genetic disorder category because of its primary ethiology (DSM V, APA, 2013).

The clinical features suggest that this disorder is the result of a primary disturbance of neuronal development (Armstrong et al., 1995), perhaps resulting in maturational arrest in selective brain regions (Armstrong, 1995). The basis of this maturational arrest could, for example, be defective neurotransmitters systems that fail to provide normal trophic factors.

From a functional point of view RTT shows a latent period in which symptoms are not almost evident and a first clinical stage in which symptoms arise. 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 mouthing (Fabio et al., 2009). Post-regression patients, even though persons with severe intellectual disabilities, often regain social interest

and some verbal communication capabilities (Antonietti et al., 2008; Castelli et al., 2013).

Genotype characterization of RTT

Currently, gene MECP2 mutations (Xq28) are found in the majority of cases (90%) of standard Rett and in 30% of cases of atypical RTT. Since there are many clinically documented RTT cases who don't present the MECP2 mutation, many scientists in this field believe that the pathology is caused by genetic heterogeneity.

MECP2 gene is particularly expressed in the neuronal tissue during specific developmental stages (Jung et al.,2003; Balmer et al., 2003; Shahbazian et al., 2002).More than 200 different mutations of the MECP2 gene have been reported in the Rett Base (IRSAMECP2 Variation Database) 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 demonstrated that no correlation exists between missense vs. truncating mutations, whereas others 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 a milder clinical score. Differences in clustering the mutations, the heterogeneity in the size of the analyzed cohorts, the selected clinical parameters, and variation in the age of the subjects are likely to explain the conflicting results.

Given that conflicting findings about genotype–phenotype relationships in RTT are still under discussion, it seems worthwhile to further look into this relationship in order to overcome some methodological flaws.

In a previous study, Fabio et al. (2014) examined the effect of MECP2 mutations on the phenotypic variability within a group of 114 RTT patients, focusing on specific methodological issues. More precisely, the study was performed taking into account what was recommended by Ham et al. (2005) concerning the weak points of the previous studies. The results showed that a specific kind of genotypes can be associated with the severity of symptoms showed by RTT patients. On the basis of these results, our study aims at providing a phenotype/genotype correlation in relation to a specific cognitive process, i.e. language production and comprehension. There are different linguistic phenotype variants in RTT. A restricted sample shows the presence of verbal speech (Preserved Speech Variant or Zappella variant (Renieri et al. 2009). This form presents a less severe clinical condition, i.e. regular skull dimensions and a relevant reduction of epileptic seizures and breathing alterations. In the present study we aim at correlating the relationships between the genetic mutations presents in 14 girls with RTT and the languagecapabilities (comprehension and production) that they still present. In our position, this relation is mediated by breathing alteration linked with genetic mutation: the locus of mutation (before or after the nuclear localization signal) is correlated with the severity of breathing phenotype and consequently with speech.

Linguistic phenotype in RTT

RTT is characterized by severe alteration in motor and speech capabilities that are considered as inclusive diagnostic criteria (Neul et al., 2010).

In particular, the residual linguistic abilities are very different and each individual could manifest various degrees of severity in language production: whereas some RTT patients completely lose their activity in verbal sound production, which is functional to communication, others preserve functional vocal sound and/or words (Budden, 1997; De Bona et al., 2000; Fabio et al., 2009; Zappella et al. 2001).

Linguistic deficits typically arise after the regression phase: indeed, RTT females are characterized by a normal language development comparable with a healthy one before the regression phase. They often exhibit babbling and phonological coupling except for the early onset variant.

It has been demonstrated that linguistic competence levels are correlated to the language acquisition stage in which individuals were at the onset of regression (Marschik et al., 2012).Few studies have evaluated a genotype/phenotype correlation between linguistic residuals and specific genotype. Uchino and colleagues (2001) tried to correlate the grade of disability in locomotion and that of microcephalus with a language disability in RTT in a preliminary study and after they correlated language RTT to the loci of MECP2 mutation. This correlation was found on the basis of a qualitative evaluation of spoken language.

This study proposes a genotype/linguistic phenotype correlation based on an articulation capability test by a phonetic evaluation test (Fanzago, 1983). Our hypothesis moves off the assumption that linguistic alterations in RTT derive from alterations in breathing and facial-laryngeal muscle' coordination rather than from general motor disease. As a consequence, genotypes producing severe breathing and facial-laryngeal muscles alteration are expected to show a severe linguistic phenotype.

Indeed, girls with RTT show a complex breathing phenotype that includes hypoventilation, hyperventilation, apnea and breath hold terminated by Valsalva maneuvers (Katz et al., 2009). It seems that modifications of subcortical nuclei in the brainstem, which regulate breathing rhythm, are connected to speech motor control (Ogier& Katz, 2008, Ramirez et al., 2013). Many studies show that the brainstem respiratory network (Trevarthen& Daniel, 2005) is affected in RTT, but to date this aspect has not yet been analyzed for speech. On the basis of previous research on residual linguistic capacity in RTT patients and on the basis of the evaluation of residual communication ability (both

comprehension and articulation) (cf. Fabio et al., 2009) real linguistic phenotype to genotype has been correlated.

In order to do this, speech ability has to be evaluated by using the Fanzago test, an Italian phonetic evaluation instrument which follows the typical degree of language acquisition phase in phonological difficulties aspects. Four parameters of the Fanzago test were used: the number of vowels spontaneously produced, the number of consonants spontaneously produced, the number of vowels with elicited denomination and the number of consonants with elicited denomination. The spontaneous production of language sounds and the presence of breathing alteration at baseline condition and during a cognitive task were evaluated.

The aims of the present study are two: the first one is to analyze the correlation between breathing dysfunctions and speech. The second one is toanalyze data related to linguistic phenotype and specific RTT genotype in a pilot study.

Methods

Participants

Twenty-one girls with a diagnosis of RTT, ranging from age 4 to 31 (mean= 16,34 years, SD=5,98), took part in the experiment. Their families had been contacted by the Italian Rett Association, which asked them to participate in the study.All of the participants were diagnosed with RTTand all of them were positive to MECP2 mutation.

A general assessment was carried out by a psychologist through the Vineland Adaptive Behavior Scale (VABS) (Sparrow et al., 1984), the standardized test for the Rett Assessment Rating Scale (RARS, Fabio et al., 2005) and Modified Colored Progressive Matrices.

The Fanzago phonetic articulation test was administered to evaluate the status of vocal sound articulation and objective production articulated voice functional to communication. Behavioural breathing parameters were evaluated: all the girls showed breathing alteration and none required any Valsalva maneuvers.

Materials

The assessment consisted in functional, cognitive and linguistic scales administering. In particular the Vineland Adaptive Behavior Scales were used for functional assessment. In order to complete the functional characterization the Rett Assessment Rating Scale (RARS) was administered. This is a standardized scale used to evaluate subjects with RTT (Fabio et al., 2005). It is constructed by following the diagnostic criteria for RTT proposed by DSM-IV-TR (APA, 2010) and recent research and clinical experience. It follows a structure similar to that used for the diagnosis of the pervasive developmental disorders included in the same nosographical category as RTT (i.e. Childhood Autism Rating Scale, CARS).

For cognitive measure, Modified Raven's Coloured Progressive Matrices were used (Antonietti et al., 2003). Differently from the standard Raven's Colored Progressive Matrices, in this adapted scale the A series was administered to girls and each table was larger (42 cm x 29,7 cm). Girls have to choose between two items (one target and one distractor) placed separately in front of them. Both items (target and distractor) were shown 3 times and the spatial position of the target and distractor was randomized. When the girl replied with two consecutive and correct answers the examiner presented the following table; when the girl replied wrongly three times, the test was interrupted. For linguistic measure, Fanzago test was used. The Fanzago phonetic articulation test is used to evaluate the articulation capabilities in children and it is a good measure of their phonetic development. This instrument is based on spontaneous/repetition elicited denomination of 114 figures grouped in 22 tables. Each table represents one image whose name starts with a vocal sound and other objects in which the same sound is placed in a second or third position or is coupled with a vowel or consonant sounds. This study uses items which represent perceptively salient and commonly used objects for RTT.

During the assessment, behavioural breathing parameters were evaluated. All the girls were video taped, two observers independently transcribed the first five minutes of each tape (one during sleep and one in the waking state). The final transcription was then coded independently by both observers for the behavioural breathing parameters. The inter-rater agreement concordance was high (Kappa index = .98).

Procedure

All the activities were performed in a setting suitable for language activity with patients: all distracting stimuli were removed so the girls focused only on the task.

After the initial assessment, each girl was evaluated at breathing baseline. After the Fanzago test was administered starting with spontaneous vocal production. In the second step girls had to produce phonemes at the request of the linguistic therapist. In this study, girls are requested to produce first vowel sounds, that are easier to articulate and that appear early on during normal development.

All correct phoneme (spontaneous/elicited) production was marked on the specific template, in which therapists can write if girls utter the entire word painted in the image during spontaneous or elicited production.

In order to check the effective results of the requested sound production task, the alteration of breathing rhythms during a cognitive, not linguistic task was evaluated in this study.

Results

Before proceeding with the analysis of the genotype-phenotype correlation, Pearson's correlation coefficient between the sum of each type of respiratory dysfunctions and the four parameters of Fanzago test were calculated Results show that breathing problems display an inverse correlation with each of the Fanzago parameters, namely with the number of vowels spontaneously produced ($r(21)= -.378$, $p<.137$), the number of consonants spontaneously

produced (r (21)= -.61, p<.001), the number of vowels with elicited denomination (r(21)= -.498, p<.03), and the number of consonants with elicited denomination (r(21)= -.29, p<.23).

To proceed with the genotype-phenotype correlation, since the number of participants was low, dichotomized scores to classify participants into a particular breathing type were used. Based on high (> median) or low (≤ median) scores on respiratory rhythm (Mdn = 1.2), mild breathing problems (Mdn = 0.6), and severe breathing problems (Mdn = 2,8), participants were placed within one of the two type categories. Since some of the girls with RTT shows only clinical features and not mutation in MECP2, only 14 patients were included in the analysis.

As shown in figure 1, patients with a truncating mutation after NLS manifested a lower degree of impairment than patients with a truncating mutation within NLS in the breathing dysfunctions ($\chi 2$ (2, N = 14) = 1.74, p<.05).

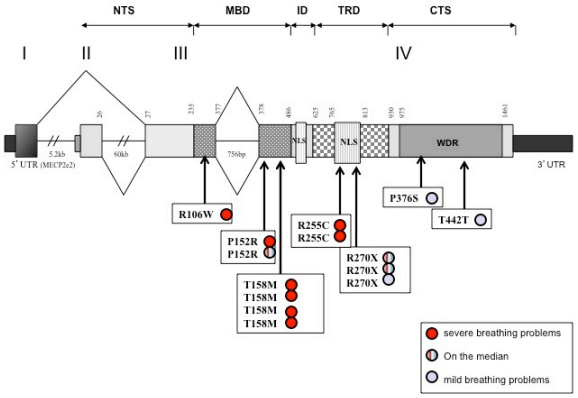

Fig. 1 Genomic structure of the MECP2 gene and localization of breathing problems in the coding regions

With reference to the number of vowels with elicited denomination, based on high (> median) or low (≤ median) scores on respiratory rhythm (Mdn = 1), low level of vowels with elicited denomination (Mdn = 0.4), and high level of vowels with elicited denomination (Mdn = 2,7), participants were placed within one of the two type categories. As shown in figure 2, patients with a truncating mutation after NLS manifested a higher level of vowels denominations than patients with a truncating mutation within NLS ($\chi 2$ (2, N = 14) = 2.15, p<.05). With reference to the number of consonants with elicited denomination (namely the syllables), only two girls (P376S and T442T) were able to repeat a high number of syllables (respectively 12 and 10). For this reason the relative figure was not produced.

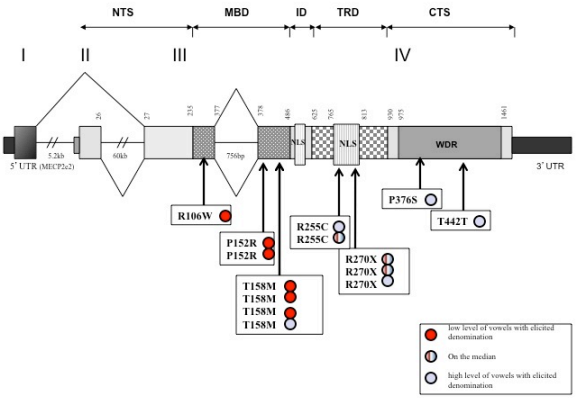

Fig. 2 Genomic structure of the MECP2 gene and localization of vowels with elicited denomination

Conclusion

In this work, a pilot study related to correlations between severity of breathing disorders and the residuals of speech capacity have been conducted. The results showed that the intensity of the parameters of the breathing dysfunction conditions speech production, in relation to the specific type of mutation in MECP2 in RTT patients. In particular comparisons between the truncating mutations differently affecting functional domains induce support for the idea that the crucial factor that leads to different phenotypes is the integrity of NLS (nuclear localization signal). Indeed, with reference to general phenotype, a milder form is linked to the possibility of protein to penetrate into the nucleus and link to Methylated CpG, maintaining its residual expression.
In this study the specific linguistic phenotype correlated to the the two kinds of mutations were clearly shown by the difference in the breathing scores and in the level of the vowels denomination with elicitation.
This pilot offers new possibility of genotype/phenotype correlation in a genetic syndrome because differently from other correlational study, the present one is focused on specific cognitive process (i.e. language) rather than the generic phenotype (i.e. both cognitive impairments and all the clinical feature damage).
The present work analyzed samples with a limited number of patients, for this reason it is just a pilot study and more data has to be collected. The most important innovation introduced in the study was the use of the correlation between breathing and language in relation to the specific genotype.

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