Rett Syndrome and Long-Term Disorder Profile

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In a cohort of 103 females clinically diagnosed with Rett syndrome (RTT), 91 had a detectable MECP2 mutation. Emphasis on details of natural history facilitated grouping of females with the same MECP2 mutation and the development of so-called disorder profiles. Some examples of disorder profiles of different recurrent MECP2 mutations are discussed. RTT females with the frequently recurrent R133C and R306C missense mutations and those with intragenic deletions in the C-terminus of MECP2 deserve more attention in larger studies as their development is different and milder in the long term. RTT females with the T158M missense mutation are often atypical with mainly behavioral characteristics in infancy and childhood but become classic RTT in adolescence after a slower, protracted course.

Key words: Rett syndrome; MECP2; disorder profile

INTRODUCTION

Since the identification of mutations in MECP2 in females with clinical Rett syndrome (RTT), numerous efforts have been made to understand phenotype–genotype relationships. Most of these studies were accomplished by looking at the type and localization of these mutations in the gene in relation to clinical severity. MECP2 mutations consist of missense (single amino acid substitution), truncating (nonsense and frame shift) mutations and complex rearrangements in MECP2. Missense mutations cluster in the methyl binding domain and truncating mutations cluster in the transcription repression domain. Such studies have produced conflicting results [Amir et al., 2000; Cheadle et al., 2000; Hoffbuhr et al., 2001; Monros et al., 2001; Huppke et al., 2002]. Some have found more severe RTT manifestations in children with truncating mutations but milder expression in the frameshift mutations located at the deletion hot spot in the C-terminal segment [Huppke et al., 2000; Smeets et al., 2005]. This was explained by the hypothesis that these “late truncating” mutations do not involve the nuclear localization signal and therefore leave residual function to the protein. Others have not found this difference in severity [Amir et al., 2000]. Therefore, in view of the age range and variable severity, it seemed unsatisfactory to look only at the type and localization of the mutation in MECP2 in trying to establish a clear phenotype/genotype relationship. Describing each RTT individual after extended follow up and grouping females with the same history and same MECP2 mutation is the most appropriate way to proceed. The purpose of our study was to establish so-called disorder profiles or subgroups in RTT based on this extended clinical follow-up and in relation to the type and localization of the MECP2 mutation.

MATERIALS AND METHODS

Complete data were obtained on 103 RTT females clinically diagnosed between 1983 and 2003 according to the international diagnostic criteria for classic and variant RTT [Hagberg et al., 2002]. Most of the adult women had been known by the first author for more than 16 years. They were re-examined in the presence of parents and/or caretakers either at the clinic, at home or in the institutions and day-care centers. Clinical evaluation included personal history of each individual, age at onset of stagnation/regression, age at clinical diagnosis, the clinical severity at the age of examination and comments on RTT manifestations and behavioral characteristics over the years. For the evaluation of clinical severity we applied two scoring systems. Score 1 is according to guidelines

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for reporting on manifestations common in RTT [Kerr et al., 2001]. In this system 20 features, encompassing growth, breathing, epilepsy, sleep, mood, orthopaedic deformities, and gross motor and cognitive abilities are scored on a severity scale from 0 to 2. The maximum score being 40, a score above 30 is considered very severe, a score between 25 and 30 as severe, and between 10 and 25 as mild to less severe. A score of less than 10 indicates the best clinical condition still recognizable as RTT. We developed a second scoring system that is shown in Table I. In this more simplified system we excluded sleep disorder and mood disturbance, breathing dysrhythmia, muscle tone, involuntary movements, head circumference and other growth parameters. There are two reasons for doing so. First of all, these manifestations are not predominantly present in milder RTT. Secondly, gross motor sitting and walking ability, residual functional hand use, speech ability, epilepsy, and neurogenic scoliosis are considered more likely to influence the long-term evolution and severity in the milder RTT as well as in females with the severe classic phenotype. A score from 0 to 3 was given for these six items as shown in Table I: 0 for the normal situation; 1 when there was impairment without total loss of motor ability, reduced hand use, some preserved speech, previous epilepsy or seizures well controlled by medication, mild scoliosis/kyphosis; 2 when there was loss of function, apraxia, no speech, uncontrolled epilepsy, severe scoliosis/kyphosis; 3 when the function was never acquired, status epilepticus, surgery for scoliosis. The maximum score is 18, below 9 the condition is considered as mild to less severe.

The routine DNA-diagnostic screening for MECP2 mutations involved DHPLC analysis followed by direct sequencing of the coding exons and immediate flanking intronic regions of the gene to confirm and identify the nature of the mutation. We analyzed initially mutation-negative cases with the multiplex ligation-dependent probe amplification (MLPA) technique to allow for detection of deletions and duplications in the MECP2 gene. Rearrangements were subsequently verified and confirmed by Southern blot analysis. Semi-quantitative PCR with markers within and flanking the gene and long-range PCR were used to characterize the rearrangements [Schollen et al., 2003]. X-chromosome inactivation (XCI) was studied in 24 of the adult RTT females [Smeets et al., 2003]. The statistical software program SPSS11.5 was used to obtain statistical and graphical results.

**RESULTS**

MECP2 mutations were detected in 91 (88%) of the 103 females with RTT: in 62 (96.8%) of the 64 classic cases and in 29 (74.3%) of the 39 variant cases. Of the latter 29 had the forme fruste, three the preserved speech variant, two the congenital onset variant, two the late regression variant, and three the infantile seizure onset variant (of whom two were monozygotic twin sisters). Only the molecularly confirmed RTT females will be considered further.

Age at time of examination ranged from 2 to 60 years, while 54 (52%) were 15 years or older. The mean age at which examination was conducted was 16.6 years. A histogram displays the age distribution in Figure 1.

Missense mutations were present in 37 females (40.7%) and truncating mutations in 54 (59.3%). The most frequent were the recurrent mutations R106W (3), R133C (5), P152R (3), T158M (9), R168X (8), R255X (9), R270X (3), R294X (8), and R306C (11). There were 13 intragenic deletions in the C-terminal segment (14.3%) and 6 other frameshift mutations: 1 (422insA) in the methyl-CpG-binding domain and 5 (705delG, 803delI, 830delC, 808delC, 860insGC) in the transcription repression domain. Gross rearrangements (5 large deletions of the same size and 1 duplication, all encompassing exon 3 and exon 4) were documented in 6 individuals with classic RTT: 3 large deletions, 7.6-kb deletion in one patient and an 8.1-kb deletion in the other patient, both including exon 3 and the coding part of exon 4, with severe to very severe RTT. The exact nature of the rearrangement in the third patient remained elusive [Schollen et al., 2003]. Two other rearrangements of the same size, had a milder RTT clinical course among whom 1 MECP2 duplication. This duplication involved intron 3, exon 3 and exon 4 up to the breakpoint. We were not able to investigate the extension of this duplication whether this was leading to a frameshift or to truncation in translation due to intragenic localization. X-chromosome inactivation was not studied in these females. X-chromosome inactivation in 24 other RTT females with mutation showed skewed X inactivation with ratios <20% or >80% in five patients. No parental material was available to determine which allele was preferentially inactivated. Two patients were not informative. The other 17 patients were classified as random X inactivation with ratios between 20% and 80%. Table II gives an overview of the type and localization of the detected MECP2 mutations in 91 females.

Score 1 was very severe for 11 females with a score 1 of 30 or more, severe for 16 with a score 1 from 25 to 29, mild to less severe for 56 with a score 1 from 10 to 24 and very mild for 8 with score 1 below 10. The results for score 2 were about the same: 63 scored mild to less severe (<9) and 28 severe to very severe (>9). The scattered plots of score 1, respectively score 2, versus age are presented in Figures 2 and 3. Figure 4 shows the mean and range of score 1 for the most...

Bar charts of the level of preserved abilities for sitting, walking, hand use and speech are presented in Figure 5. Of the 91 RTT females with a MECP2 mutation about 60% still sit and walk. Only 23% have lost ambulation and 17.5% have never walked. Hand use is preserved or reduced in 44%, the remaining having complete apraxia. Speech (meaningful use of babbles or words or word sentences) has been lost in the majority (87%), and has never been acquired by one.

The presence of epilepsy and scoliosis among these patients is displayed in Figure 6. Epilepsy was problematic (uncontrolled by medication or status epilepticus) in about 30%. Scoliosis, severe kyphosis or a combination of both was present in 27%, needing surgery in 13%.

**DISCUSSION**

The detection rate for MECP2 mutations in 91 of the 103 females (88%) is consistent with other studies [Amir et al., 1999; Sung Jae Lee et al., 2001; Huppke et al., 2002], as is the high detection rate in classic RTT (96.8%) versus the lower rate in variant RTT (74.3%) by additional MLPA analysis [Schollen et al., 2003].

The plots of severity scores 1 and 2 (Figs. 2 and 3) demonstrate more variation at younger ages. There are low as well as high scores in the younger girls, though it seems that with advancing age not only do the very low scores disappear but the high scores as well. This supports the clinical experience that, in the long-term evolution of RTT, preservation of cognitive skills and motor abilities together with milder epilepsy and less severe spine deformation were more predictive in determining the overall clinical severity than other RTT manifestations. Another explanation could be that this is probably due to survival in a less severe condition. However there is also a possibility that older RTT women are less likely to carry this diagnosis and thus still remain under-diagnosed or under-ascertained.

The guidelines for reporting manifestations common in RTT (score 1) are a practical tool in the evaluation of severity in clinically diagnosed RTT females. It is recommended that it be used uniformly by all clinicians involved in the study of RTT to facilitate comparison between studies, even though this scoring system, like any other system, is prone to subjective interpretation. When successive evaluation is performed by the same experienced clinician this will contribute to a uniform approach in clinical follow-up. Then it may appear that severity does not always increases in time. Scoring only the preservation or loss of gross motor abilities, speech, hand use and secondary manifestations like epilepsy and spine deformation (score 2) is only useful for the evaluation of overall severity at the time of clinical examination. It does not reveal the evolution in time or the prognosis of a single mutation type. Nevertheless, both score systems can be used at follow up. Using type of mutation and its localization in the gene together with clinical severity at the time of examination as variables in the study of genotype–phenotype relationship remains particularly

**TABLE II. Type of Mutation and Localization in MECP2 in 91 RTT Females**

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>NT</th>
<th>MBD</th>
<th>TRD</th>
<th>CTS</th>
<th>[n] 91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>R133C</td>
<td>E100V</td>
<td>L100V</td>
<td>P225R</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>R168X</td>
<td>L106W</td>
<td>R106W</td>
<td>R306C</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>R255X</td>
<td>Q128P</td>
<td>Q128P</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>R270X</td>
<td>R133C</td>
<td>R133C</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>R294X</td>
<td>P152R</td>
<td>P152R</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>T158M</td>
<td>T158M</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>R168X</td>
<td>R168X</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Truncating</td>
<td>R168X</td>
<td>L270X</td>
<td>R255X</td>
<td>S373X</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R270X</td>
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<td></td>
<td></td>
<td></td>
<td>R294X</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Frameshift</td>
<td>1</td>
<td>5</td>
<td>1 complex + 12 &quot;hot spot&quot;</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Gross rearr.</td>
<td></td>
<td></td>
<td></td>
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<td>6</td>
</tr>
</tbody>
</table>
troublesome in predicting the outcome of a specific MECP2 mutation found in a young girl. A girl with mild classic RTT may not differ much from one with a forme fruste at the age of 4 years, except for behavior and the eventual decline in head circumference. The variants where age of onset or preservation of speech are dominating the disorder profile may be or become classic in many ways, milder or severely affected with advancing age. Some of the milder cases stay as such far into adulthood, with slow neuromotor impairment, cognitively alert and still learning from their surroundings and new persons [Hagberg et al., 2003; Smeets et al., 2003, 2005]. Severity thus changes with age, but not necessarily for the worse. The inherent clinical variability in RTT combined with the inconsistent application of diagnostic criteria for classic RTT and the subjective application of scoring systems in females of different ages complicates the comparison of data between studies. This makes it difficult to distinguish more specific genotype–phenotype relationships. The fact that milder or variant forms of RTT until now have been defined according to preservation of skills or to time of onset seems unhelpful in understanding the ongoing pathology of the syndrome. RTT can be recognized as such, whether congenital or of later onset, with or without speech, and may even be found to become “classic” at a certain stage or age. Therefore a complete description of each individual with all of its manifestations over a longer follow-up period is very important. Grouping the individuals according to similar disorder profiles will contribute more to the understanding of the ongoing pathology relative to the specific character of the involved MECP2 mutation. Moreover it might help to distinguish between or to discover other etiologies of “new” developmental disorders that are similar to the
Rett disorder. The twins with infantile seizure onset variant appeared later to have a CDKL5 mutation [Tao et al., 2004].

Some disorder profiles are given below as examples of this approach. The R255X, R270X and the R294X stop codon mutations, as truncating mutations in the transcription repression domain, cause a classic Rett disorder profile. Clinical diagnosis is often suspected before the age of 2 years and confirmed by rapidly increasing clinical severity with advancing age. They present many if not all the characteristic manifestations of the syndrome and survival beyond 30 years in this group of stop codon mutations is rare. Although a milder disorder profile is said to be found in the R294X mutation [Colvin et al., 2004], we could not confirm this. The range in severity score 1 was the largest in the R294X group. The highest mean score 1 was obtained in the R255X and R270X. However, numbers are too small to distinguish between mutations that include nuclear localization signals (TRD-NLS) like the R255X and the R270X and those in the rest of the transcription repression domain (R294X).

The T158M missense mutation in methyl CpG binding domain is very common in Rett. All were very lively with hyperactive and odd behaviors in childhood with a relatively slow protracted course into adolescence. Ambulation is relatively well preserved far into adulthood in spite of persistent axial hypotonia and slowly developing kyphosis and/or severe scoliosis. They all have predominant epilepsy in some period of their lives and/or obvious breathing irregularities but no vacant spells. Severity scores climb slowly with advancing age and ambulation is lost in adulthood. The profile in infancy is relatively atypical, mainly behavioral, but becomes classic in adolescence. Many of them are diagnosed in childhood as atypical or forme fruste.

The R133C genotype is known to often cause very mild Rett. All but one of our patients maintained a milder form. As infants they were suspected of having a pervasive developmental disorder with hyperactivity and autistic behavior. In childhood, dystonic features were discrete and they were defined as forme fruste because of normal head circumference and a slow protracted course. Their behavior remained hyperkinetic, playful and joyful, with bouts of unexplained yelling, with the preservation of a good general physical condition. There was one case suffering from severe inoperable scoliosis causing her to loose postural control as an adult. None of these patients used words or phrases but apparently understood some routine conversation. None presented visible breathing irregularities or other obvious signs of autonomic dysfunction. Epilepsy was either never present or controlled by medication. This group obtained the lowest scores because their physical condition was the best among those still recognizable as Rett. Others described the milder phenotype in females with this R133C missense mutation presenting more preserved hand use and speech ability [Zappella et al., 2001; Leonard et al., 2003]. One case showed skewed X-inactivation (XCI) but clinically did not differ in severity from two other cases where random patterns of XCI were seen.

The R306C missense mutation is very common in Rett and is the only one recurrently occurring in the transcription repression domain. The profiles of females with this mutation are very interesting since they were defined as forme fruste in childhood and as classic Rett in young adulthood. The main characteristic seems to be a slow progression of neurological symptoms with marked spasticity in the lower limbs, starting with tiptoe walking in infancy evolving to fixed equinus feet [Smeets et al., 2003; Colvin et al., 2004]. They seldom developed epilepsy or when they did they became seizure free on medication. Their spine deformation was mild requiring no bracing or surgery. Walking became impaired because of slow progressive spasticity in the lower limbs causing the
spine to bend forward with severe kyphosis and imbalance at an older age. They remained however, relatively stable through adolescence into adulthood, even above 40 years of age. They are remarkably quiet in their temperament. One case had a severe score because of life-threatening breathing irregularities and severe autonomic vacant spells, but was otherwise phenotypically like the others with the R306C mutation (Table II).

As a group the C-terminal segment hot spot deletions represent more then 14% of this series. The course of their disorder in all cases was more protracted in time, with more preserved cognitive abilities in adolescence and adulthood. Their main clinical problem was a rapidly progressive spine deformation due to dystonia and asymmetrical posturing from childhood on. With advancing age they became more impaired in walking and at least slower and more passive in general motor performance. They developed more inappropriate hand use and tended to use less verbal expression (in preserved speech variants). This slow neuromotor regression together with a smaller stature (as expected in classical RTT), a rigid posturing with pronounced kyphosis or scoliosis, abiotrophic changes in skin and underlying muscles, gave them a “pre-aging” appearance, at least looking older than their chronological age. In contrast with this decline in motor performance was the preservation of simple communicative and cognitive abilities. These females were still able to recognize and to learn about new persons and situations in their daily surroundings far into adult life [Smeets et al., 2005]. Hagberg et al. [2003] described a single case of CTS hot spot deletion and refers to the ongoing neuromotor impairment as a decline “at the output-side” in contrast to a better functioning “at the input-side.” This may generally hold true for the RTT person, but is more surprising in these initially milder, atypical cases.

CONCLUSION

Application of the international diagnostic criteria by clinicians involved in the clinical evaluation is imperative not only for early diagnosis but also when larger phenotype-genotype studies are conducted. The availability of molecular testing may lead to early confirmation of clinical suspicion of RTT in an infant under 2 years of age. To arrive at an internationally accepted scoring system, score 1 is the best tool for a uniform approach. The simplified scoring system of this study is useful in the ad hoc evaluation of clinical severity at the time of examination but of lesser prognostic value. Older RTT females above the ages of 30–40 years have survived longer because of a milder disorder profile about which we still know little. We did not encounter RTT in females above 33 years with truncating mutations, stop codons and frame shifts except for the CTS intragenic deletions. There is also a possibility that older RTT women are less likely to carry this diagnosis and thus still remain under-diagnosed or under-ascertained. In the present cohort of 103 females with clinical RTT the MECP2 detection rate was high in both classical and variant phenotypes. We confirm that RTT females with early truncating mutations in general have the more severe and classic RTT phenotype recognizable at a younger age. And most of the RTT females in relatively good physical and neurological condition at adult age had missense mutations at different locations in the gene or deletions in the C-terminal segment as late truncating mutations.

We discovered further that different profiles in development and behavior existed. A profile was obtained by describing the clinical history and behavioral manifestations on follow up over the years. The individual clinical and molecular data were compared according to the long-term history. Therefore grouping of individuals with the same mutation (type and localization) and according to their individual long-term history at follow up tells us more about the phenotype-genotype relationship than looking only at type and localization of the mutation in the gene. The females with the frequently recurrent R133C and R306C missense mutations and those with intragenic deletions in the C-terminus of MECP2 deserve more attention in larger studies because they seem to develop differently in the long term and remain “milder” far into adult life. This accounts also for the frequently occurring missense mutation T158M that leads to classic RTT in a more protracted way.

REFERENCES


