MECP2 mutations are an infrequent cause of mental retardation associated with neurological problems in male patients

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Abstract

Mutations in the methyl-CpG-binding protein 2 (MECP2) gene located on Xq28, cause Rett syndrome (RTT) in female patients. Meanwhile, nonmosaic MECP2 mutations unknown in girls have been found in an increasing number of male patients with a normal 46, XY karyotype. They can cause a broad spectrum of neurodevelopmental disorders which often show a combination of mental retardation (MR) with neurological symptoms. We present the results of MECP2 analysis in a group of 72 male patients with an unexplained combination of MR and neurological features, and review the mutational reports published on male patients since the discovery of the MECP2 gene. Analysis included sequencing of exon 1 which thus far was mostly omitted from DNA screening. One pathogenic mutation has been found in a patient with Rett variant, in addition to an unclassified variant and a series of nonpathogenic changes. No changes have been found in exon 1. Criteria for testing of male patients are classic RTT, severe neonatal encephalopathy, and RTT variant which may be clinically underrecognized. Testing can also be considered in males with a combination of unexplained MR and (progressive) neurological manifestations although the yield of MECP2 analysis is probably low in this situation. Based on the literature, MECP2 testing in males with MR only is debatable.

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1. Introduction

Rett syndrome (RTT, OMIM #312750) is a clinically defined neurodevelopmental entity occurring mainly in females. Diagnostic criteria have been established for classic RTT leaning upon a characteristic developmental profile, and for variant forms of RTT [1]. RTT is thought to be the second most common cause, after trisomy 21, of severe mental retardation (MR) in females [2]. In up to 90% of individuals with RTT, mutations in the MECP2 (methyl-CpG-binding protein 2) gene can be found [3–5]. For further review of the clinical and genetic aspects, the reader is referred to a recent publication by Weaving et al. [6].

In the last few years, the phenotypic spectrum of MECP2 mutations has expanded considerably and mutations have also been reported in an increasing number of male patients, reviewed by others and us [7,8]. Apart from males with RTT caused by a MECP2 mutation and X-chromosome aneuploidy or somatic mosaicism of the mutation, and from male patients with severe neonatal encephalopathy due to a known MECP2 mutation, reported cases consist of a group of patients with a broad spectrum of neurodevelopmental disorders. They have MR of various degrees, mostly in combination with neurological features, sometimes associated with psychiatric disorders, up to mild MR only [8]. These individuals often have familial mutations, which are unknown to occur in females affected with RTT.

In order to study the phenotypic spectrum, the MECP2 gene has been screened for mutations in various cohorts of mentally retarded males. The initial suggestion that MECP2
mutations might be a significant cause of MR in males, comparable even to FMR1 mutations [9], could not be confirmed by further studies as reviewed in detail recently [10]. In the latter study, no pathogenic mutation was found in a group of 103 males with unspecific MR. Interestingly, however, the majority of published male patients with MECP2 mutations presented with MR in combination with various neurological signs. In addition, MECP2 studies in male patients did often not include analysis of exon 1. In this study, a group of 72 carefully examined male patients with an unexplained combination of MR and neurological symptoms were tested for MECP2 mutations, including exon 1 analysis. Aim of the study was to further elucidate the phenotype of disorders caused by MECP2 mutations in males and to reconsider the criteria for mutation analysis.

2. Patients and methods

2.1. Patients

A group of 72 male patients with MR and various neurological symptoms aged 2–83 years, with a mean age of 29.8 years, was tested for mutations in the MECP2 gene. All patients had undergone a careful clinical evaluation by a pediatrician or a physician for persons with intellectual disabilities, and by a clinical geneticist trained in dysmorphology. Cytogenetic analysis was performed in all and additional investigations (e.g. FMR1 analysis) in most cases. Patients with known diagnoses, as well as those with dysmorphism, an unexplained MR/multiple congenital anomalies syndrome or with indications for an external (e.g. perinatal) cause of their MR, were not included in the study group. The disorder was reported to be progressive in 12 patients including two with a clinical diagnosis of Rett variant. The neurological features were further unselected, and the patients were affected by one or more of the following features: different forms of epilepsy or seizures, spasticity, hypotonia, dystonia, choreoathetosis, ataxia or other cerebellar signs, tremor, rigidity, and other signs. The most frequent neurological features were epilepsy in 40/72 patients, including five males with West or Lennox Gastaut syndrome, and spasticity in 34/72 patients.

2.2. Methods

Total genomic DNA was extracted from blood samples using the Wizard Genomic DNA purification kit (Promega). All 4 exons of MECP2, including intron–exon boundaries, were amplified by PCR (in overlapping fragments for the exons 3 and 4) in each individual. Purified PCR fragments were screened for mutations by bi-directional sequencing, using the Big Dye Terminator cycle sequencing kit (Applied Biosystems) and analysis on an ABI 3100 DNA automated Sequencer.

The presence of an identified sequence change was tested in a control panel of 100 X-chromosomes, which derived from 50 healthy male and 25 healthy female individuals.

3. Results

In total, seven sequence changes of the MECP2 gene were identified (see Table 1).

The c.674C>T mutation (p.P225L) occurred in a 22 year old male with RTT variant. He had an apparently normal early infantile development, showed hypotonia from 4 months of age emerging into severe spasticity, became severely retarded and lost his initial motor abilities. At age 22 years, he had severe MR, spastic tetraplegia, dystonia, complete apraxia, neurogenic scoliosis, breathing irregularities and a good visual interactive behavior highly suggestive for RTT. His data have been published in detail elsewhere [8]. This missense mutation has not been found in DNA of his mother and two brothers, nor in the control panel.

The c.1214C>T mutation (p.P405L) was found in a young man with severe MR. His psychomotor development was retarded from the beginning. He had infantile convulsions and developed generalized epilepsy from age 16 years on, autistic behavior and characteristic, stereotypic hand movements. When seen at the age of 29 years, he had

### Table 1
Sequence changes identified in the MECP2 gene in the present study

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Aminoacid change</th>
<th>Domain</th>
<th>Control panel</th>
<th>Segregation in family</th>
<th>Conclusion</th>
<th>Clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.674C&gt;T</td>
<td>p.P225L</td>
<td>TRD</td>
<td>Neg</td>
<td>De novo mutation M &amp; S carrier</td>
<td>Pathogenic</td>
<td>Male RTT variant, MR, epilepsy, autism</td>
</tr>
<tr>
<td>c.1214C&gt;T</td>
<td>p.P405L</td>
<td>CTS</td>
<td>Neg</td>
<td>Healthy M &amp; GF carrier</td>
<td>Unclassified variant</td>
<td></td>
</tr>
<tr>
<td>c.378-74C&gt;T</td>
<td>–</td>
<td>Intrinsic</td>
<td>–</td>
<td>Healthy M &amp; GF carrier</td>
<td>Nonpathogenic</td>
<td></td>
</tr>
<tr>
<td>c.378-17delT (2X)</td>
<td>–</td>
<td>Intrinsic</td>
<td>3 females, 2 males</td>
<td>–</td>
<td>Nonpathogenic</td>
<td></td>
</tr>
<tr>
<td>c.602C&gt;T</td>
<td>p.A201V</td>
<td>IDR</td>
<td>–</td>
<td>Healthy GF carrier</td>
<td>Nonpathogenic</td>
<td></td>
</tr>
<tr>
<td>c.815C&gt;T</td>
<td>p.P272L</td>
<td>TRD</td>
<td>–</td>
<td>Healthy M carrier</td>
<td>Nonpathogenic</td>
<td></td>
</tr>
</tbody>
</table>

CTS, C-terminal region; IDR, inter domain region; TDR, transcription repression domain; GF, grandfather; M, mother; S, sister.

*a Registered as nonpathogenic in the IRSA database [11].
no speech, could walk and showed neither further neurological signs such as hypotonia or spasticity, nor breathing irregularities; his visual interactive behavior was poor. His head circumference was normal and he had no scoliosis. The family history was complicated by borderline intelligence in both parents and several other paternal and maternal family members. The sequence change was found also in the mother and the patient’s sister who was of borderline intelligence and had epilepsy. It has not been detected in the control panel. Male family members were unavailable for testing.

The c.815C>T change (p.P272L) has been registered as polymorphism, unfortunately without reference [11], because it has been found in male and female relatives of a girl with RTT. Very probably, it thus is a nonpathogenic variant. In four other males, changes were classified as nonpathogenic variants because they were also found in the healthy maternal grandfather (c.602C>T) or in our male control panel (2×c.378-17delT), respectively.

4. Discussion

In this paper, we report on the results of MECP2 analysis in 72 male patients with MR and associated neurological features. Adding to a previous review on MECP2 related disorders in males, we want to update the knowledge on the phenotypic spectrum of MECP2 mutations in males with MR, and we critically review clinical features thought to be suggestive for a MECP2 mutation in males.

In the reported cohort of 72 male patients with MR and neurological features, seven sequence changes have been found, of which five were probably nonpathogenic variants (see Table 1). The c.674C>T mutation is thought to be a pathogenic change, as it occurred de novo, is so far unreported in male control panels, and affects a highly conserved residue in the transcriptional repression domain (TRD) [8]. At the same position, C>G transitions have been reported repeatedly as pathogenic mutations. The c.1214C>T mutation is hitherto unreported and affects a conserved residue in the C-terminal segment (CTS). It occurred as a familial mutation, male family members were unavailable for testing and the family history made an unmistakable segregation study impossible. Up to now, it has to be regarded as an unclassified variant. The low yield of (possibly) pathogenic changes suggests that a MECP2 mutation does not seem to be a common cause of neurodevelopmental disorders even in this clinically highly selected group.

Recently, a new MECP2 isoform has been discovered encoded by exons 1, 3 and 4, but not exon 2, and named MECP2-B [12–14]. It was shown to be more abundant in human brain and to be preferentially translated as compared to the first described MECP2-A isoform, which is encoded by exons 2–4 [12]. It has therefore been suggested that mutations in exon 1 could possibly be responsible for yet unexplained RTT cases. So far, they have, however, rarely been found in females with classic or atypical RTT testing negative for mutations in exons 2–4 [13–15]. A recent report indicates that exon 1 mutations are not a common cause of X-linked MR in male patients [16].

Table 2
Overview of MECP2 mutations in published male patients

<table>
<thead>
<tr>
<th>Nr</th>
<th>Base change</th>
<th>Amino acid change</th>
<th>Domain</th>
<th>Clinical findings</th>
<th>Family history</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.410A&gt;G</td>
<td>p.E137G</td>
<td>MBD</td>
<td>MR of different degrees 7 δ in 2 G affected (MRX16)</td>
<td>All obligate carrier ♀: normal intelligence; Obligate carrier ♂: at least 2 with essential tremor, normal intelligence; random XCI</td>
<td>[9,39,40]</td>
</tr>
<tr>
<td>2</td>
<td>c.499C&gt;T</td>
<td>p.R167W</td>
<td>MBD/TRD</td>
<td>Mild MR + neurological features 5 δ in 4 G affected (T36)</td>
<td>De novo mut.</td>
<td>[9,39]</td>
</tr>
<tr>
<td>3</td>
<td>c.674C&gt;T</td>
<td>p.P225L</td>
<td>TRD</td>
<td>RTT variant</td>
<td>Present study [8]</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>c.810_816dup7</td>
<td>p.G273fsX</td>
<td>TRD</td>
<td>RTT variant</td>
<td>De novo mut.</td>
<td>[41]</td>
</tr>
<tr>
<td>5</td>
<td>c.1161_1400del240</td>
<td>p.P388_P467del80</td>
<td>CTS</td>
<td>Severe to mild MR 3 δ in 2 G affected (T44)</td>
<td>Carrier ♀: asymptomatic, SXCI</td>
<td>[20,39]</td>
</tr>
<tr>
<td>6</td>
<td>c.1216C&gt;T</td>
<td>p.Q406X</td>
<td>CTS</td>
<td>Severe MR + neurological features. Uncle (a) + nephew (b) affected</td>
<td>1 ♀ mut. carrier: asymptomatic; 1 ♀ mut. carrier: borderline intelligence, mild facial hypotonia; both random XCI</td>
<td>[42,43]</td>
</tr>
<tr>
<td>8</td>
<td>c.1415_1416del2</td>
<td>p.E472fsX</td>
<td>CTS</td>
<td>Moderate MR, hypotonia, obesity, gynaecomastia</td>
<td>De novo mut.</td>
<td>[19,44]</td>
</tr>
</tbody>
</table>

MBD, methyl-CpG-binding domain; TRD, transcriptional repression domain; CTS, C-terminal segment; RTT, Rett syndrome; MR, mental retardation.

* Insufficient clinical documentation; M, mother; G, generation; mut., mutation; SXCI, X-chromosome inactivation; SXCI, skewed X-chromosome inactivation; δ, male(s); ♀, female(s).
*MECP2* studies in males, the present study included exon 1 analysis; so far, it does not indicate that the discussed neurodevelopmental disorders in males may be associated with exon 1 mutations. Because of the possibly crucial function of the *MECP2-B* protein, it can in particular not be excluded that exon 1 mutations may be involved in more severe conditions such as neonatal encephalopathies in males.

Table 2 presents an overview of eight published cases of neurodevelopmental disorders in males due to presumably pathogenic *MECP2* mutations. Three families were identified via the European Consortium for X-linked MR (families MRX16, T36, T44), two of which with nonsyndromic MR (MRX) and one with associated progressive neurological symptoms (MRXS). One further family also showed severe MR and a progressive neurological disorder. Two patients with de novo mutations, including the patient of the present study, had RTT variants. Finally, there were two sporadic patients with mutations in the CTS: one with a de novo mutation and clinical features unreported in all other patients (case 8), and one who clinically poorly documented (case 7). The recessive value of the mutations of these cases is difficult to explain by the type of the mutation and/or the location within the gene, mainly due to the small sample size. Compared to *MECP2* mutations causing RTT in females, there are more missense mutations, but nonsense mutations also occur. It is noteworthy that both de novo mutations leading to severe Rett variant, occurred in the TRD and resemble known recurrent mutations in females with RTT: the p.P225L mutation occurred at the same position as the recurrent p.P225R mutation; and the p.G273fsX mutation encodes for a protein comparable to the product of mutations c.806delG (p.269fs) and c.808C>T (p.R270X) which occur in 3 and 6% of females with RTT, respectively. Mutations that occurred in the CTS (cases 5–8) all affect the functionally essential WW domain binding region which extends from residue 337 to the C terminus [17]. The recent report of a boy with severe retardation and features suggestive of RTT and a microduplication Xq28 involving the *MECP2* gene suggests that in some cases, also overexpression of *MECP2* might be pathogenetically involved [18].

Several clinically defined cohorts, including male individuals, have been screened for *MECP2* mutations. In cohorts of patients with MR only, collected by the XLMR consortium or testing negative for fragile X syndrome or clinically carefully selected as patients with unspecific MR [10,19,20], a low frequency of mutations has been found. The two patients identified in these studies to have a *MECP2* mutation, are included in Table 1 as patients five and eight. Based on our current knowledge, MR alone thus is a debatable criterion for *MECP2* analysis [10].

In view of the clinical overlap between RTT and Angelman syndrome (AS), one further group of interest have been male and female patients with presumed AS who tested negative for 15q11–q13 abnormalities [21–25]. In

these studies, pathogenic *MECP2* mutations have been found only in a small percentage of female patients suspected to have AS [21–24]. Remarkably, they partly developed clinically a phenotype consistent with or suggestive of RTT [21–23]. No *MECP2* sequence changes of pathogenic value have been found in male patients with a clinical diagnosis of AS so far. Though two patients with somatic mosaicism of a known *MECP2* mutation have been ascertained by testing individuals negative for methylation defects in 15q11–q13, they clinically had RTT variant and classic RTT, respectively [21,24]. Altogether, *MECP2* mutations probably are much less frequently associated with a phenotype consistent with AS than presumed.

As the behavior of patients with RTT might be mistaken for an autism spectrum disorder, and as RTT is classified as a form of autism in the DSM-IV [26], several studies have also been initiated to screen for *MECP2* mutations in a cohort of male and female individuals with autism, with or without MR. Most studies found no convincing evidence that *MECP2* mutations might contribute to the pathogenesis of autism [27–30], although in one study, pathogenic *MECP2* mutations have been found in 2 out of 69 autistic females [31]. However, the behavior of RTT patients most often is distinctly different from autistic behaviors, although low intensity in contact, odd behaviors, attention deficit and hyperactivity may predominate the disorder profile in infants for a long time. RTT is characterized at long term by the dissociation between diminishing neuromotor abilities, and a relatively good visual interactive behavior and preserved personality.

Combining the results of our study and the literature, *MECP2* analysis should be considered in particular in males with a history suggestive of RTT (RTT variant). As a detailed knowledge of the patient’s history and awareness of the behavioral characteristics of RTT, in particular of the visual interactive abilities, is important for the diagnosis of a RTT variant, this disorder is possibly underdiagnosed in male patients. Preliminarily, testing may likewise be considered when MR is associated with a progressive neurological disorder, in particular in the lack of information on the patient’s early history, although the yield of testing in this situation seems to be low.

In concurrence with other studies, the detected *MECP2* sequence changes comprised a high proportion of non-pathogenic polymorphisms or unclassified variants [7,10,19]. The validation of hitherto unreported nucleotide changes leading to amino acid changes, shows some general problems. It is often hampered by the familial character of the mutation, inaccessibility of male family members for investigation, and by the availability of only very few functional studies [32,33]. The change of a highly conserved amino acid or the rareness of a variant which remains undetected in a control panel, are helpful but insufficient arguments to assess pathogenicity. We fully agree with Laccone and coworkers who reclassified two previously published cases of presumably pathogenic mutations as
nondisease related variants and therefore appealed to “... proceed with caution” [34]. The p.A140V mutation has been reported in six other, familial cases with remarkably diverse phenotypes [9,35–38], but so far is of unknown value [11,34].

In conclusion, a synopsis of the current knowledge on MECP2 related disorders in mentally retarded males has been given. For the time being, appropriate criteria for MECP2 testing in male patients are: male RTT, classic or atypical, and severe neonatal encephalopathy negative on testing for other causes. A combination of unexplained MR and (progressive) neurological manifestations, in particular of extrapyramidal and/or pyramidal origin, seems to be a less certain criterion.

References


