Short Report

Screening for MECP2 mutations in Spanish patients with an unexplained mental retardation


Rett syndrome (RTT) is an X-linked progressive encephalopathy. Mutations in the MECP2 (methyl-CpG-binding protein) gene have been found to cause RTT. In the past few years, the role of MECP2 mutations in patients with mental disorders other than RTT has been studied, finding that mutations in MECP2 also contribute to non-syndromic entities. More recently, it has been demonstrated that RTT shares clinical features with those of Angelman syndrome, another neurodevelopmental disorder. These observations must be confirmed in a large series, to better understand the criteria needed for justifying a molecular test. Consequently, we have searched for MECP2 mutations in 294 patients (43 Angelman and Prader–Willi like included) with mental retardation (MR) of unknown aetiology. We found six polymorphisms (three novel, three previously reported) in 10 patients, one novel unclassified silent change (p.V222V) in a man, and one causative mutation in a girl with MR. Once this case was clinically reviewed, the girl presented symptoms of atypical RTT. The mutation (p.Y141C) lies within the methyl-binding domain, and has only been reported once in another atypical RTT. Our results show that the MECP2 mutations account for a low frequency (1/416 chromosomes = 0.24%) among mentally retarded individuals, which imply that it is necessary to perform an exhaustive clinical examination of patients before determining whether analysis of MECP2 is required or not.

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Mental retardation (MR) is a condition that affects 2–3% of the general population in developed countries. It is due to a number of different factors, and mutations in X-linked genes play a big role. To date, it has been reported that more than 66 different genes are involved in X-linked MR (XLMR) (1).

Among these genes, mutations in the methyl-CpG-binding protein 2 (MECP2) gene, located in Xq28, were reported to account for the majority of typical cases of Rett syndrome (RTT) (2). RTT is a neurodevelopmental disorder characterized by MR, regression of development, hand stereotypes and progressive microcephaly (3). Although first thought to affect only females, MECP2 mutations have also been found in males with other phenotypes such as severe encephalopathy (4), progressive spasticity (5), Angelman and
Prader–Willi-like phenotypes (6, 7), and non-syndromic XLMR (8).

Criteria regarding the need to study mutations in the MECP2 gene in these phenotypes other than classic RTT have been controversial. Some authors have suggested that it could be one of the main genes involved in XLMR with the highest mutation frequency (5, 8, 9), but more recently, other studies have not found a similar frequency of mutations (7, 10, 11). It has also been suggested that one mutation in the MECP2 gene – the A140V mutation – could be a hot spot of mutation, resulting in moderate to severe MR in males (12). These observations must be confirmed in large series, to clarify better the criteria needed for justifying a molecular test. For this reason, the aim of this study was to search for MECP2 mutations in a large sample of non-syndromic MR (NSMR) patients and in clinically diagnosed cases of Angelman syndrome (AS) or Prader–Willi syndrome (PWS) from the Spanish population in order to add more information relating to the involvement of MECP2 mutations in unexplained MR, and to help clinicians when they request a MECP2 molecular study.

Materials and methods

Patients

MECP2 was completely tested for mutations in 294 patients showing MR of unknown aetiology, who had normal karyotype and who were negative for FMR1 CGG repeat expansion. These 294 patients were from the North of Spain and were subdivided into four groups: (i) 150 unrelated NSMR male patients; (ii) 101 unrelated NSMR female patients; (iii) 16 (8 women and 8 men) sporadic cases with clinical traits of AS because they presented developmental delay, speech impairment, frequent laughter and a short attention span but no cytogenetic or molecular abnormality involving chromosome 15q11-13 could be identified and these patients have normal methylation patterns at SNURF-SNRPN exon 1 and no mutations in UBE3A; and (iv) 27 Prader–Willi-like patients (PWS) (13 women and 14 men) with no methylation defect on chromosome 15q11-13 and with growth retardation, hyperphagia, obesity, dysmorphic features, genital hypoplasia and MR. In summary, the only common feature of all these patients selected for the MECP2 study was cognitive impairment. We studied only the A140V mutation in another group of 70 unrelated MR male patients. Informed consent was obtained from all patients.

Methods

The four coding exons (exon 1 included) of MECP2 were amplified by PCR with primers and conditions as previously described (2, 13). To screen the gene for sequence variations, we used two protocols: in the first protocol, for males, we used the single-strand conformation polymorphism (SSCP), and in the second protocol, for females, we used heteroduplex analysis by conformation-sensitive gel electrophoresis (CSGE). When an aberrant migration pattern was detected, PCR amplification product was directly sequenced. Finally, for the group of 70 MR patients specifically analysed for the A140V mutation, we used a PCR–RFLP (Restriction Fragment Length Polymorphism) assay that creates an AccI restriction site when the mutation is present (12).

Results

We studied a total of 416 X-chromosomes for the MECP2 gene, from 294 patients (172 men and 122 women) and found six polymorphisms (three novel, three previously reported) in 10 patients, one novel silent change (p.V222V) with unknown significance in a male, and only one pathogenic missense mutation (Table 1) in a girl from the group of NSMR females. In groups of 15q-negative Angelman or Prader–Willi-like patients,
we did not observe mutations or polymorphisms. The specific assay performed in 70 additional MR males, in order to identify the recurrent mutation A140V, did not reveal any patients with this change.

The mutation found is a c.422A>G transition, which leads to a change of amino acid p.Y141C in one of the functional domains of the encoded protein – the methyl-CpG-binding domain (MBD) – probably disrupting partly its ability to bind methylated DNA (14). Neither of her parents showed this mutation, demonstrating that it is a de novo mutation. Furthermore, we did not find this mutation in 276 unrelated normal male X-chromosomes taken from the same regions of Spain as a normal control population.

The total frequency of the sequence variants by chromosome was 2.88% (12/416), but if we only count causative mutations, the frequency dropped to 0.24% (1/416). No important differences were found between polymorphisms in males (3/172 = 1.74%) and in females (8/244 = 3.27%) (p = 0.515).

We screened all the polymorphisms listed in Table 1 in the 276 unrelated normal male X-chromosomes taken from the same regions of Spain as a normal control population. We found that the deletion of a T nucleotide in intron 3 (c.378-17delT) is the most frequent with a frequency of 1.81% (5/276). In all the other cases, except one, we assume that they are polymorphisms because they were found in at least one normal man, either in the father for females or in a normal male control population. The exception is the case of the novel silent change in the coding sequence c.666C>G (p.V222V) because we have not found it in the control population, and although the amino acid does not change, the hybrid computational/experimental method RESCUE-ESE (15) reveals that it may create a candidate sequence for the termed exonic splicing enhancer (ESE).

Discussion

In the past few years, the role that mutations in MECP2 could have among individuals showing MR with unknown aetiology is under continuous study and we wanted to contribute our own work towards a better understanding of the criteria needed for justifying a molecular test. So, we report on the screening of a large number of samples for mutations in the MECP2 gene.

Mutations were screened using SSCP and CSGE techniques, although this gene is small and sequencing would have been the method of choice. Nevertheless, the advantage of these methods for small hospital-based clinical laboratories is that they are inexpensive and straightforward for technicians to use due to the minimal standardization required. Furthermore, it has been demonstrated that the combination of these mutation detection methods with DNA sequencing enhances the efficiency of identifying pathogenic mutations (16, 17).

Some authors have suggested that MECP2 could be one of the main genes involved in XLMR with the highest mutation frequency (8, 9), but on the contrary, there are other more recent studies that do not find a similar frequency of mutations (7, 10, 11). The findings of the present study are consistent with these more recent studies. We could not find the A140V mutation [which was suggested to be a hot spot of mutation resulting in MR in males (12)] in 220 MR males (the 150 samples completely screened and the 70 only studied for this mutation), indicating that such an analysis is not necessary.

Our results also agree with the authors who indicated that many of the DNA changes reported in MECP2 are silent polymorphisms instead of disease-causing mutations (18): while six non-pathogenic variants have been found, just one mutation affects the function of the encoded protein. Further familial investigations and control population studies allowed us to rule out the pathogenic effect for these six polymorphisms. Regarding the deletion of a T nucleotide in intron 3 (c.378-17delT) that has been demonstrated to be a benign polymorphism (19), it is a frequent polymorphism in our population (1.81%), although such a DNA change had not previously been found in two different normal population screens (19).

It is important to pay attention to the fact that the novel silent change in the coding region, c.666C>G (p.V222V), identified in a male might have a pathogenic effect because we have not found it in the control population and the hybrid computational/experimental method RESCUE-ESE (15) reveals that the new sequence could be an ESE. These ESE are specific short oligonucleotide sequences that enhance pre-mRNA splicing when present in exons and play important roles in constitutive and alternative splicing. Consequently in our case, this ESE theoretically might alter the expression of the gene. The patient carrying this variation had initially been referred to our DNA diagnostic laboratory to test fragile X-syndrome because he had MR of an unknown origin. Unfortunately, the complete phenotype in this patient has not been determined yet because the patient lives far from our laboratory and we could not clinically re-examine
him nor take another blood sample to study the mRNA. This is why in Table 1 this variant appears as ‘unclassified’ because we could not definitely say if it is a polymorphism or a disease-causing mutation. In conclusion, we have not found any clear pathogenic mutation in the 150 NSMR males. These results suggest that routine testing for MECP2 mutations in this group of patients is probably unwarranted in the absence of good clinical indications, such as for men who are born with congenital encephalopathy or men with an RTT phenotype (20).

Regarding the females studied, we found only one pathogenic missense mutation: a c.422A>G transition that leads to a change of amino acid p.Y141C in one of the three functional domains of the encoded protein, the MBD. The MBD, at position 0-174 of the MeCP2, exclusively binds to symmetrically methylated CpGs; therefore, the mutation probably partly disrupts this function (14) causing the phenotype. Furthermore, neither of her parents showed this mutation, demonstrating that it is a de novo mutation. We also did not find this mutation in the 276 unrelated normal male X-chromosomes taken from the same regions of Spain as a normal control population.

A recent review of RTT (21) shows that patients with mutations in the MBD retained more voluntary hand use and show later onset and less severe language retardation, than those with mutations in the other two domains of the protein. Probably, the fact that our patient has an incomplete phenotype, with preserved speech and almost normal hand use, was the reason for being misdiagnosed, but further complete clinical examination demonstrated clear features of atypical RTT (Table 2). Kammoun et al. (11) previously reported one case with the same mutation in a group of atypical cases with RTT. Members of this group lacked at least one positive necessary criterion but had at least three out of six positive necessary criteria and possibly other minor criteria supportive of RTT as defined by Harberg et al. (22). This is exactly what our patient has. The study by Kammoun et al. (11) found a 47% of mutations in females with atypical RTT, demonstrating the importance of requesting MECP2 screening when clinical symptoms of female patients fall into this group.

Some authors have described mutations in MECP2 in patients diagnosed with AS and no 15q11-13 abnormalities (6, 7). They have hypothesized that since MECP2 is involved in transcriptional silencing, mutations in this gene could disrupt the normal expression of genes located in the 15q region and this would explain the phenotypic overlap between these two conditions. More recently, it has been demonstrated that MeCP2 deficiency causes epigenetic aberrations at the PWS/AS imprinting centre that affects UBE3A expression (23). Furthermore, it was also suggested that MECP2 might have a role in regulating genes involved in body habitus (7) because some of the patients with mutations in MECP2 have generalized obesity. For these reasons, we have screened for MECP2 mutations in patients with clinical features of AS or PWS without the molecular abnormalities on chromosome 15q. No mutations have been detected in either group, in accordance with a recent publication (24), although the number of cases we have studied was low. This is why we prefer to follow the recommendations of Kleefstra et al. (7), when they say that it is important to test MECP2 mutations in AS without molecular abnormalities on chromosome 15q11-q13, but not in Prader–Willi-like patients.

To summarize, in the Spanish patients studied, MECP2 mutations do not represent a major cause for NSMR, either in males or in females. This tells us that the implementation of a routine MECP2 mutations screening in these patients is not necessary unless they have some clinical symptoms of RTT. In conclusion, we recommend to the general practitioners and paediatricians doing

<table>
<thead>
<tr>
<th>Table 2. Clinical characteristics of the girl carrying the mutation: c.422A&gt;G (p.Y141C)</th>
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<tr>
<td>Main criteria for atypical RTT</td>
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<tr>
<td>Absence or reduction of hand skills</td>
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<tr>
<td>Reduction or loss of babble speech</td>
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<tr>
<td>Monotonous pattern of hand stereotypes</td>
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<tr>
<td>Reduction or loss of communication skills</td>
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<tr>
<td>Decrease of head growth from early childhood</td>
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<tr>
<td>Regression followed by recovery of interaction</td>
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<tr>
<td>Supportive criteria</td>
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<tr>
<td>Breathing irregularities</td>
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<tr>
<td>Bloating/air swallowing</td>
</tr>
<tr>
<td>Bruxism, harsh sounding type</td>
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<tr>
<td>Abnormal locomotion</td>
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<tr>
<td>Scoliosis/kyphosis</td>
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<tr>
<td>Lower limb amyotrophy</td>
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<tr>
<td>Cold, purplish feet, usually growth impaired</td>
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<tr>
<td>Sleep disturbances including night screaming</td>
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<tr>
<td>Laughing/screaming spells</td>
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<tr>
<td>Diminished response to pain</td>
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<td>Intense eye contact/eye pointing</td>
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MECP2 study in Spanish disabled patients
an exhaustive clinical examination of patients with MR before requesting the molecular MECP2 test or any other molecular test.

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References