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Molecular analysis of MECP2 gene mutations in Moroccan patients with Rett syndrome

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Abstract

Background: Rett syndrome (RTT) is a severe and progressive neurodevelopmental disorder, affecting 1/10,000–15,000 girls. It is one of the most common causes of mental retardation in females. This disorder results in 80% of cases from mutation of MECP2 gene (methyl-CpG binding protein 2), a transcriptional repressor involved in chromatin remodeling and the modulation of RNA splicing. MECP2 aberrations result in a constellation of neuropsychiatric abnormalities, whereby both loss of function and gain in MECP2 dosage lead to similar neurological phenotypes. Identified mutations are almost in de novo and familial cases are rare and due to X-chromosomal inheritance from a carrier mother.

Material Methods: This study represents the only MECP2 molecular analyze done on Moroccan patients with RTT, in which bidirectional sequencing of the entire gene coding and the flanking intronic sequences in 8 female patients provisionally diagnosed to have RTT was carried out.

Results: Four different pathogenic mutations c.397C>T [p.Arg133Cys], c.[916C>T(;)1208dupC], p.[Arg306Cys(;)Glu404X], c.1158_1186del [p.Pro387ArgfsX8], were identified in three patients and one polymorphism c.1-220dupC. All of them were located in exon four.

Conclusion: The present study allowed identification of mutations of MECP2 gene in Moroccan cohort and gave an appropriate genetic counseling to the mutation-positive patients.

Keywords: Rett Syndrome, Moroccan population, molecular analysis, MECP2 gene

Introduction

Rett syndrome (RTT [MIM 312750]) is an X-linked neurodevelopmental disorder that affects mainly female [1-4]. It is characterized by mental retardation, loss of acquired skills [especially purposeful hand use], and deceleration of head growth. A normal prenatal and postnatal development that lasts 8–30 months is followed by developmental stagnation and regression of mental and motor abilities. Diagnostic criteria and disease stages for RTT were established in 1985 [3]. Common clinical features include stereotypical hand movements, hyperventilation, seizures, growth retardation, scoliosis, and autonomic dysfunction. The prevalence of the disease is estimated to be 1/10,000–1/15,000 female births [3].

In addition to the classic form of RTT, five atypical variants have been delineated on the basis of clinical criteria. Each variant lacks some of the necessary criteria of the classic form and can be milder or more severe.

Most females with RTT survive to the middle age [5].

The RTT gene on Xq28 was identified as MECP2, which encodes the methyl-CpG–binding protein 2 that is normally involved in transcriptional silencing [6]. Loss of MeCP2 functions in the brain leads to reduction in neuronal size and number of dendrites causing deficits in synaptic formation and/or transmission [7].

Approximately 99.5% of RTT cases are sporadic. In the few familial cases, the mutation is either present in the asymptomatic mother or due to germline mosacisim in one of the parents.

MECP2 mutations were identified in 80% of patients throughout the coding region of the gene including missense mutations, nonsense mutations, small insertions or deletions, splicing mutations, and large rearrangements (duplications or complex deletions) [8].

Thus, in order to define, for the first time, the spectrum of mutations and clinical features in Moroccan children with RETT Syndrome, we carried out mutation screening of the entire coding region of MECP 2 gene in 8 Moroccan patients with RTT.

Materials and methods

RETT Syndrome was diagnosed by the presence of the major clinical characteristics in seven female patients. After obtaining informed

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Table 1: Theclinical phenotypes of the studied female patients

Patient number	Age at regression	Ability to walk	Ability to speak	Ability to use hand	Stereotypical hand movements	Postnatal microcephaly	Seizures	Respiratory disorders	Behavior abnormalities
P1	18 months	Walked with aid	lost	No hand use	Abnormal hand movements	Yes	No	No	No
P2	12 months	Never walked	lost	No hand use	Abnormal hand movements	Yes	No	No	Autistic features
P3	12 months	Never walked	No speech	Impaired hand use	Abnormal hand movements	Yes	Generalized seizures	No	No
P4	10 months	Walked with aid	No speech	lost	Abnormal hand movement	Yes	No	Yes	Autistic features
P5	16 months	Walked with aid	lost	Impaired hand use	Abnormal hand movement	Yes	No	Yes	Autistic features
P6	12 months	Never walked	lost	No hand use	Abnormal hand movement	Yes	No	Yes	Autistic features
P7	14 months	Started Walking late	lost	Impaired hand use	Abnormal hand movement	No	Tonico clonic epilepsy	No	No
P8	18 months	Walked with aid	lost	Impaired hand use	Abnormal hand movement	No	No	No	No

Table 2: RTT checklist for studied female patients (The checklist is quoted from Huppke et al. [10])

Clinical criterion	Patients with MECP2 mutation			Patients without MECP 2 mutation			
	P4	P5	P6	P1	P2	P3	P7
Normal prenatal and peri natal period	1	1	1	1	1	1	1
Normal psychomotor development during the first 6 months	1	1	1	1	1	1	1
Normal head circumference at birth	1	ND	ND	1	ND	1	1
Deceleration of head growth	1	1	1	1	1	1	0
Hand skills (1 if never, 2 if lost)	1	1	1	0	0	1	1
Stereotypic hand movements	1	1	1	1	1	1	1
Communication dysfunction and social withdraw	1	1	1	0	1	0	0
Acquired language(1 if never, 2 if lost)	2	2	1	1	1	1	1
Sever psychomotor retardation	1	1	1	1	1	1	1
Impaired or absent locomotion	1	1	1	1	1	1	1
Score	11	10	10	8	8	9	9

ND : not documented

Table 3: Mutations of the MECP2 gene detected in the studied RTT patients

Patient	Nucleotide change	Amino acid change	Mutation type	Domain
P 1	c.1-220dupC	No amino acid change	polymorphism	
P 4	c.397C>T	p.Arg133Cys	Missense	MBD
P 5	c.1158_1186del	p.Pro387ArgfsX8	Nonsense	
P 6	c.916C>T	p.Arg306Cys	Missense	TRD
	1208dupC	p.Glu404X	Nonsense	

consent, peripheral blood was obtained from each patient, and genomic DNA samples were extracted from blood lymphocytes using Gene Catcher™ Magnetic Beads Kit (Invitrogen).
For each patient, the three coding exons (exons 1, 3 and 4) and the flanking intronic sequences of MECP2 were amplified in overlapping fragments. Primers used for exons 3 and 4 were previously reported by Bienvenu et al. [9]. We have designed other set of primers to generate shorter fragments for sequencing.
The PCRs were performed in a 25-ml reaction volume containing 100 ng genomic DNA, 5 U Taq (invitrogen), 20 pmol each primer, 25 mM $MgCl_2$, 2.5 mM dNTP, and 10 x PCR Buffer (invitrogen), through use of a Veriti™ 96-well Thermal Cycler 9902 (Applied Biosystems). the PCR cycling condition was: 94°C for 6 min; 35 cycles of 94°C for 45 s, 63°C (Exon 2, 3A, 3B, 3C, 4A, 4B, 4C, 4D) and 58° (intron and exon 1) for 45 s, and 72°C for 45 s; 72°C for 10 min.
Bidirectional direct sequencing of purified PCR products was performed using the BigDye Terminator V1.1 Cycle Sequencing Kit (ABI Prism™) and an Applied Biosystems 3500Dx Genetic Analyzer. The chromatogram was analyzed by the Sequencing Analysis SeqA v.5.4 (Applied Biosystems). And the sequences obtained have undergone Bioinformatics Analysis using "nucleotide blast" Alignment Program (http://blast.ncbi.nlm.nih.gov).

Results

Clinical features

All patients were, basically, autistic, microcepphalic and showed repetitive stereotypic hand movements. Therefore, they were referred with a provisional diagnosis of Rett syndrome and requesting MECP2 molecular analysis. The Clinical criteria of the studied patients are summarized in Table 1and the score achieved by each patient according to RTT checklist described by Huppke et al.[10] is showed in Table 2.
Mutation analysis of the MECP2 gene
Molecular analysis of MECP2 gene in those patients revealed four different mutations in three unrelated patients. All the identified mutations are located in exon 4; one were within the MBD and one in the TRD (Fig. 1). These mutations have previously been reported as disease-causing mutations. The (table 3) below summarizes mutation screening result. Moreover, one other subject have shown a c.1-220dupC polymorphism in the exon 3.

Discussion

Clinical features
Our study covers 8 cases of Rett syndrome followed in neuropeadiatric consultation. Comparing our 8 cases among themselves and with the literature data, we can note that:

- The perinatal period and the development during the first months of life was normal. The same concept is reported in the literature.
- The beginning of disorders is between 6

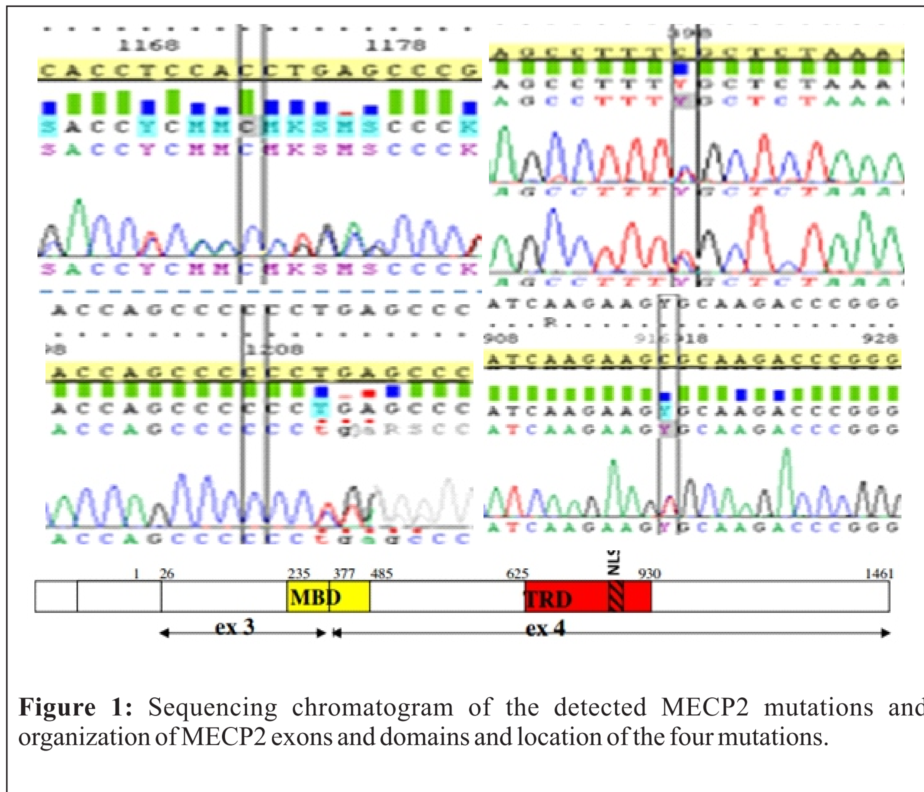

Figure 1: Sequencing chromatogram of the detected MECP2 mutations and organization of MECP2 exons and domains and location of the four mutations.

and 18 months according to what is described in the literature, sometimes preceded by a stabilization of acquisitions.

- Most of our patients are referred for psychomotor regression, microcephaly or stereotypic hand movements.

According to Fig 1:

- All of our patients have trouble walking, speech disorder and hand stereotypies.
- 80% of patients present disorders of hand use, postnatal microcephaly and autism.
- 37.5% of patients present respiratory disorders. Hyperventilation with respiratory blockage is indicated in patients 4, 5 and 6. These respiratory disorders are frequently found in various published observations They occur in waking state, especially when the child is under emotional stress,
- 25% of patients had scoliosis. According to Bassett and Tolo, scoliosis is present in two thirds of patients with Rett syndrome, in an age between 11 and 15 years. Other authors report a frequency between 42 and 83%.
- Two of our patients report the concept of epilepsy.

Mutation analysis

RTT is one of the most common causes of mental retardation in females with a prevalence estimated to be 1 in each 10,000:15,000 female births [11, 12]. MECP2 gene mutations were identified to be the major cause of RTT. They are found in 80–90% of classic RTT patients and in 20–40% of patients with RTT variants [10]. Detection of the underlying cause in RTT patients will confirm the diagnosis, helping clinicians to manage their patients better and to offer precise counseling. Furthermore, it may provide insight regarding genotype-phenotype correlation. This study represents the first molecular analyses of MECP2 gene in Moroccan patients with RTT. Direct sequencing of the three coding exons (exons 1, 3 and 4) and the flanking intronic sequences of MECP2 of 8 female patients that included in this study revealed four different pathogenic mutations in three unrelated patients; two missense and two non-sense. Generally, MECP2 mutations were detected in about 80% of RTT patients [13, 14]. However, some previous studies showed relatively low rate of mutation detection and this mainly might depend on the clinical selection of the studied patients. Xiang et al. [15] screened the MECP2 gene for mutations by direct sequencing in 68 RTT cases and only a total of 27 patients (40%) were found to have mutations in the MECP2 gene. Raizis et al.[16] analyzed the MECP2 coding region by both direct automated DNA sequencing and MLPA in 74 patients with global developmental delay and mental retardation from New Zealand. The MeCP2 mutations among this selected group were only 20%. The checklist for RTT described by Huppke et al. [10] seems to be effective in giving a better screening tool. According to this checklist, molecular analysis should be carried out only in patients achieving a score of 8 or more out of 12. In this study, patients with detected MECP2 mutations had a score of 10 at least.

Most MECP2 mutations are de novo. The leading hypothesis holds that MeCP2 dysfunction resulting from mutation in the TRD or MBD disrupts the delicate precision of gene expression during development. Some mutations affect residues that are important for DNA binding, whereas others may disrupt the native structure of the protein and/or its interactions with other proteins. The documented nonsense, frameshift, and splicing mutations, most of which are distal to the MBD, likely result in premature termination of the protein. Truncated proteins may still bind methylated DNA but be unable to interact with the corepressor Sin3A; it is also possible that mutations in the carboxy terminus of the protein disable DNA binding [17]. In either case, the silencing complex would not be properly assembled and the target genes could not be properly silenced.

The four mutations identified in this study were p.Arg133Cys, p.Pro387ArgfsX8, p.Arg306Cys, and p.Glu404X. In previous reports, p.Pro387ArgfsX8 and p.Glu404X showed low recurrence rate. In contrast, R133C and R306C are of the most commonly occurring MECP2 mutations accounting for about 5.4% and 6.4% of RTT patients respectively [18]. Generally, it has been shown that the missense mutations were associated with milder phenotypes than truncating mutations [19,20]. Ham et al. [21] revised the mutations detected in 45 patients reported in 4 studies [22–25] with the milder RTT variants. They found that those patients mainly had carboxyl-terminal truncations and eight missense mutations. Of these missense mutations were P127L, R133C and R306C. The other five missense mutations identified were E10Q, T158 M, T158A, R168X and P302A. Hence, they inferred that a patient with a mild phenotype is likely to disclose either a carboxyl-terminal truncation or one of these

missense mutations [21]. In our study, the mutations R133C and R306C were associated with classical course of RTT rather than a milder variant.
Leonard et al., 2003 [26] studied 24 patients having R133C to examine the phenotype associated with this mutation specifically and they found that the phenotype of a patient with R133C mutation is overall milder with better ambulation, hand use and a greater likelihood of being able to use speech [27]. Subsequently, Neul et al. [28] studied a large cohort of 542 patients with typical RTT. They identified R133C in 12 patients and reported that it was associated with relatively mild phenotype. Most patients with R133C preserved some hand use (92%), a large percentage was able to walk alone (75%) and a significant proportion spoke words (50%). On the other hand, R306C was identified in another 21 patients and it was shown that a large set of patients with R306C could walk (67%) and retained some hand use (52%), but very few were able to use words. In our study, both patients with R133C and R306C showed lack of their ability to walk and use their hands.
Patient 6 with R306C didn't walk she has two pathological mutations and presents sever psychomotor retardation.
Previous reports documenting that truncating (nonsense and frameshift) mutations, in general, are associated with a more severe disease phenotype than missense mutations [19, 20]. They suggested that mutations leading to a truncation of the NLS produce proteins that will remain in the cytoplasm with more loss of protein function. Our patient with Nonsense mutations showed a classical phenotype with normal development at the first months of life and no seizures in early life denoting that she is not a case of congenital or early onset seizures RTT variant. However, she assessed a relatively high combined severity score for the abilities of hand use, speech and walking. They also present respiratory dysfunction.
Among the non-mutated patients, patient 2 and 3 where monozygotic twins with typical table of Rett syndrome.They also present bilateral blindness. We could achieve this in a consanguineous family homozygosity mapping to search of a new area or a new candidate gene for RTT.

Conclusion

This is the first genetic studies of Rett syndrome in Morocco. it is important for clinicians to be aware of this disorder because increased identification will help in greater understanding of this disorder and proper guidance will help the patient and family, and reduce the burden of care on the parents. If possible, genetic analysis or gene mapping should be carried out. Thus, it is suggested that all female children presenting with low intelligence and autistic symptoms should be suspected of having Rett's syndrome until proved otherwise.

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