MECP2 mutation in male patients with non-specific X-linked mental retardation

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Abstract In contrast to the preponderance of affected males in families with X-linked mental retardation, Rett syndrome (RTT) is a neurological disorder occurring almost exclusively in females. The near complete absence of affected males in RTT families has been explained by the lethal effect of an X-linked gene mutation in hemizygous affected males. We report here on a novel mutation (A140V) in the *MECP2* gene detected in one female with mild mental retardation. In a family study, the A140V mutation was found to segregate in the affected daughter and in four adult sons with severe mental retardation. These results indicate that *MECP2* mutations are not necessarily lethal in males and that they can be causative of non-specific X-linked mental retardation.

Key words: X-linked mental retardation; *MECP2* gene; Missense mutation; Development

1. Introduction

X-linked mental retardation is a common clinical condition that can be caused by defects in any of a number of genes [1–7]. Among the well characterized X-linked conditions causing mental retardation, mutations in the methyl-CpG binding protein 2 (*MECP2*) gene on Xq28 have been found in patients with Rett syndrome (RTT), a neurological condition [8,9] which, in addition to other symptoms, severely affects higher cognitive functions in females. A wide degree of phenotypic variability has been observed in patients with *MECP2* gene mutations [10–13], and it has been explained either by skewed X chromosome inactivation (XCI) or by the type and the relative position of the mutation observed. The phenotypic variability in these patients ranges from display of all symptoms of RTT to essentially only mild mental retardation [10–13]. To further investigate the occurrence of *MECP2* mutations in mental retardation, we performed a mutation analysis of the *MECP2* gene in 76 female patients with various types of mental retardation. Novel and known mutations in the *MECP2* gene were found in patients with classical and variant RTT and in a patient who presented with full classical autism and some of the signs of RTT. In addition, a novel mutation (A140V) in the *MECP2* gene was detected in one female with mild mental retardation. The A140V mutation was found to segregate in the affected daughter and in four adult sons with severe mental retardation. These findings indicate that *MECP2* mutations are not necessarily lethal in males and can be causative of non-specific X-linked mental retardation.

2. Materials and methods

Genomic DNA from leukocytes of peripheral venous EDTA blood or from lymphoblastoid cell lines was extracted by standard procedures [14]. The androgen receptor polymorphism was always analyzed in DNA from peripheral blood leukocytes. All the exons and the flanking regions of the *MECP2* gene were amplified by PCR as described [10]. Heteroduplex analysis was performed on a WAVE™ denaturing high-performance liquid chromatography (DHPLC) instrument (Transgenomic). The WAVE utility software was used to determine the correct temperature for mutation scanning based on the sequence of the wild-type DNA. Direct sequencing of the PCR products was performed using the automated fluorescence-based dideoxynucleotide termination method (model 310 ABI, PE Biosystems, Foster City, CA, USA). Both strands were sequenced to confirm all the mutations detected. Sequencing results were compared with the reference human *MECP2* sequence (GenBank accession number X99686).

3. Results

In order to further analyze the role of *MECP2* gene mutation in RTT and general mental retardation, 76 unrelated females with mental retardation were screened for mutations in this gene. Towards this aim, all the exons and the flanking regions of the *MECP2* gene were amplified by PCR and analyzed for heteroduplex formation by DHPLC and by direct DNA sequencing. In the course of the study, three silent polymorphic variants were found. These include a previously reported [10] silent conservative polymorphism (656C→T) found in two unrelated patients (X119 and X226), and two novel polymorphisms, (1145C→T), changing codon 357 from AGC to AGT, both encoding serine, found in patient X388,

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and a IVS2 −61C→G, which does not alter the site of splicing, found in patient X231.

Mutations in the *MECP2* gene were found in three of the five patients with clinical features that fulfilled the criteria for RTT [15–16]. In patient CL90, a 502C→T mutation was found that results in R168X. In patients CL82 and CL91 a 763C→T mutation resulting in R255X was observed (Table 1). Both mutations have been previously reported [11]. The R255X mutation was also identified in one patient (C4) out of four who were clinically assessed as RTT variants, as they did not show the full clinical spectrum of RTT symptoms [17–19]. Patient C4 is an 11-year-old girl. The first 20–24 months of her psychomotor development were reported to be normal. After the age of 2, she became less reactive and began to show stereotyped hand movements and teeth grinding (bruxism). Currently, she presents with severe mental retardation and speech impairment and displays an unusually aggressive character. She is able to ambulate independently and to run and can use her hands adequately for her mental age. There is no evidence of kyphoscoliosis, microcephaly, hyperventilation, or seizures. As a whole, the clinical features had suggested that this case was an atypical and milder RTT variant. The XCI pattern of patient C4 was evaluated, as females with completely skewed XCI present a closer to normal phenotype [10–13,20,21]. Androgen receptor polymorphism detection [20,21] of patient C4 revealed a skewed ratio of androgen receptor alleles of more than 80% (data not shown).

A novel *MECP2* missense mutation was detected in one of 42 patients with autistic disorder diagnosis. The affected patient, X44, is a severely retarded girl who presents a full autistic picture with impairment of speech and communication. She is a 12-year-old girl, adopted at 2 years of age from a Romanian institute and little is known about her early development. At 5 years she had an EEG with bouffees of theta waves which could be compatible with a third stage of RTT syndrome EEG. Now she presents a full autistic picture, shows 'squeezing' stereotypic activities of her hands, which are frequently clapped together, and bruxism. She has significant difficulties in social interaction and communication, although in recent years she has learned to pronounce a few words. She walks without assistance and has good use of her hands; there is no evidence of seizures, kyphoscoliosis and microcephaly. The diagnosis of autism was based on clinical features and evaluated by diagnostic criteria from the Autism Behavior Checklist and DSM-IV [22,23]. Following a more recent clinical evaluation, the girl was also found to display five of the diagnostic criteria for RTT syndrome, which would also qualify her as a RTT variant, in addition to autistic features. The mutation (682C→T) found in this patient results in the substitution T203M. The amino acid residue at position 203 is not conserved among different species. In human it is T, in mouse A, in chicken G, and in *Xenopus laevis* K [24,25]. Nevertheless, the 682C→T mutation was not observed in 200 normal X chromosomes, and, as it occurs near the transcription repression domain start site (codons 207–

Table 1
MECP2 mutations and genotype–phenotype relationships

Patient	Nucleotide change	Amino acid change	Phenotype
CL90	C502T	R168X	RTT
CL82	C763T	R255X	RTT
CL91	C763T	R255X	RTT
C4	C763T	R255X	RTT variant
X44	C682T	T203M	Autistic/RTT variant
X309	C493T	A140V	Mild non-specific MR
MR49	C493T	A140V	Mild non-specific MR
X307	C493T	A140V	Severe non-specific MR
MR48	C493T	A140V	Severe non-specific MR
MR50	C493T	A140V	Severe non-specific MR
X308	C493T	A140V	Severe non-specific MR
MR47	–	–	Normal

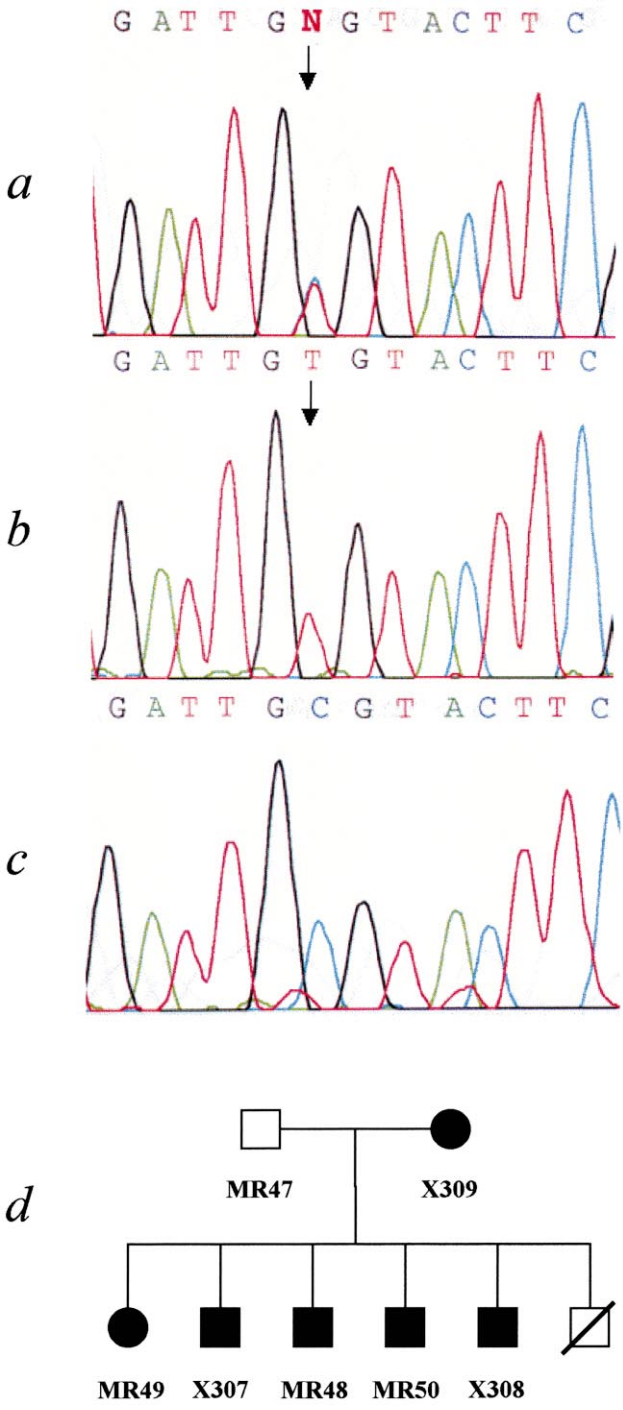

Fig. 1. Electrophoretograms showing 493C→T found in the family of patient X309: (a) X309 (affected heterozygote); (b) X307 (affected hemizygote); (c) MR47 (normal hemizygote); (d) pedigree of the family of patient X309.

310), it may result in a protein with altered function [10–13]. The XCI pattern of this patient was found to be close to random (data not shown).

In a third group of 25 females considered to be mildly mentally retarded, a novel missense mutation in *MECP2* was found in patient X309 (Fig. 1). This patient is a 63-year-old mildly retarded female with microcephaly, asternic habitus with poorly muscled build, speech difficulties, genu valgum and gait disturbance. The mutation (493C→T) results in an A140V substitution. The alanine in position 140 is located in the methyl-CpG binding domain (MBD) and is strictly conserved from mammals to *Xenopus laevis* [10]. Five missense mutations causing classical RTT have been already identified in the MBD (R106W, R133C, F155S, F155I and T158M), but mutations of residue 140 have not been found in any of the patients with classical RTT and RTT variant (approx. 250) previously described [10–13,26]. The MBD of MeCP2 can recognize a single symmetrically methylated CpG in the major groove of DNA. This domain consists of a wedge-shaped structure with an α-helix constituting one face of the wedge and with four antiparallel β-strands constituting the other face of the wedge (Protein Data Bank ID 1QK9 [27]). Interestingly, A140 is located in the middle of the α-helix. Using several programs for secondary structure prediction (http://pbil.ibcp.fr), the substitution of a valine for alanine at position 140 appears to shorten the α-helix length by half. We therefore propose that A140V has a subtle effect on MeCP2 function by altering the wedge-shaped structure of the MBD.

Investigation of the family of patient X309 revealed that she had five living children. Of these, four sons presented with severe mental retardation and movement disorders. The single daughter is a 41-year-old woman, who presented with a mild mental handicap with clinical features similar to those of her mother. The four affected sons, aged 27–40 years, have a normal head size, severe mental retardation with friendly personalities, impaired expressive language development, resting tremors, and slowness of movements. These subjects did not show a history of regression of higher brain functions after an initial normal development. In particular, there was no deterioration in language skills, which always appeared very poor. Affected males put only some words together and communicate in short and simple sentences, and the relatively better speech of affected females appears related only to simpler aspects of their everyday life. None of the affected members had autistic-like behaviors. An apparently unaffected son died accidentally when he was 4 years old. Following detection of the A140V mutation in the mother, the family was clinically re-evaluated and the *MECP2* genotype determined in all available family members. All the affected sons (X307, MR48, MR50 and X308) and the daughter (MR49) were found to carry the A140V mutation. The mutation was not detected in the normal father (MR47) nor in 300 X chromosomes from normal individuals. XCI patterns of patient X309 and her daughter (MR49) were analyzed at the androgen receptor locus and revealed a close to random pattern of inactivation (data not shown).

4. Discussion

The finding of a *MECP2* mutation in a patient with a diagnosis of autism, together with similar findings reported elsewhere [11,28], suggests that females with unexplained mental retardation, especially individuals with autistic-like behavior, should be screened for mutations in the *MECP2* gene. As suggested by Cheadle et al. [13], missense mutations, like A140V, could correlate with milder diseases than those sustained by truncating mutations, possibly through the presence of a residual protein function. Previous to this report, RTT alleles were thought to be lethal for males [29]. The lack of surviving affected males in the few known RTT families and the absence of spontaneous cases of affected males with classical RTT supported this contention [10,11,29]. Further evidence that non-functional *MECP2* alleles were lethal for males has also been provided by experimental work with embryonic stem (ES) cells carrying the null *MECP2* allele. Deletion of the *MECP2* gene in male murine ES cells affects the ability of these cells to progress through development, indicating that a functional *MECP2* gene is essential for cell development and differentiation during embryogenesis [30]. In this context, the analysis of the family of patient X309, where a mutation in *MECP2* is observed in mentally retarded adult males, inherited from the affected mother, indicates for the first time that not all mutations in *MECP2* are necessarily lethal in males and indicates that the *MECP2* gene is a potential candidate in non-specific X-linked mental retardation in both female and male patients.

Clinical evaluation of these patients agrees with the A140V being a mild mutation, as the female patients, who have a random XCI pattern (at least in peripheral blood leukocytes), only present with a mild form of mental retardation, while the more severe form of mental retardation in the sons is likely due to their hemizygous condition. Therefore, the A140V mutation in males appears to allow the execution of the developmental program involved in birth and survival of male individuals, yet it can still cause a severe form of mental retardation in these patients.

Thus the *MECP2* gene is a potential candidate in non-specific X-linked mental retardation (MRX) in both female and male patients, and a likely candidate for those MRX families (MRX3, MRX16, MRX25 and MRX28) previously mapped to loci on Xq28 [3,31]. Our findings support that the phenotypic spectrum due to *MECP2* mutations can extend beyond the traditional RTT diagnostic boundaries. As more becomes known about the interacting and/or target proteins associated with MeCP2, we envisage that molecular analysis of the different *MECP2* alleles and especially of the A140V mutation could provide insight into the roles and epigenetic mechanisms of the *MECP2* gene product in various developmental programs.

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