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Mutations in the *MECP2* Gene Are Not a Major Cause of Rett Syndrome-Like or Related Neurodevelopmental Phenotype in Male Patients

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Rett syndrome is a genetic neurodevelopmental disorder that affects mainly girls, but mutations in the causative *MECP2* gene have also been identified in boys with classic Rett syndrome and Rett syndrome-like phenotypes. We have studied a group of 28 boys with a neurodevelopmental disorder, 13 of which with a Rett syndrome-like phenotype; the patients had diverse clinical presentations that included perturbations of the autistic spectrum, microcephaly, mental retardation, manual stereotypies, and epilepsy. We analyzed the complete coding region of the *MECP2* gene, including the detection of large rearrangements, and we did not detect any pathogenic mutations in the *MECP2* gene in these patients, in whom the genetic basis of disease remained unidentified. Thus, additional genes should be screened in this group of patients.

Keywords: autism; neurodevelopment; Rett

Rett syndrome (MIM 312750) is a neurodevelopmental disorder affecting mainly girls that combines mental retardation, autism, and neurological features.[1] Amir and colleagues[2] identified as the genetic cause of Rett syndrome mutations in the X-linked methyl CpG-binding protein 2 gene (*MECP2*), which encodes the MeCP2 protein, that binds methylated DNA and is thought to repress transcription of target genes. Classically, patients with this syndrome present an apparently normal initial developmental period (6 to 18 months of age) followed by an arrest and then regression of development, with deceleration in head growth, psychomotor delay and loss of acquired social and motor skills, and appearance of stereotypies.[1] The atypical forms of the disorder may present as either milder or more severe phenotypes. Given the very low frequency of male cases with Rett syndrome, the disorder was initially thought of as an X-linked dominant disorder, usually fatal in males. However, the low frequency of male cases could also be due to the fact that most mutations in *MECP2* occur *de novo* in paternal transmissions.[3] In the small number of males with Rett syndrome described in the literature, classical Rett syndrome is associated with (1) a somatic mosaicism of the mutation, (2) a Klinefelter syndrome (47, XXY), or (3) a hypomorphic *MECP2* allele.[4-7] As happens with other X-linked dominant disorders, the hemizygous males most often exhibit a more severe phenotype and most of the mutations that cause classical Rett in females originate a very different clinical presentation in males.[3,8] The most common phenotype associated with *MECP2* mutation in males is a severe neonatal encephalopathy (see supplementary review Table S1).[4,8,15-17,20,23,24,28]

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Table S1. Phenotypes Exhibited by *MECP2* Mutation-Positive Male Patients Described in the Literature

Domain	Mutation	Somatic Mosaicism	Clinical Presentation	Reference
	Missense			
MBD, ATRX	**E137G**	**Nonmosaic**	**MR**	1
	A140V	**Nonmosaic**	**MR, Schizophrenia/psychosis**	2-6
	F157I	NA	Progressive neonatal encephalopathy	7
		Nonmosaic	**Neonatal encephalopathy**	8-11
	T158M	NA	Angelman syndrome, neonatal encephalopathy	
		Klinefelter syndrome	Classical RTT	
interdomain	**R167W**	**Nonmosaic**	**MR**	1
TRD	P225L	NA	Atypical RTT	12
	K284E	NA	MR	3
WW	P399L	NA	MR	3
	P405L	**Nonmosaic**	**MR**	12
	K417M	**Nonmosaic**	**Neonatal encephalopathy**	7
	G428S	**Nonmosaic**	**Nonfatal, nonprogressive encephalopathy**	13
	R453Q	NA	MR	3
	Truncating			
All	P81fsX89	Somatic mosaicism; NA	Encephalopathy; Angelman syndrome-like	14,15
TRD-NLS	**E258X**	**Nonmosaic**	**Neonatal encephalopathy**	16
	G269fs	NA	Neonatal encephalopathy	7, 17
	R270X	Somatic mosaic	Classical RTT	18
	R270fs	NA	Neonatal encephalopathy	7
TRD	**P273fs**	**Nonmosaic**	**Congenital RTT**	19
	X288	NA	Neonatal encephalopathy	20
WW	**P384fs**	**Nonmosaic**	**Neonatal encephalopathy**	8
	L387fsXM466	**Nonmosaic**	**MR**	21
	G406X	**Nonmosaic**	**MR + progressive spasticity**	22
	R471fs	NA	Prader-Willi syndrome-like	23

MR, mental retardation; RTT, Rett syndrome; NA, not available.

However, mutations in this gene have been identified in patients with X-linked mental retardation associated with neurological features, such as limb spasticity with truncal and facial hypotonia,[19,26,27,29] with nonspecific mental retardation[12,14,30,31] and autism.[32] *MECP2* mutations have also been identified in male patients with language disorder and schizophrenia,[11] with an X-linked syndrome, including psychosis, pyramidal signs, and macro-orchidism,[13] and with Prader-Willi and Angelman syndrome-like phenotypes.[18,22,27] For this reason, in a research context, the molecular testing of *MECP2* gene in males with a wide spectrum of neurodevelopmental disorders such as unexplained early neonatal death, neonatal encephalopathy, or mental retardation, with or without neurological signs, has been proposed, even in the absence of familial history of similar disease, to further characterize the role of *MECP2* as an etiologic factor in this wide range of diseases.

The aim of the current study was to identify the extent to which mutations in the *MECP2* gene could explain the neurodevelopmental phenotypes presented by a group of 28 male patients of Portuguese origin displaying neurodevelopmental phenotypes compatible with those previously associated with *MECP2* mutations, including 13 patients with "Rett-like" features.

Material and Methods

Patients

The 28 Portuguese male patients selected for this study were sent to our laboratory by clinical geneticists, pediatricians, or pediatric neurologists to be tested for *MECP2* gene mutations. A questionnaire asking for clinical, molecular, and familial information was filled in by the clinicians requesting the diagnosis; a summary of the clinical presentation for all the patients is presented in Table 1. Analysis of this information suggested to us that 13 of the patients had a clinical presentation compatible with a variant form of Rett syndrome (Rett syndrome-like), according to the criteria defined by Hagberg and colleagues[33] (group I, patients 1 to 13 in Table 1); whereas the others did not fulfill the criteria but had clinical manifestations previously seen in *MECP2* mutant males, namely mental retardation of unknown etiology with one or more of the following features: autism, microcephaly,

Table 1. Clinical Manifestations Presented by 28 Boys With a Neurodevelopmental Disorder

Patient	Psychomotor Developmental Delay	Deceleration in Head Growth (age, months)	Microcephaly	Period of Regression (age months)	Autism (Presently?)	Type of Hand Stereotypies					
						Washing	Wringing	Mouthing	Patting/ Clapping	Flapping/ Waving	Other
1	Yes	Yes (3)	Yes	No	Poor social contact	Yes	No	Yes	No	No	No
2	Yes	No	No	No	Yes	Yes	No	No	No	Yes	No
3	Yes	No	Yes	No	No	No	No	No	Yes	Yes	Hyperventilation
4	Yes	Yes (6)	Yes	No	Yes	Yes	No	No	No	No	No
5	Yes	Yes (12)	Yes	Yes (12)	No	Yes	No	Yes	Yes	No	No
6	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No
7	Yes	Yes (6)	Yes	Yes (3)	Yes	No	No	No	Yes	Yes	No
8	Yes	No	No	Maybe (18)	Yes	No	No	No	No	Yes	No
9	Yes	Yes (6-12)	Yes	Yes (6-7)	Yes	No	No	Yes	No	No	No
10	Yes	Yes (6-12)	No	Yes (5)	Yes	Yes	No	No	Yes	No	No
11	Yes	No	No	No	No	No	No	Yes	No	Yes	No
12	Yes	No	No	No	Yes	Yes	No	Yes	Yes	No	Inferior limbs
13	Yes	Yes (4)	Yes	Yes (6)	Yes	No	No	Yes	No	Yes	No
14	Yes	Yes (11)	Yes	Yes (11)	No	Yes	No	No	Yes	Yes	Laughing spells
15	Yes	No	No	Yes (18)	Yes	No	No	No	Yes	Yes	No
16	Yes	Yes (8)	Yes	No	Yes	Yes	No	No	No	No	No
17	Yes	Yes (9)	Yes	No	Yes	No	No	Yes	Yes	Yes	Hyperventilation
18	Yes	No	No	No	Yes	Yes	No	No	No	No	No
19	No	No	No	No	No	Yes	No	No	No	No	No
20	Yes	No	No	No	Yes	No	No	No	No	No	No
21	Yes	No	No	No	Yes	No	No	No	No	No	No
22	Yes	No	No	No	No	No	No	No	No	No	No
23	Yes	No	No	No	No	No	No	No	No	No	No
24	Yes	na	Yes	na	No	na	na	na	na	na	na
25	Yes	na	na	na	na	na	na	na	na	na	na
26	No	No	No	No	No	Yes	No	No	No	No	No
27	Yes	No	No	No	No	Yes	No	No	No	No	No
28	Yes	Yes (6-12)	No	Yes (6)	Yes	No	No	Yes	Yes	No	No

Table 1. (continued)

Patients	Fine Motor Skills Loss?	Epilepsy	Affected Language	Dysmorphic Face	Familial History of Disease
1	No	Yes	Yes	No	No
2	No	Yes	No	Yes	No
3	No	Yes	Yes	Yes	No
4	No	Yes	Yes	No	No
5	Yes	No	Yes	No	Yes
6	Never acquired	Yes	Yes	No	No
7	Yes	No	Yes	No	No
8	No	Yes	Yes	No	No
9	Yes	Yes	Yes	Yes	No
10	Yes	Yes	Yes	No	No
11	Never acquired	No	Yes	No	No
12	No	No	Yes	No	No
13	No	Yes	Yes	No	No
14	No	Yes	Yes	Yes	No
15	No	No	No	No	No
16	No	Yes	Yes	Yes	No
17	No	Yes	Yes	No	No
18	No	No	No	No	No
19	No	Yes	No	No	No
20	No	No	No	No	No
21	No	No	No	No	No
22	No	No	No	No	Yes
23	No	No	No	No	No
24	Na	Yes	No	No	No
25	na	Yes	No	No	No
26	No	Yes	No	No	No
27	No	No	No	No	Yes
28	Yes	No	Yes	No	No

na, not available.

Table 2. Age, Karyotype and Molecular Exclusions of 28 Boys With Neurodevelopmental Disorder

Case ID	Age	Karyotype	Angelman Syndrome	ATR-X	Fragile X	Other
1	8	46, XY	Negative		Negative	
2	6		Negative			
3	19		Negative			
4	5		Negative			
5	5				Negative	FISH (subtelomeric probes)
6	11		Negative		Negative	
7	15		Negative			
8	5				Negative	
9	17		Negative	Negative	Negative	FISH (subtelomeric probes)
10	17		Negative		Negative	FISH (subtelomeric probes)
11	12		Negative			
12	7		Negative			
13	4					FISH (subtelomeric probes)
14	13		Negative			
15	19				Negative	
16	8		Negative			
17	4		Negative		Negative	
18	15				Negative	
19	7					
20	15				Negative	
21	7				Negative	
22	21				Negative	
23	8					
24	12					
25	13					
26	8		Negative			
27	5		Negative			
28	na		Negative			FISH (subtelomeric probes)

FISH, fluorescent *in situ* hybridization; na, not available.

epilepsy, manual apraxia, and/or stereotypies (group II, patients 14 to 28 in Table 1). Patients 1, 2, 4, 8, 9, 10, 16, 26, and 28 presented an epileptogenic encephalopathy. Patients 14 and 15 presented with visual and hearing deficits, not typical of Rett syndrome, and so they were not included in group I. Metabolic studies were performed for all patients and did not reveal any alterations. Informed consent for the genetic study was obtained from all patients or their legal representatives.

Molecular analysis:

Genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA isolation kit (Gentra, Minneapolis, Minn). The coding region and exon–intron boundaries of the *MECP2* gene were amplified by polymerase chain reaction (PCR); the amplified fragments were automatically sequenced and analyzed for point mutations or small rearrangements. Sequence changes were confirmed by re-amplification with genomic DNA and sequencing in the opposite direction. The RD-PCR method[34] was used for the detection of large duplications of the *MECP2* gene. Primers used, PCR mixtures and cyclic conditions, are available on request.

Results

Table 1 presents a summary of the clinical presentations of the patients we analyzed. The patients' age at the last observation ranged from 4 to 21 years (mean 11 years). Some of the patients had previous molecular exclusion of other clinical conditions and their karyotypes had all been assessed as normal (Table 2). Analysis of the DNA sequence of the *MECP2* gene in all patients (group I and group II) did not reveal any potentially pathogenic mutation. We found a silent mutation in the *MECP2* gene in one patient (897C>T; T299T) and an intronic deletion of one nucleotide (IVS3-17delT) in another patient, both already described as polymorphisms in the literature (http://mecp2.chw.edu.au/mecp2/). We also searched for large duplications or deletions in the *MECP2* gene in both groups of patients but did not find any alteration in the exons or gene dosage.

Discussion

Male patients with neurodevelopmental disorders present a wide spectrum of phenotypes and share a combination of symptoms, which encompass mental retardation, autism,

epilepsy, and movement disorders. In most cases the genetic basis of the pathology is unknown and *MECP2* is an interesting candidate gene to be analyzed.

Mutations in *MECP2* are found in female patients with heterogeneous clinical presentations; contributing to this fact are the effects of X-chromosome inactivation patterns as well as a potentially significant influence of epistatic, epigenetic, or environmental modifiers. Furthermore, mutations known to be Rett syndrome-causing in females do not produce similar phenotypes in males, due to the X-linked dominance of the disorder[6] and possibly to differences in the above-mentioned modifier effects. The heterogeneity of phenotypes associated with *MECP2* mutation in males (supplementary Table S1) opened the possibility that mutations in this gene could underlie a variety of X-linked neurodevelopmental phenotypes.

We were not able to identify any mutation in the *MECP2* in our sample of boys with Rett syndrome-overlapping (Rett syndrome-like) phenotypes, including the large duplications of this gene, which were described to be frequent in mentally retarded males with progressive neurological symptoms.[29] Mutations in the noncoding regions of *MECP2,* introns and regulatory 5′untranslated region and 3′untranslated region, might have been missed since these regions were not covered in this study. However, although Shibayama et al[35] reported that 3′untranslated region variants in the *MECP2* gene seemed to be more frequent in autism patients than in the general population, we have previously searched for mutations in the 3′untranslated region of the *MECP2* gene in a group of Portuguese Rett-syndrome female patients and we did not find any pathogenic mutation[36] suggesting that this must be a rare cause of Rett syndrome.

Our data suggests that, prior to the indication of systematic molecular testing of *MECP2* in all males with neurodevelopmental pathologies, the study of larger population series should be performed. In fact, the majority of male patients with Rett syndrome-like symptoms do not present mutations in the *MECP2* gene, which is in favour of the hypothesis that mutations in other gene(s) may be involved in this disorder.[37] Even using a stricter phenotype definition, there are males with a clinical diagnosis of Rett syndrome without identified *MECP2* mutation.[5]

There are a number of genes in which mutations have been found in patients with pathologies that partially overlap Rett syndrome, which it would be interesting to test in patients with Rett syndrome or a Rett syndrome-like clinical presentation without a *MECP2* mutation: neuroligin 3 and neuroligin 4, mutated in patients with autism, mental retardation or Asperger syndrome,[38] the aristaless-related homeobox[39] and the serine/threonine kinase 9 genes, mutated in patients with West syndrome [40] and ubiquitin-protein ligase E3A (involved in Angelman syndrome)[41] are interesting candidate genes. Brain derived neurotrophic factor and distal-less homeobox 5; two downstream target genes of MeCP2 regulation,[42-44] would also be potentially important candidates for future analysis. Nevertheless the possibility remains that additional novel genes may be identified as the molecular basis of disease in these patients.

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