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MECP2 analysis in mentally retarded patients: implications for routine DNA diagnostics

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Rett syndrome (RTT) is one of the most common neurodevelopmental disorders in females. The disease is caused by mutations in the methyl-CpG-binding protein 2 gene (*MECP2*), and various mutations have been reported. The phenotypic spectrum in both female and male patients is diverse, ranging from very mild to congenital encephalopathy and prenatal lethality. In this study, the question was addressed as to whether implementation of systematic screening of *MECP2* in patients with an unexplained mental retardation in DNA diagnostics would be reasonable, and the spectrum of phenotypes resulting from mutations in this gene was further explored. Mutational analysis of *MECP2* was performed in mentally retarded female patients who were negative for *FMR1* CGG repeat expansion, in male and female patients with clinical features suggestive of either Angelman or Prader-Willi syndrome without methylation defects on chromosome 15q11–q13. In the cohort of females negative for the molecular Fragile-X studies ($N=92$), one nonsense mutation (p.Q406X) was found. In the cohort of Angelman-negative patients ($N=63$), two missense mutations (p.R133C in a female patient and a mosaic p.T158M in a male patient) were found, which have been reported many times in patients with classical RTT syndrome. In the Prader-Willi-negative group ($N=98$), no pathogenic mutations were found.

The results support testing of patients with features suggestive of Angelman syndrome, but without methylation defects on chromosome 15q11–q13 for mutations in *MECP2*. In the remaining patients with unexplained mental retardation, additional clinical features should determine whether analysis of *MECP2* is indicated.

European Journal of Human Genetics (2004) 12, 24–28. doi:10.1038/sj.ejhg.5201080
Published online 15 October 2003

Keywords: MECP2; routine diagnostics; unexplained mental retardation

Introduction

Heterozygous mutations in the X-linked *MECP2* gene have been first reported in Rett syndrome (RTT), a progressive neurologic developmental disorder, occurring almost exclusively in females.¹ In approximately 95% of patients,

these mutations occur *de novo*, and it has been shown that in most cases they are of paternal origin.²

Recent studies indicate that females with RTT appear to represent a more heterogeneous phenotype than was first realized.^{3–5} There are cases with a very mild phenotype, with preserved speech variant or with a congenital, early seizure onset. It is hypothesized that these differences are mainly due to the genotype, variation in X-inactivation patterns and probably other polygenic modifiers.^{3,6}

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Received 7 March 2003; revised 1 July 2003; accepted 9 July 2003

Unlike previous thoughts, *MECP2* mutations are not necessarily prenatally lethal in males, and are the cause of a variable phenotype, ranging from lethal congenital encephalopathy to Angelman-like phenotype and mild nonspecific X-linked mental retardation (MRX).^{7–12} Since the complete phenotypic spectrum of *MECP2* mutations in both male and female patients is presently unknown, more studies are required. Previously, we reported on *MECP2* mutation analysis in a cohort of 475 mentally retarded males who were negative for *FMR1* CGG repeat expansion, and found one mutation that correlated with a Prader-Willi-like phenotype.^{13,14} It was suggested that *MECP2* might have a role in regulating genes involved in body habitus. Since some patients with features of Angelman syndrome also had mutations in the *MECP2* gene,^{10,11} genes in the region 15q11–q13 could be target genes downstream of *MECP2*. Therefore, it was suggested that it was worthwhile to evaluate the prevalence of *MECP2* mutations among patients with features suggestive of the Prader-Willi syndrome, but without the characteristic molecular abnormalities on chromosome 15q.

To answer the question whether implementation of systematic screening of *MECP2* in patients with an unexplained mental retardation in DNA diagnostics would be reasonable, and to explore further the spectrum of phenotypes resulting from *MECP2* mutations, we performed mutational analysis in three additional patient groups: in mentally retarded female patients ($N=92$) who were negative for *FMR1* CGG repeat expansion, in male and female patients with a phenotype suggestive of Angelman syndrome ($N=63$) without methylation defects in chromosome 15q11–q13 and in male and female patients with a phenotype suggestive of Prader-Willi syndrome ($N=98$), also without methylation abnormalities on chromosome 15q11–q13.

Subjects and methods

Patients

The patients had been referred to our DNA diagnostic division during the last 5 years. The patients were subdivided in three panels according to their previous request for DNA testing for Fragile-X syndrome, Angelman syndrome or Prader-Willi syndrome. The panels were called Fragile-X-negative (92 female patients), Angelman-negative (30 females and 33 males) or Prader-Willi-negative (41 females and 57 males). If the patient's DNA had been analysed for Angelman syndrome or Prader-Willi syndrome and Fragile-X syndrome, the patient was included in the Angelman-negative or Prader-Willi-negative panel. For all patients, informed consent was obtained.

DNA analysis

DNA was extracted from peripheral blood as described previously.¹⁵ The three coding exons of *MECP2* were

amplified using PCR and genomic DNA as a template. Both strands were sequenced. Sequencing reactions were run on an Applied Biosystems 3730 automated sequencer.

X-inactivation studies

X-inactivation studies were carried out on DNA isolated from lymphocytes from the p.Q406X patient, as described by Allen *et al.*¹⁶ However, the analysis was performed on an automated sequencer (ABI 3100 Prism) instead of Southern hybridisation.

Results

DNA studies

The results of mutation analysis of all the three panels are shown in Table 1a and b. In the Fragile-X-negative panel, two sequence changes were found, one known polymorphism c.1126C→T (p.P376S) and one nonsense mutation c.1216C→T (p.Q406X). The mutation was not found in the parents. X-inactivation studies in the patient lymphocytes showed a 100% skewed pattern.

In the Angelman-negative group, two known pathogenic missense mutations were found: c.397C→T (p.R133C) in a female patient and a mosaic c.473G→T (p.T158M) in a male patient, and one novel change c.1497G→C in a female patient. The c.1497G→C is in the 3' UTR of the gene, and was also found in the mother of the patient. The known polymorphism c.1126C→T (p.P376S) was also found in this cohort in a female patient and in her father. The p.R133C was not found in the mother. The mother of the male patient with the mosaic p.T158M mutation did not have this mutation.

In the cohort of Prader-Willi-like patients one novel silent change c.393C→G (p.A131A) was found.

Clinical studies of patients with a *MECP2* mutation p.Q406X

This girl was born at the term after an uneventful pregnancy and delivery with a birth weight of 2550 g. During early life she was a passive baby. Psychomotor development was delayed; she walked at 3 years and spoke her first words at the age of 5 years. She had no EEG abnormalities.

At the age of 16 years, she attended an institute for child psychiatry because of behavioral problems. The degree of mental retardation was moderate. Chromosomal analysis and DNA studies of the Fragile-X syndrome were normal.

At the age of 22 years, she was re-examined. Her height was 160 cm (–1.5 SD), her weight was 57 kg (0 SD), and her skull circumference was 56 cm (0.5 SD). She had a face with a child-like appearance and a strabismus of the left eye. She spoke with short sentences. Her gait was slightly unsteady.

Table 1 (a) Number of pathogenic mutations and (b) polymorphisms

(a) Cohort	N	Nucleotide change	Amino-acid change	N mutations	Region	Sex
Fragile-X-negative	92	c.1216C→T	p.Q406X	1	Cter	F
Angelman-negative	63	c.397C→T c.473G→T (mosaic)	p.R133C p.T158M	2	MBD MBD	F M
Prader-Willi-negative	98			0		
(b) Cohort	N	Nucleotide change	Amino-acid change	N polymorphisms	Region	Sex
Fragile-X-negative	92	c.1126C→T	p.P376S	1	Cter	F
Angelman-negative	63	c.1497G→C c.1126C→T	p.P376S	2	3'UTR Cter	F F
Prader-Willi-negative	98	c.393C→G	p.A131A	1	TRD	M

p.R133C

This girl was born at the term after an uneventful pregnancy and delivery, with a birth weight of 3500 g. After 6 months of normal development, there was developmental stagnation, and only a slow further progression. No developmental regression was noted. She walked at 24 months and spoke her first words at 5 years of age.

On investigation at the age of 7 years, she had normal height and head circumference, and was slightly obese. She was a severely retarded, friendly and happy person, and spoke with two-words sentences. She showed teeth grounding and had a wide-based gait. She was insensitive to pain. Since the age of 7 years, there were severe sleep disturbances. Daytime EEG studies showed epileptic activity in less than 1%, whereas during the night more than 75% disturbed EEG patterns were observed. In spite of EEG abnormalities, no convulsions had been observed.

Routine chromosomal studies and molecular studies of the Angelman syndrome were negative.

At the age of 14, she was examined again (Figure 1). She was a friendly person with growth retardation (height of 137 cm (< -2.5 SD)), generalised obesity (weight of 60 kg (> +2 SD)) and hypotonia. Head circumference was 53.5 cm (-1 SD). She revealed the same signs as discussed above. Moreover, thoracic scoliosis, cold extremities and tapering of the fingers were noticed. Her gait was unsteady and wide-based, but she could walk and climb stairs unaided. In addition, although with some spilling, she was able to eat and drink.

p.T158M

This boy was born after an uneventful pregnancy and delivery, with a birth weight of 3440 g. During the first year

**Figure 1** Female patient with the p.R133C mutation in *MECP2* at the age of 14 years.

of life, he developed completely normal. Thereafter, his development stagnated followed by a period of regression. He lost his capability of purposeful hand use and walking along the table. At the age of 2 years and 9 months, he was a passive boy with autistic features and hardly any speech. Stereotypic movements like handclapping and teeth grounding were noted. There were convulsions and diffuse EEG abnormalities. Later on, he developed periods of yelling and shouting and sleeping disturbances. No metabolic defects were found. Routine chromosomal studies and DNA studies of the Angelman and Fragile-X syndrome were normal.

At the age of 11 years, he was re-examined (Figure 2). He had a weight of 11 kg (-2 SD) and a skull circumference of



Figure 2 Male patient with the p.T158M mosaic mutation in *MECP2* at the age of 11 years. He shows the classical Rett syndrome phenotype.

51.5 cm (-2 SD). His height was not measured. In addition to the previous features, he was wheelchairbound, and a severe thoracic scoliosis was observed. Feet were small and cold.

Discussion

We report on three large patient cohorts in which *MECP2* mutation analysis was performed to ascertain whether routine screening of this gene in patients with unexplained MR in a DNA diagnostics setting is indicated, and to further explore the phenotypic spectrum of *MECP2* mutations.

In the cohort of females negative for the molecular Fragile-X studies, we found two nucleotide changes. The nonsense p.Q406X mutation that was found in this cohort has been reported once in a family where both the maternal uncle and son had mental retardation and spasticity.¹² The two carrier females had a borderline intelligence, but no other symptoms. X inactivation in these two carriers showed random patterns. In our patient, however, the mutation was *de novo*. She was moderately retarded, had EEG abnormalities, behavioural problems and autistic features without spasticity. Differences in these two phenotypes might be explained by differences in the

X-inactivation patterns, or by currently unknown genetic modifiers. X-inactivation analysis in this patient showed completely skewed X inactivation. Unfortunately, it was not possible to analyse which allele was the active or inactive one, but most likely the normal allele was inactivated. This might explain why heterozygosity of the p.Q406X in our patient leads to a severe phenotype, whereas in the two other individuals it results in a nearly normal phenotype. Since X inactivation is performed in lymphocytes, it will not be conclusive for X inactivation in brain cells. Therefore, the question still remains as to whether the differences in the two phenotypes are explained by X-inactivation differences. The other nucleotide change that was found in this cohort (p.P376S) has been reported as a polymorphism.⁵

In the cohort of 63 Angelman-negative patients, we found three nucleotide changes. Two changes were known missense mutations, one p.R133C and one mosaic p.T158M. The *de novo* p.R133C mutation has been reported many times in patients with classical RTT syndrome (<http://www.ed.ac.uk/~skirmis>). Though our patient did meet several criteria for classical RTT syndrome, there was no history of regression; she did not have the acquired microcephaly and stereotypic handmovements. In addition, she showed extreme obesity, while most RTT patients had decreased body fat. Owing to this atypical appearance of Rett syndrome, this clinical diagnosis was not considered initially.

The p.T158M mutation found in mosaic form in a severely retarded male patient is a frequently reported mutation in classic RTT syndrome as well. The percentage of mutant *versus* wild-type allele was estimated to be 25%. Since this is analysed in lymphocytes, it is uncertain whether in brain tissue this percentage will be equal. In males, this mutation has been reported in a Klinefelter syndrome patient¹⁷ and post mortem in two brothers with congenital encephalopathy.⁷ Males with other mosaic *MECP2* mutations have been reported as well.^{18,19} Since they all exhibit features of classic RTT syndrome, it is suggested that an *MECP2* mutation associated with RTT syndrome in females could lead to a similar phenotype in males as a result of somatic mosaicism. The clinical diagnosis of Rett syndrome was considered previously, but it was believed at that time that this syndrome did not occur in males.

The c.1497G \rightarrow C that has not been reported so far, was in the 3' UTR of the gene, and was also found in the mother of the patient. Therefore, this change is considered a polymorphism.

Finally, in the panel of Prader-Willi-negative patients, one silent change in the coding sequence was found. Although this change has not been reported so far, it is unlikely to have a pathogenic effect.

In female patients with unexplained MR but without features of classic RTT syndrome, the mutation detection

rate found here is 1/92. It is believed that the X-inactivation patterns observed in patients with RTT syndrome show a standard distribution. The percentage of possible cases with a *MECP2* mutation on both tails of the distribution (neonatal death *versus* mild neurologic/cognitive defects) is expected to be very low and therefore difficult to find.³ A routine search for *MECP2* mutations in female patients with unexplained MR without features of classical Rett syndrome, therefore, might not be worthwhile.

In the Angelman-negative cohort, our mutation rate (2/63) is lower than the ones found by Imessaoudene *et al* (6/78)¹⁰ and Watson *et al* (5/47).¹¹ Although the patient cohorts in these studies were possibly clinically better defined than in the present study, our results still indicate that *MECP2* mutational analysis in patients with a clinical diagnosis of Angelman syndrome but without molecular abnormalities on chromosome 15q11–q13, is worthwhile.

Recently, we suggested that *MECP2* might have a role in regulating genes involved in body habitus.¹⁴ Since some patients with features of Angelman syndrome also had mutations in the *MECP2* gene,^{10,11} genes in the region 15q11–q13 might be target genes downstream of *MECP2*. Therefore, we evaluated the prevalence of *MECP2* mutations among patients with features suggestive of the Prader-Willi syndrome, but without the characteristic molecular abnormalities on chromosome 15q11–q13. However, based on the current studies, routine screening of *MECP2* in Prader-Willi-like patients might not be worthwhile, since we only found one silent change in a panel of 98 patients.

Acknowledgements

This work was supported by grants from ZonMw.

References

- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY: Rett syndrome is caused by mutations in X-linked *MECP2*, encoding the methyl-CpG-binding protein 2. *Nat Genet* 1999; **23**: 185–188.
- Trappe R, Laccone F, Cobilanschi J *et al*: *MECP2* mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. *Am J Hum Genet* 2001; **68**: 1093–1101.
- Wan M, Lee SS, Zhang X *et al*: Rett syndrome and beyond: recurrent spontaneous and familial *MECP2* mutations at CpG hotspots. *Am J Hum Genet* 1999; **65**: 1520–1529.
- Hoffbuhr K, Devaney JM, LaFleur B *et al*: *MECP2* mutations in children with and without the phenotype of Rett syndrome. *Neurology* 2001; **56**: 1486–1495.
- Sung Jae Lee S, Wan M, Francke U: Spectrum of *MECP2* mutations in Rett syndrome. *Brain Dev* 2001; **23**: 138–143.
- Hoffbuhr KC, Moses LM, Jerdonek MA, Naidu S, Hoffman EP: Associations between *MECP2* mutations, X-chromosome inactivation and phenotype. *Ment Retard Dev Disabil Res Rev* 2002; **8**: 99–105.
- Villard L, Kpebe A, Cardoso C, Chelly J, Tardieu M, Fontes M: Two affected boys in a Rett syndrome family: clinical and molecular findings. *Neurology* 2000; **55**: 1188–1193.
- Orrico A, Lam CW, Galli L *et al*: *MECP2* mutation in male patients with non-specific X-linked mental retardation. *FEBS Lett* 2000; **481**: 285–288.
- Couvert P, Bienvenu T, Aquaviva C *et al*: *MECP2* is highly mutated in X-linked mental retardation. *Hum Mol Genet* 2001; **10**: 941–946.
- Imessaoudene B, Bonnefont JP, Royer G *et al*: *MECP2* mutation in non-fatal, non-progressive encephalopathy in a male. *J Med Genet* 2001; **38**: 171–174.
- Watson P, Black G, Ramsden S *et al*: Angelman syndrome phenotype associated with mutations in *MECP2*, a gene encoding a methyl CpG binding protein. *J Med Genet* 2001; **38**: 224–228.
- Meloni I, Bruttini M, Longo I *et al*: A mutation in the Rett syndrome gene, *MECP2*, causes X-linked mental retardation and progressive spasticity in males. *Am J Hum Genet* 2000; **67**: 982–985.
- Yntema HG, Kleefstra T, Oudakker AR *et al*: Low frequency of *MECP2* mutations in mentally retarded males. *Eur J Hum Genet* 2002; **10**: 487–490.
- Kleefstra T, Yntema HG, Oudakker AR *et al*: De novo *MECP2* frameshift mutation in a boy with moderate mental retardation, obesity and gynaecomastia. *Clin Genet* 2002; **61**: 359–362.
- Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
- Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW: Methylation of *HpaII* and *HhaI* sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992; **51**: 1229–1239.
- Leonard H, Silberstein J, Falk R *et al*: Occurrence of Rett syndrome in boys. *J Child Neurol* 2001; **16**: 33–38.
- Topçu M, Cemaliye A, Sayi A *et al*: Somatic mosaicism for a *MECP2* mutation associated with classic Rett syndrome in a boy. *Eur J Hum Genet* 2002; **10**: 77–81.
- Clayton-Smith I, Watson P, Ramsden S, Black GCM: Somatic mutation in *MECP2* as a non-fatal neurodevelopmental disorder in males. *Lancet* 2000; **356**: 830–832.