European Journal of Human Genetics (2005) 13, 523–524

www.nature.com/ejhg

LETTER

Mutations in exon 1 of *MECP2B* are not a common cause of X-linked mental retardation in males

European Journal of Human Genetics (2005) **13,** 523–524. doi:10.1038/sj.ejhg.5201399
Published online 16 March 2005

Mutations in the *MECP2* gene result in a neurological disorder called Rett syndrome (RTT, OMIM 312750) characterized in girls by a normal development followed by developmental stagnation and then regression of fine motor and language skills. Mutations in *MECP2* account for the great majority of RTT cases and are also involved in a broad spectrum of phenotypes, including mild intellectual difficulties in female subjects and neonatal encephalopathy in males surviving to birth.[1,2] More recently, *MECP2* mutations such as E137G, A140V R167W or E406X associated with severe forms of nonsyndromic mental retardation were reported in several families.[3–7]

Originally it was thought that MeCP2 consisted of a single isoform with a translational start site beginning in exon 2. Exon 1 was therefore not included in genetic tests for RTT and X-linked mental retardation. However, two recent studies have described a new isoform of MeCP2 and shown that it is the predominant form of the protein in the human brain. The new isoform named MECP2B has an alternative N-terminus, transcribed from exon 1 of *MECP2*.[8,9] Three reports have described mutations or deletions in typical and atypical Rett patients that affect exon 1 (c.62 + 1delGT, c.47_57del11, c.47_48insAGG).[8,10] It remains possible that exon 1 mutations might account for a proportion of males with mental retardation.

The aim of this study was to test a large sample of mental retardation individuals for mutations in exon 1 of *MECP2B* in order to evaluate its involvement in nonspecific mental retardation and to assess the frequency of mutations associated with this condition.

We analysed four large families with XLMR linked to Xq28 according to the recent version of the Euro-MRX database. Cognitive impairment is the only common feature between patients of these families. We also studied a panel of 406 clinically well-characterized small families with at least two boys affected with MR. All patients were of European origin. CGG expansions involved in Fragile X syndrome, assessed by Southern blot analysis using DNA digested with *Eco*RI/*Eag*I restriction enzymes and an StB12-3 probe corresponding to FRAXA locus, as well as mutations in *MECP2A* and cytogenetic abnormalities were excluded in these patients.

Genomic DNA was extracted from peripheral blood lymphocytes according to standard protocols and was used to amplify exon 1 of *MECP2B*. The resulting PCR product is 222 bp. Primer sequences used were: 1B1F 5′-cggagag agggctgtggtaa-3′ and 1B1R 5′-aggagggacgccatccgcca-3′. Reactions were performed in a volume of 50 μl containing 50 mM Tris–HCl (pH 8.4), 1.5 mM $MgCl_2$, 200 mM of all four deoxynucleotides, 100 pM each of the primers, 10 μl betaine, 2.5 U of *Taq* Polymerase (Platinum, Invitrogen) and 100 ng template DNA. Then, 40 cycles were then performed with denaturation for 30 s at 94°C; annealing for 30 s at 58°C, and elongation for 30 s at 72°C. The DNA synthesis step of the final cycle was extended to 7 min. The amplified DNA was then heated at 94°C for 7 min, and at 55°C for 4 min to favour the formation of heteroduplexes. Mutation analysis of the MR patients was performed by using denaturing high-pressure liquid chromatography (DHPLC; Wave DNA fragment analysis system, DNASep column; Transgenomics). DHPLC conditions were chosen according to the Wavemaker program (Transgenomics, Santa Clara, CA, USA) (68.8 and 69.4°C). PCR products were subjected to chromatography using appropriate temperatures and acetonitrile gradients. PCR products were eluted with a linear acetonitrile gradient at a flow rate of 0.9 ml/min, and those showing an abnormal DHPLC profile were directly sequenced on an automated sequencer (ABI 377, Perkin-Elmer) using the Dye Terminator method.

Among 410 patients with X-linked mental retardation, no abnormal pattern of chromatographic migration could be detected. We did not observe mutations or polymorphisms in exon 1 of *MECP2B*. The mutations, deletions or polymorphisms described in some RTT patients were thus absent in the male mental retardation population tested. This observation rules out the possibility that the exon 1 of *MECP2B* is mutated in a significant fraction of male patients with mental retardation. Recently, screening of this region in cases of autism (100 probands) or mental retardation in males (seven males referred through the Medical Genetics Clinic for RTT) also did not detect relevant mutations in exon 1.[11,12] Furthermore, data obtained from all French laboratories studying RTT suggest that mutations located in exon 1 of *MECP2B* only represents 0.49% of *MECP2* mutation and does not seem

to explain a great majority of typical or atypical RTT patients (data not shown). As our figures were obtained from a large European cohort, one can conclude that the systematic screening of exon 1 of *MECP2B* should not be considered as a priority in unexplained cases of mental retardation.

Acknowledgements

This study was supported by grants from AFSR, INSERM and GIS-Institut des maladies rares.

Karine Poirier[1], Fiona Francis[1], Ben Hamel[2], Claude Moraine[3], Jean Pierre Fryns[4], Hans H Ropers[5], Jamel Chelly[1] and Thierry Bienvenu[1]

[1]*Institut Cochin – INSERM U567, CNRS UMR8103, Université Paris V René Descartes, 24 rue du Fbg St Jacques, Paris, France;*
[2]*Department of Human Genetics, University Hospital, Nijmegen, The Netherlands;*
[3]*Service de Génétique – INSERM U316, CHU Bretonneau, Tours, France;*
[4]*Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium;*
[5]*Max Planck Institut für Molekulare Genetik, Berlin, Germany*
Correspondence to: Thierry Bienvenu, E-mail: bienvenu@cochin.inserm.fr

References

1 Clayton-Smith J, Watson P, Ramsden S, Black GC: Somatic mutation in MECP2 as a non-fatal neurodevelopmental disorder in males. *Lancet* 2000; **356**: 830–832.
2 Moog U, Smeets EE, van Roozendaal KE *et al*: Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (MECP2). *Eur J Paediatr Neurol* 2003; **7**: 5–12.
3 Orrico A, Lam C, Galli L *et al*: MECP2 mutation in male patients with non-specific X-linked mental retardation. *FEBS Lett* 2000; **481**: 285–288.
4 Couvert P, Bienvenu T, Aquaviva C *et al*: MECP2 is highly mutated in X-linked mental retardation. *Hum Mol Genet* 2001; **10**: 941–946.
5 Winnepenninckx B, Errijgers V, Hayez-Delatte F, Reyniers E, Frank Kooy R: Identification of a family with nonspecific mental retardation (MRX79) with the A140V mutation in the MECP2 gene: is there a need for routine screening? *Hum Mutat* 2002; **20**: 249–252.
6 Kleefstra T, Yntema HG, Nillesen WM *et al*: MECP2 analysis in mentally retarded patients: implications for routine DNA diagnostics. *Eur J Hum Genet* 2004; **12**: 24–28.
7 Gomot M, Gendrot C, Verloes A *et al*: MECP2 gene mutations in non-syndromic X-linked mental retardation: phenotype–genotype correlation. *Am J Med Genet* 2003; **123A**: 129–139.
8 Mnatzakanian GN, Lohi H, Munteanu I *et al*: A previously unidentified MECP2 open reading frame defines a new protein isoform relevant to Rett syndrome. *Nat Genet* 2004; **36**: 339–341.
9 Kriaucionis S, Bird A: The major form of MeCP2 has a novel N-terminus generated by alternative splicing. *Nucleic Acids Res* 2004; **32**: 1818–1823.
10 Evans JC, Archer HL, Whatley SD, Kerr A, Clarke A, Butler R: Variation in exon 1 coding region and promoter of MECP2 in Rett syndrome and controls. *Eur J Hum Genet* 2005; **13**: 124–126.
11 Harvey C, Alfred SE, Mnatzakanian GN *et al*: Screening for mutations in exon 1 of the Rett syndrome gene, MECP2, among individuals with autism and with mental retardation. *Am J Hum Genet* 2004; abstract 2274.
12 Bleoo SL, Bridge PJ, Innes AM, Parboosingh JS: Mutation analysis of MECP2A and MECP2B in patients referred for Rett syndrome. *Am J Hum Genet* 2004; abstract 2626.