Classic Rett Syndrome in a Boy as a Result of Somatic Mosaicism for a *MECP2* Mutation

Judith Armstrong, MsC,[1] Pilar Póo, MD, PhD,[2] Mercè Pineda, MD, PhD,[2] Elena Aibar, MsC,[1] Esther Geán, MsC, MD,[1] Vicenç Català, MsC, PhD,[3] and Eugènia Monrós, MsC, PhD[1]

Rett syndrome (RTT) is a neurodevelopmental disorder affecting females in a sporadic manner. In a high proportion of patients the disease is caused by de novo dominant mutations at *MECP2* gene (Xq28).[1,2] The existence of RTT males has been discussed extensively, and less restrictive diagnostic criteria have been proposed to include this variant, which should be considered when evaluating boys with idiopathic developmental regression, autistic features, and loss of hand function. Nevertheless, no *MECP2* analysis has been reported from Rett-like males to date. Analysis of 2 familial cases showed that boys carrying the same *MECP2* mutation that caused RTT in their sisters suffered from severe–fatal neonatal encephalopathy.[2,3] Recent data, however, demonstrate that the clinical spectrum of *MECP2* mutations is wider than previously expected. With a frequency comparable to that of fragile X syndrome, recessive nonspecific X-linked mental retardation can be caused by missense mutations at *MECP2*, different than those causing RTT.[4] Mutations have also been described in patients with congenital nonprogressive encephalopathy and in some cases of Angelman syndrome (AS), the only reported AS boy being a somatic mosaic for a *MECP2* truncating mutation.[5]

We document the first *MECP2* analysis of a boy with classic RTT and a normal 46,XY karyotype. The patient is 14 years of age and fulfils eight of nine necessary criteria, seven of eight supportive criteria, and no exclusion criteria, according to the Rett Syndrome Diagnostic Criteria Work Group. Genetic informed consent was obtained and the study was approved by the Ethical and Investigation Commissions of our hospital.

MECP2 sequencing of two independent patients' DNA samples from peripheral lymphocytes revealed the presence of a heterozygous change 398G→A, causing an R133H substitution. The mutation had been previously described in 2 RTT female patients.[6] As the boy had a normal karyotype, heterozygosity could be explained by (1) a low frequency mosaicism for a Klineffelter syndrome, discarded by FISH on prophasic nuclei; (2) a *MECP2* locus duplication, rejected by high-resolution karyotype and observation of hemizygosity for X-linked markers and two intragenic *MECP2* polymorphisms; or (3) somatic mosaicism for the mutation. To test this last hypothesis, DNA was prepared from the patient's oral mucosa and sequenced. The normal sequence, with only a small amount of the mutated allele, was observed. These results demonstrated that the RTT boy is a somatic mosaic for the R133H mutation and seemed to indicate that the mutation is present in a high proportion of lymphocytes but at a lower frequency in oral mucous. To specifically test and semiquantify the heterozygous status of the mutation, an amplification–refractory mutation system (ARMS) experiment was designed. Both the normal and mutated alleles were amplified in the patient's lymphocytes and oral mucosa (Fig). Relative differences in band intensities corroborated the different degree of mosaicism between the two tissues.

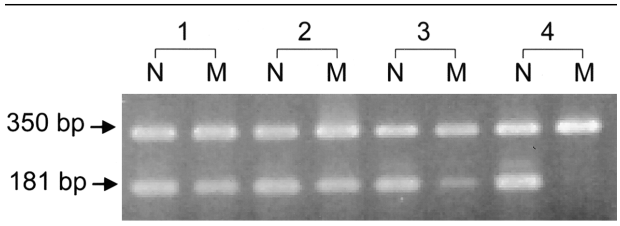

Fig. ARMS analysis of the 398G→A mutation. The 350 bp band corresponds to fragment 2b from exon 2 of MECP2 (according to Amir et al),[1] used as an internal control of amplification in a multiplex PCR reaction. The 181 bp fragment was designed to specifically amplify the normal or the mutated base at position 398. Pairs of adjacent lines correspond to normal (N) and mutated (M) specific amplifications of the same DNA sample: (1) patient's DNA from peripheral blood lymphocytes, first blood extraction; (2) patient's DNA obtained from peripheral blood lymphocytes, second blood extraction; (3) patient's DNA from oral mucous; (4) DNA from a control male individual.

The infrequent clinical picture of this patient is thus explained by the existence of somatic mosaicism for an RTT-causing *MECP2* mutation. Concerning X-linked diseases, this phenomenon in males mimics the result of lyonization naturally occurring in females and may explain the classic Rett syndrome phenotype of the boy.

This work was supported by the Asociación Valenciana y Catalana de Síndrome de Rett, and by Fondo de Investigación Sanitaria (FIS99/0235 to J.A.).

Sequencing runs were done at Serveis Cientificotècnics, Universitat de Barcelona, and at Sistemas Genómicos, Valencia.

[1]Genetics Section and [2]Neurology Service, Hospital Universitari Sant Joan de Déu, Barcelona; and [3]Prenatal Genetics, Barcelona, Spain

References

1. Amir RE, Van den Veyver IB, Wan M. Tran CQ, et al. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2. Nature Genet 1999;23: 185–188.
2. Wan M, Sung Jae Lee S, 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.
3. Villard L, Kpebe A, Cardoso C, et al. Two affected boys in a Rett syndrome family. Neurology 2000;55:1188–1193.
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. 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.
6. Hoffbuhr K, Devaney J, LaFleur B, et al. Genotype and phenotype evaluation of MeCP2 mutations in Rett syndrome. Neurology 2001;56:1486–1495.

