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SHORT REPORT

Variation in exon 1 coding region and promoter of *MECP2* in Rett syndrome and controls

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Mutations in *MECP2* are a cause of Rett syndrome. Recently, a new isoform of MeCP2 was described, which has an alternative N-terminus, transcribed from exon 1. We screened exon 1 and the promoter region of *MECP2* in 97 mutation-negative Rett syndrome cases. We found two sequence variants, but there was no evidence that they are pathogenic. Mutations in exon 1 and the promoter of *MECP2* are not a common cause of Rett syndrome.

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Introduction

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.[1,2] The new isoform has an alternative N-terminus, transcribed from exon 1 of *MECP2*. Exon 1 was previously thought to be noncoding and is therefore not included in genetic tests for Rett syndrome.

Mutations in *MECP2* can cause Rett Syndrome,[3] a neurodevelopmental X-linked disorder that predominantly affects female populations and is the most common genetic cause of profound intellectual disability in female subjects. Typically, there is a period of near-normal development followed by a regression period with loss of social, motor and communication skills. Other features of Rett syndrome include autonomic disturbance, scoliosis, feeding difficulties and epilepsy. Most cases of Rett syndrome are sporadic, with the majority being caused by a *de novo* mutation on the paternal copy of *MECP2*.[4]

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Two cases have been described in which Rett syndrome was caused by mutation or deletion of *MECP2* exon 1 (from a sample of 19 classical Rett *MECP2* mutation-negative cases).[1] An additional case has been described where exons 1 and 2 were deleted.[5] It is possible that exon 1 mutations account for a significant proportion of the ~15% of classical Rett cases who do not have mutations in exons 2, 3 or 4 of *MECP2*. We assessed the sequence variation in exon 1 and the promoter region of *MECP2* in 243 individuals: 97 cases of Rett syndrome with no mutation found in exons 2, 3 or 4 of *MECP2* and 146 controls.

Methods

Samples

DNA samples were obtained from 97 Rett syndrome patients who have previously had a negative *MECP2* test (by sequencing exons 2, 3 and 4). Patients were defined as classical Rett ($n = 37$) or atypical Rett ($n = 60$) according to the criteria described in Kerr *et al*.[6] The control DNA samples were obtained from 146 anonymous, healthy adults.

MECP2 exon 1 sequencing

PCR was carried out using the primers EX1_F 5′-gcactcggtgcatctgtggacagag-3′ and EX1_R 5′-catccgccagccgtgtcgtccgac-3′ in a $[NH_4]_2SO_4$-based buffer with 3.7 mM. $MgCl_2$, 750 μM dNTPs, 0.5 M betaine, 5% DMSO and Platinum Taq (Invitrogen). The annealing temperature was 65°C. Sequencing was performed with a BigDye v3.1 sequencing kit (Applied Biosystems) according to the manufacturer's instructions except for the addition of 1 M betaine. The template was sequenced from each end using the PCR primers and from the middle using two internal primers: INT1_F 5′-caattgacggcatcgccgctgagac-3′ and INT1_R 5′-cgtcattggctgtgatggc-3′.

Multiplex ligation-dependent probe amplification

The girls with classical Rett syndrome were tested for deletions using a *MECP2* Multiplex ligation-dependent probe amplification (MLPA) kit (MRC Holland) according to the instructions provided.

Assay for −219_220insC

This 1 bp insertion creates a *Bsa* I restriction site. Samples were amplified with primers EX1_F and EX1_R. The PCR products were digested overnight at 50°C with 5 U *Bsa* I (New England Biolabs) and run on a 2% agarose gel. Normal DNA remains undigested (size 672 bp), DNA with the insertion is digested to 270- and 402- bp fragments.

Assay for 3_4insGCCGCC

Samples were amplified using primer EX1_R and a fluorescent INT1_F primer; the PCR products were run on an ABI 3100 (Applied Biosystems). The normal PCR product size is 419 bp and with the insertion it is 425 bp.

X-inactivation

Aliquots of DNA were pre-digested with the methylation-sensitive enzymes *Hpa*II and *McrBC* (New England Biolabs). The HUMARA locus was then amplified using fluorescent primers and the allele peak areas analysed using an ABI 3100 and Genotyper software (Applied Biosystems).

Results

We sequenced *MECP2* exon 1 and approximately 400 bp of the promoter region in a total of 97 individuals with Rett syndrome who do not have mutations in exon 2, 3 or 4. We found two changes from the normal sequence and we assessed their frequency by genotyping 146 controls (Table 1, Figure 1, Supplementary Figure). The first variant is a 1- bp insertion at position −220 relative to the start codon in exon 1 (g.-219_220insC, Figure 1a). Note the reference sequence, AF030876, has the insertion (GenBank/EMBL/DDBJ). The second variant is a 6- bp insertion in the $[GCC]_6$ trinucleotide repeat at the start of exon 1 (c.3_4insGCCGCC, Figure 1b).

Table 1 Frequency of the *MECP2* exon 1 and promoter variants in Rett syndrome cases and controls

Sample group	*g.-219_220insC*	*c.3_4insGCCGCC*
Classical Rett, $n = 37$	1	1
Atypical Rett, $n = 60$	2	—
Total Rett, $n = 97$	3	1
Female controls, $n = 73$	1	—
Male controls, $n = 73$	3	—
Total controls, $n = 146$	4	—

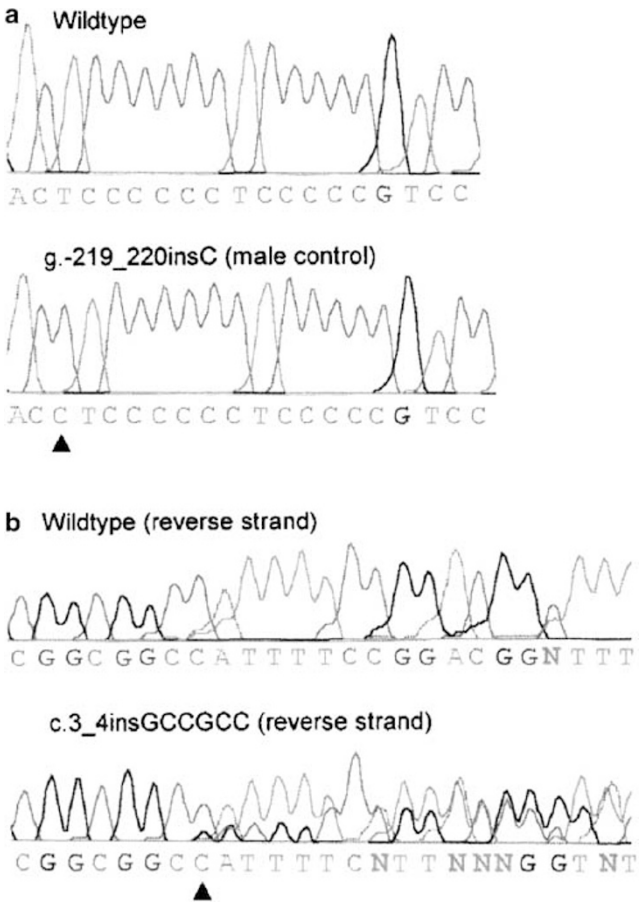

Figure 1 Sequence variants identified in *MECP2* promoter and exon 1.

Deletion analysis by MLPA was successful in 20 classical Rett girls. One girl was found to have a deletion of exon 4 and she was therefore excluded from this study. A further six of the classical Rett girls have previously had deletions in exon 2, 3 and 4 excluded by quantitative fluorescent PCR (data not shown). We were unable to exclude deletions in the 12 remaining classical Rett girls due to variable DNA quality. Based on our experience in diagnostic testing for *MECP2* mutations, we would expect to

find a deletion in approximately 5% of similar *MECP2*-negative Rett patients. Deletions were excluded in 29 of the atypical Rett girls by MLPA.

Discussion

The 1-bp insertion (g. -219_220insC) appears to be a rare polymorphism since it was seen in 3% of Rett cases and 3% of controls (Table 1). Three of the controls with the insertion were male and are therefore hemizygous for *MECP2*. This confirms that the 1-bp insertion is not pathogenic.

The 6-bp insertion (c.3_4insGCCGCC) is predicted to result in insertion of an extra two alanine residues in the polyalanine sequence at the N-terminus of the recently described *MeCP2* splice variant. It was found in one girl with classical Rett syndrome and her unaffected mother. Skewed X-inactivation can sometimes mask the pathogenic effect of a *MECP2* mutation,[7] but in this case X-inactivation was random in lymphocyte DNA from both the girl and her mother (X-inactivation was 54% in the mother and 60% in the girl). It is possible that there is skewed X-inactivation in the brain of the girl or the mother, but it is not feasible to test this. It seems unlikely that c.3_4insGCCGCC is pathogenic, although we cannot rule out this possibility because we did not find this variant in the controls.

Exon 1 of *MECP2* contains two trinucleotide repeat tracts, $[GCC]_6$ and $[GGA]_5$. It has been suggested that expansion of these repeat tracts could be pathogenic, similar to other triplet repeat disorders such as fragile X syndrome.[2] We observed a small expansion (c.3_4insGCC GCC) in the first repeat tract. This change is probably not pathogenic, but it demonstrates that expansions can occur. It is interesting to note that the mutation described by Mnatzakanian *et al*[1] was also in one of the repeat tracts (an 11-bp deletion in the $[GGA]_5$ repeat).

We conclude that mutations in exon 1 and the promoter region of *MECP2* are not a common cause of Rett syndrome. In a substantial number of classical Rett cases, the cause remains unknown.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg).