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RESEARCH ARTICLE

Two sisters with Rett syndrome and non-identical paternally-derived microdeletions in the *MECP2* gene

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Abstract The unique case of two sisters with symptoms of RTT and two quite distinct, novel, and apparently de novo microdeletions of the *MECP2* gene is described. One sister possessed an 18 base-pair (bp) deletion (c.1155_1172del18) within the deletion hotspot region of exon 4, whereas the other sister exhibited a 43 bp deletion at a different location in the same exon (c.1448_1461del14+29). Although these lesions occurred on the same paternally-derived X chromosome, this is probably due to chance co-occurrence owing to the relatively high mutation rate of the *MECP2* gene rather than to a constitutional mutator phenotype.

Keywords Rett syndrome · *MECP2* gene · Familial non-identical mutations

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Introduction

Rett syndrome (RTT; RTT MIM#312750) is an X-linked dominant neurological disorder of development which affects 1 in 10,000–15,000 females (Percy 2002; Chahrour and Zoghbi 2007). Classic RTT has a characteristic presentation with subtle abnormalities in development from birth, a period of stagnation, followed by regression with loss of hand and social skills and development of hand stereotypies, deceleration in head growth, severe learning difficulties and gait dyspraxia (Weaving et al. 2005; Williamson and Christodoulou 2006).

Mutations in the *MECP2* gene (MIM# 300005) were first reported in RTT by Amir et al. (1999) and are identifiable in ~80% of classic RTT cases, although less frequently in atypical RTT (Huppke and Gärtner 2005). The *MECP2* gene contains four exons and encodes methyl-CpG-binding protein-2 (MeCP2), a multifunctional protein with at least four different functional domains: (i) a methyl-CpG-binding domain, (ii) an arginine-glycine repeat RNA-binding domain, (iii) a transcriptional repression domain and (iv) an RNA splicing factor binding region (WW group II binding domain) [Fan and Hutnick 2005]. Two distinct isoforms have been reported; MECP2A is encoded by exons 2–4 whereas MECP2B is encoded by exons 1, 3 and 4 (Mnatzakanian et al. 2004). To date, more than 200 different nucleotide substitutions have been reported in the *MECP2* gene as a cause of RTT (RettBASE, http://mecp2.chw.edu.au; Miltenberger-Miltenyi and Laccone 2003; Williamson and Christodoulou 2006). In addition, ~10% of RTT patients possess microdeletions which cluster within a ~150 bp exon 4 hotspot region, between nucleotides 1050 and 1200 (Williamson and Christodoulou 2006; Bienvenu and Chelly 2006; Philippe et al. 2006).

The vast majority of RTT cases are sporadic (>99%), but some familial cases have been described which are usually caused by either maternal or paternal germline mosaicism (Evans et al. 2006; Venâncio et al. 2007) or skewing of X-inactivation towards the wild-type *MECP2* allele in the asymptomatic carrier mother (Villard et al. 2001; Knudsen et al. 2006; Dayer et al. 2007). In such cases, affected relatives invariably possess the same molecular lesion. Here, we describe the unique case of two sisters with symptoms of RTT who were found to have two quite distinct, and apparently de novo, microdeletions on the same paternally-derived *MECP2* allele.

Case report

Two sisters with Rett syndrome were born to healthy non-consanguineous Irish parents (Fig. 1). Case 1, now aged 11, was born after an uncomplicated pregnancy, weighing 3.6 kg. The maternal age was 36 years, and the paternal age 40 years. Feeding was initially poor, although this improved on bottle feeds, and she gained weight rapidly. She was described as a very placid baby, and from the age of 7 months, concerns were raised about significant muscular hypotonia, regressing attentiveness and slowing of head growth. At around the same time, she started to develop seizures upon waking, as well as ritualistic table-banging. She did not achieve a stable sitting posture, and never developed recognisable speech. Classical 'hand-wringing' stereotypies were not, however, observed. Head growth stalled at 47.3 cm at the age of 3 years 7 months (0.4 cm below the 0.4th centile). Other growth parameters were within the normal range. MRI brain scan was reported as normal, but electroencephalograms (EEGs) showed frequent central and Sylvian epileptiform discharges. The karyotype was normal as was testing for Prader-Willi and Angelman syndromes.

Case 2, now 4 years of age, weighed 3.24 kg at term. The pregnancy was largely uncomplicated, although occasional periods of fetal inactivity were noted by the mother (now aged 47 years; father 51 years). She was noted to be quite floppy during feeding but was able to sit at 9 months, and walked unsteadily from 18 months. Developmental progression was slow, with no recognisable speech, although she did babble. She did not develop imaginative play, and engaged in frequent hand-wringing and clapping. On assessment at age 2 years 4 months, she was markedly hypotonic but non-dysmorphic. Height and weight were on the 25th and 75th centiles, respectively, but head circumference was on the 9th centile, and had stalled at 48 cm by 3 years 4 months (~2nd centile). Further developmental regression had been noted from the age of 2 years 6 months, with loss of stacking skills and spoon handling ability. She did not have seizures. MRI brain scan was normal but an EEG has not yet been carried out. The affected girls have two apparently normal sisters.

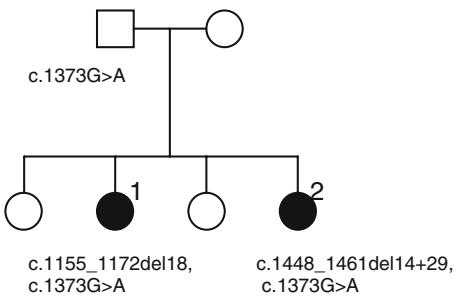

Fig. 1 Pedigree of the affected family; The clinically affected sisters (Cases 1 and 2) are denoted by filled circles. The MECP2 genotypes of the sisters are given. The paternal inheritance of the c.1373G>A transition is indicated

Materials and methods

DNA sequencing

Polymerase chain reaction (PCR) amplification of the four exons of the *MECP2* gene was performed using oligonucleotide primers and amplification conditions described by Cheadle et al. (2000). PCR products and plasmid clones were sequenced using Thermosequenase (Amersham Pharmacia, Little Chalfont, UK) according to the manufacturers' instructions. PCR-amplified genomic fragments were then sequenced in both directions using the primers used for PCR amplification.

Cloning of PCR amplified fragments

Exon 4 of the *MECP2* gene was PCR amplified from DNA derived from both sisters and their parents as described above. PCR fragments were cloned into pGEM-T (Promega, Southampton, UK). Four clones from each individual were then sequenced in both directions using vector-based primers pGEMT5B (5′ CAGTCACGACGTTGTAAAACG 3′) and pGEMT3B (5′ ATGACCATGATTACGCCAAGC 3′) using BigDye v3.1 (Applied Biosystems, Warrington, UK) and analysed on an ABI 3100 sequencer (Applied Biosystems, Warrington, UK).

Results

Sequencing of the *MECP2* gene in Case 1 revealed a novel 18 base-pair (bp) deletion, c.1155_1172del18, within the deletion hotspot region in exon 4 (Fig. 2). Although this microdeletion interrupts codon 385, it is effectively

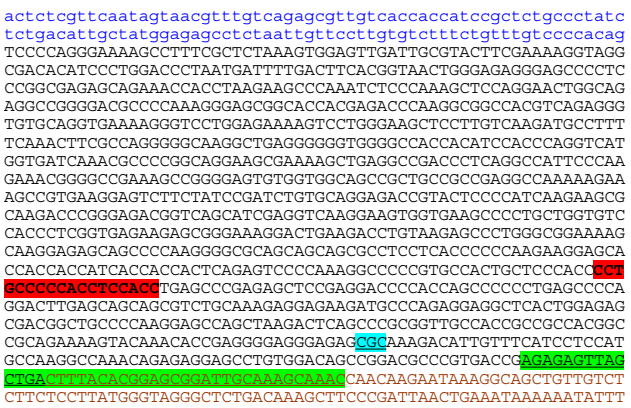

Fig. 2 Sequence of exon 4 of the *MECP2* gene showing the locations of the two microdeletions and the missense mutation detected in the reported family

in-frame and would be predicted to lead to the loss of amino acid residues 386–391. When sequencing was performed on the younger sister (Case 2), a quite different 43 bp deletion was detected which spans the junction between exon 4 and the 3′ UTR viz. c.1448_1461del14+29 (Fig. 2). This frameshift deletion would be predicted to lead to the synthesis of an elongated protein product (in which the last four amino acids of MeCP2 would be replaced by a novel stretch of 14 amino acids at the C-terminal end of the protein). However, in the absence of protein studies, it remains unclear if a stable protein product would actually be produced. Neither microdeletion was found by PCR/direct sequencing analysis to be present in the constitutional (lymphocyte) DNA of the parents, indicating that these lesions probably occurred de novo. According to the *MECP2* Variation Database (RettBASE; http://mecp2.chw.edu.au/), neither microdeletion has been previously reported, either as a pathological lesion or as a polymorphic variant.

Both sisters also carried a novel missense variant in exon 4 of the *MECP2* gene, c.1373G>A (p.Arg458His; Figs. 2, 3). No examples of this nucleotide substitution have previously been reported (RettBASE; http://mecp2.chw.edu.au/). This G>A transition is located within a potentially hypermutable CpG dinucleotide and is compatible with a mutational mechanism of methylation-mediated deamination of 5-methylcytosine. Arg458 is conserved in both mouse and *Xenopus laevis* MeCP2 (Amir et al. 1999) and such evolutionary conservation often implies biological function. However, the Arg458His substitution is also present in the constitutional DNA of the clinically normal father indicating that, irrespective of its possible functional relevance, it is most unlikely to be of pathological significance. This notwithstanding, the presence of this rare paternal c.1373G>A variant in both affected sisters renders the twin possibilities of sample mix-up and non-paternity in a diagnostic context highly unlikely.

The rare c.1373G>A variant serendipitously provided a marker for the paternal *MECP2* allele in this family. The sequencing of cloned exon 4-containing fragments in the two affected sisters demonstrated that both microdeletions

a)

CTGCTC**CCACC**cctgccccacct**ccacc**TGAGCCCG

b)

CC**Gag**a**gag**ttagctgactttacacggagcggattg**caa**agcaaac**CAA**CAA

c)

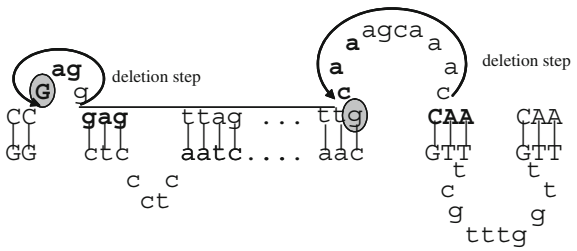

Fig. 3 Repetitive sequence elements in the vicinity of the microdeletion breakpoints and the secondary structures postulated to have been involved in the genesis of the two mutations. Lower case letters denote deleted nucleotides. (**a**) Repetitive sequence elements (shown in bold) found in the vicinity of the breakpoints of the 1155_1172del18 microdeletion. (**b**) Repetitive sequence elements (shown in bold) found in the vicinity of the breakpoints of the 1448_1461del14+29 microdeletion. (**c**) Schematic representation of the postulated non-B DNA slipped structures at the breakpoint of the 1448_1461del14+29 microdeletion. The nucleotides shown in bold type correspond to the direct repeats shown in (b). Nucleotides circled in grey denote the homology between two breakpoint junctions. The arrows indicate the sequences deleted

had occurred in *cis* to the paternal c.1373G>A variant. We may therefore conclude that both microdeletions occurred independently on the same paternal allele. This is consistent with the observations of Trappe et al. (2001) that most de novo *MECP2* gene lesions in RTT arise on the paternally derived X chromosome.

Complexity analysis (Gusev et al. 1999) was used to examine the potential role of the local DNA sequence environment in mediating the two microdeletions in the *MECP2* gene. Several repetitive sequence elements were identified which could have mediated the two mutational events. Deletion 1155_1172del18, which occurred within the deletion hotspot region, could have been mediated by two CCACC direct repeats via slipped mispairing (Fig. 2a). However, the 1448_1461del14+29 deletion appears to be more complex and may have originated via either one of two distinct deletions both of which could have been mediated by direct repeats. These repeats, GAG and CAA (Fig. 2b), appear to be capable of forming slipped structures (Fig. 2c). Such slipped structures can adopt non-B DNA conformations which are known to be susceptible to double strand breaks and hence are also highly mutagenic (Wang and Vasquez 2006).

Discussion

The vast majority of cases of Rett syndrome (RTT) are female and sporadic. However, a number of familial cases of RTT, exhibiting recurrent RTT within a sibship, have been reported and have generally been explained either by parental gonadal mosaicism or by a clinically unaffected carrier mother with skewed X-inactivation; in such cases, affected relatives have invariably possessed the same molecular lesion (Villard et al. 2000; Mari et al. 2005; Archer et al. 2007; Hardwick et al. 2007; Venâncio et al. 2007). As far as we are aware, the independent occurrence of two non-identical *MECP2* gene lesions in the same family, as reported here, is unprecedented. The question therefore arises as to whether the X chromosome upon which the microdeletions arose could somehow be predisposed to mutation. Although both microdeletions occurred on the same paternally inherited chromosome and within the WW group II binding domain, only c.1155_1172del18 occurred within the deletion hotspot region in exon 4. Moreover, the two micro-deletions appear to have arisen via entirely different mutational mechanisms. Finally, inspection of the sequence of the affected *MECP2* allele failed to identify any private sequence characteristics that could account for a particular predisposition to deletional mutagenesis on this paternally-derived chromosome. We therefore conclude that, in all likelihood, this unique occurrence of two non-identical *MECP2* gene lesions within a sibship probably represents a chance event.

This case is reminiscent of our previous report of a family with three individuals displaying clinical evidence of neurofibromatosis type 1 (NF1) who were found to possess three different heritable and pathological mutations in their *NF1* genes (Upadhyaya et al. 2003). Although several possible explanations for this unique finding were considered, including the possibility that the *NF1* alleles segregating in the family might be unstable, on balance we concluded that it probably represented chance co-occurrence (Upadhyaya et al. 2003). These cases are not altogether without precedent. Indeed, other such examples of independently segregating non-identical pathological gene lesions have involved the *NF1* gene in a second quite unrelated family with NF1 (Klose et al. 1999), the *RB1* gene in a family with retinoblastoma (Munier et al. 1993) and the *APC* gene in a family with adenomatous polyposis coli (Davidson et al. 2002). Examples of this phenomenon have also been reported in the X-linked recessive conditions haemophilia A (Tizzano et al. 2005) and Duchenne/Becker muscular dystrophy (Laing et al. 1992; Mostacciuolo et al. 1994; Morandi et al. 1995; Zatz et al. 1998). Irrespective of whether these cases represent examples of mutationally unstable alleles or simply chance co-occurrence owing to the relatively high mutation rates exhibited by some of these genes, such reports should serve as a stark reminder that it is unwise to extrapolate from the detection of a single mutation in a specific individual to the assumption of an identical molecular genetic aetiology in other clinically affected members of the same family.

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