Short Report

Germline mosaicism for a MECP2 mutation in a man with two Rett daughters


Rett syndrome is a severe neurodevelopmental disorder that is caused by mutations in the X-linked gene, methyl-CpG binding protein 2 (MECP2). The majority of cases are sporadic, but rarely germline mosaicism can lead to familial cases. Here, we report the first case where germline mosaicism for a MECP2 mutation has been shown in a man. He has two affected daughters who are half-sisters, and both have the c.808delC mutation. We show that this mutation is present at a low level in DNA extracted from the patient's semen. This case has implications for genetic counseling, and pre-natal testing should be offered for the partners of men who have a daughter with Rett syndrome.

Materials and methods

Case history

The subject is a healthy adult male. He has three daughters from two relationships. Two of his daughters, who are half sisters, have been diagnosed with Rett syndrome (see pedigree in Fig. 1). The diagnosis was confirmed using molecular genetic testing in both the girls, and they were reported to have the same rare MECP2 mutation, c.808delC. At this point, the father requested genetic testing to assess his risk of having more affected children. The father and both the mothers gave an informed consent for this study. Ethical approval was obtained for this study; Multicentre Research Ethics Committee (MREC, Wales) Ref. 02/9/33.

DNA extraction

Genomic DNA was extracted from blood samples using standard methods. In addition, we
collected buccal cells (saliva) and semen sample from the father. Saliva was collected using an Oragene\textsuperscript{TM} collection kit (DNA Genotek, Ottawa, Canada), and DNA was extracted according to the manufacturer’s instructions. Semen samples were incubated at 55°C for 2 h in buffer containing 10 mM Tris–HCl (pH 8.0), 10 mM ethylenediamine tetracetic acid (EDTA), 100 mM NaCl, 2% sodium dodecyl sulphate (SDS), 40 mM dithiothreitol (DTT) and 250 μg/ml proteinase K. Semen DNA was recovered using a standard phenol/chloroform purification method followed by ethanol precipitation.

Confirmation of family relationships

Family relationships were confirmed using a PowerPlex\textsuperscript{®} 1.2 System (Promega, Madison, WI, USA). This is a forensic kit containing eight short tandem repeat loci. Analysis was performed according to the manufacturer’s instructions.

MECP2 sequence analysis

The polymerase chain reaction (PCR) primers 5’-TCCACCCAGGTCTAGGTGATC-3’ and 5’-TGAGTCTTAGGCTCCTTG-3’ were used to amplify a fragment of MECP2 exon 4 that contains the c.808delC mutation. Sequencing was performed using the same primers and a BigDye v1.1 kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions.

Allele-specific PCR

To determine the parental origin of the mutations, intron 3 and the 3’ untranslated region (UTR) of MECP2 were sequenced in the father and his affected daughters. An informative single nucleotide polymorphism (SNP) was identified in one of the daughters (IVS3 + 648A>G, rs3850326 in individual II-3). Allele-specific PCR primers were designed so that the base on the 3’ end of each primer was complementary to only one allele of the 648A>G SNP. The two forward primer sequences were 5’-GCAGTGTGACTCTCGTTCAA-3’ and 5’-GCAGTGTGACTCTCGTTCA-3’, and the exon 4 return primer sequence was as above. Allele-specific PCR was performed to amplify fragments including the c.808delC mutation. The two allele-specific PCR products were sequenced to determine which haplotype contained the mutation. It was then possible to determine the parent of origin using the father’s genotype at the 648A>G SNP.

Results

We present the case of a father who has two daughters with Rett syndrome. The girls have different mothers but diagnostic genetic testing showed that they share the uncommon MECP2 mutation, c.808delC. Family relationships were confirmed, and we were able to show that the mutation had been transmitted from the father to one of his daughters using allele-specific PCR (see Fig. 1, the other daughter was not informative). The father himself is healthy, and we were not able to detect the mutation in his lymphocyte or buccal DNA, implying paternal germline mosaicism. When we sequenced DNA extracted from his semen, it was possible to see a low level of the c.808delC MECP2 sequence underneath the normal sequence (Fig. 2). By comparing the appearance of the sperm sequence trace with the traces obtained from mixtures of the daughter’s DNA and normal control DNA in various ratios, we estimate that approximately 5% of the father’s semen DNA contains the mutation.

Discussion

This is the first case where germline mosaicism for a MECP2 mutation has been shown in a man. It is difficult to provide this man with a precise risk for having more affected daughters. The proportion of semen DNA carrying the mutation was approximately 5%. The actual proportion of sperm carrying the mutation could be higher because semen contains a mixture of cell types. In this case, it appears that the mosaicism is restricted to the germline because the mutant sequence could not be detected in the lymphocytes or buccal cells. It is possible that somatic mosaicism was present at a level too low
to be detected by sequencing, but given that he did not show any symptoms of this X-linked condition, we believe this is unlikely.

Cases where germline mosaicism for a MECP2 mutation was suspected have been reported previously (1, 4–7). In four of these cases, the pedigree implied that the mosaic parent was the mother, and in one case, the mosaic parent was not identified (7). Also, Rett syndrome is an X-linked condition, so when germline mosaicism is suspected, it is easy to assume that the mother is the carrier. Here, we show that MECP2 mosaicism can occur in a man. Genetic counseling and pre-natal testing should be available for men with an affected child who may be starting a family with a new partner.

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References