CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2014: 86: 295–297
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CLINICAL GENETICS
doi: 10.1111/cge.12287

Letter to the Editor

A novel deletion to normal size in the sperm of a fragile X full mutation male

To the Editor:

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability (ID) and the leading monogenic cause of autism spectrum disorders, affecting approximately 1 in 4000 newborn boys. CGG-repeat instability is the most common mutational mechanism that disrupts *FMR1* expression and accounts for 95–99% of symptomatic mutations

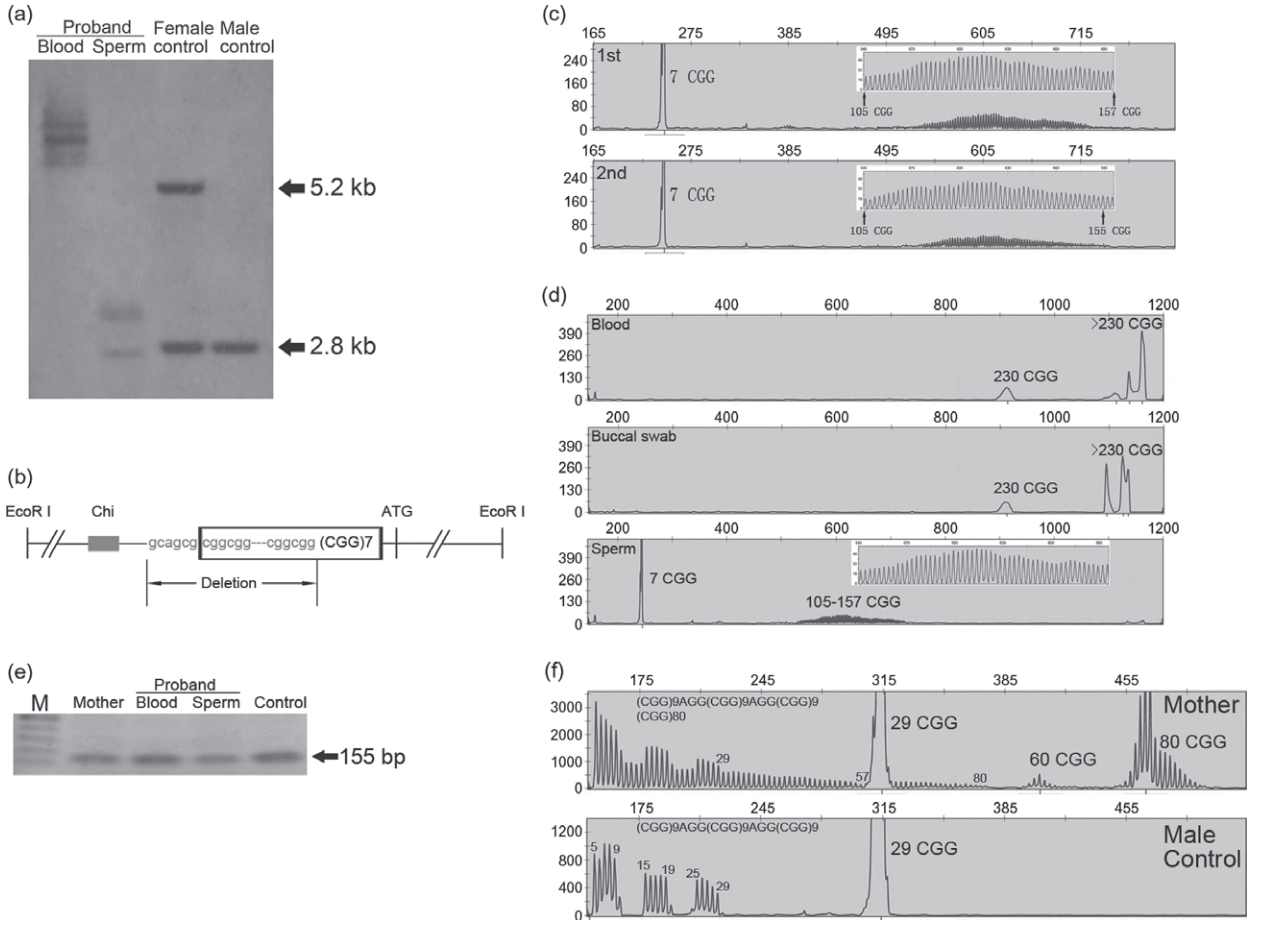

Fig. 1. A deletion occurred in the sperm of a fragile X male. The proband is a 26-year-old Chinese male with moderate intellectual disability. Physical examination of the patient showed typical facial dysmorphism, including prominent large ears, elongated face, high-arched palate, crowded teeth, and macro-orchidism. Behavioural characteristics (poor eye-contact, hyperactivity/attention deficit hyperactivity disorder, seizure disorder, tactile defensiveness, aggressive behaviour and speech delay) were noted. (**a**) Southern blot of the blood DNA shows full mutations as three dominant bands above 5.8 kb whereas the sperm DNA shows a mosaic for premutation and a deletion. (**b**) Schematic representation, not drawn to scale, of the deletion found in the sperm. Flanking sequences of 6 bp at the 5′ end of the CGG repeat and part of the CGG repeat region were deleted (indicated by lower case letters), leaving seven pure CGG repeats consequently accompanying with intact ATG site. The EcoR I sites are indicated. The horizontal arrows indicate the extent of the deletion. The box indicates the CGG repeats that show partial deletion and that consists of pure CGG repeats. The ATG site and Chi-like element are indicated. (**c**) Polymerase chain reaction PCR analyses of duplicate sperm samples received at different time points (4 months away). The upper electropherogram shows mosaic for a premutation and deletion in the sperm and the bottom one shows the same mosaic pattern in another batch of sperm. (**d**) (PCR) analyses of DNA from the blood, buccal swab, and sperm of the subject. In the blood and buccal swab sample, products are obtained only from the full mutation alleles. In the sperm sample, however, both premutations ranging from 105 to 157 repeats and reduced alleles upto seven CGG repeats are obtained. (**e**) Exclusion of deletion in the premutation sized sperm. Lane 1 is the blood DNA sample from the patient's mother. Lane 2 is the blood DNA from the patient. Lane 3 is the sperm sample from the patient. Lane 4 is a female control sample. PCR for lanes 1 to 4 is with primers specifically designed. The expected 155-bp fragment is successfully amplified in all samples, including the sperm DNA (lane 2). (**f**) CGG repeat primed PCR analysis of the maternal blood DNA. Capillary electrophoresis analysis shows that the mother is a premutation carrier of 80 CGG repeats with no AGG interruptions.

(1). A deletion or point mutation in *FMR1* may also cause FXS in a minority of cases; more than 30 articles have documented such instances in patient peripheral blood (2). Males with full mutations only have premutations in their sperm, as determined by an examination of CGG-repeat size in sperm samples from four males with FXS (3). However, in this study, we describe a male patient with full mutation in his blood but a deletion to normal size in one-third of his sperm.

Southern blot analyses showed completely expanded methylated alleles ranging from 5.8 to 7.0 kb in the patient's peripheral blood, but a 3.1-kb band that corresponded to the contracted premutation alleles and an unexpected band similar to the normal unmethylated 2.8-kb band in the sperm, with intensities of 62% and 38%, respectively, which was measured using IMAGE J; (National Institutes of Health; http://rsb.info.nih.gov/ij/; Fig. 1a). A sequencing analysis of the shortened fragment showed that the deletion spanned 6-bp flanking sequences at the 5′ end of the CGG repeat and part of the CGG repeat region, consequently leaving seven pure CGG repeats. A schematic representation of the deletion breakpoints is shown in Fig. 1b. A follow-up examination 4 months later identified the same deletion pattern (Fig. 1c).

A buccal swab was collected to investigate tissue-to-tissue variations. A sensitive *FMR1*-specific polymerase chain reaction (PCR) analysis (Asuragen, Austin, TX) of the blood and buccal swab DNA showed the same repeat-size full mutation with no traceable sign of premutation or deletion, whereas a size mosaic for the premutation, ranging from 105 to 157 repeats, and a deletion (upto seven repeats) were detected in the sperm, which was consistent with Southern blot analysis results (Fig. 1d). The timing of deletion may be predicted to specifically occur in the male germ line after segregation of primordial germ cells at approximately day 18–19 of embryonic development. To investigate whether the deletion also existed in the premutation-sized sperm, a specific primer pair was designed to amplify a 155-bp segment upstream of the CGG repeat region, with the six deleted bases located at the 3′ end of the antisense primer. The amplification would fail if the deletion was present. The expected 155-bp band was amplified from the sperm DNA, showing that the premutation-sized alleles in the sperm did not carry the deletion (Fig. 1e).

To date, it is widely accepted that the presence of AGG interruptions could reduce the risk of expansion during maternal premutation carrier transmission (4). Nevertheless, AGG interruptions have never been discussed in reported cases of deletion. The maternal AGG interruption was investigated with CGG repeat-primed PCR analyses (Asuragen) to identify the proband sequence context, as the AGG pattern in the full mutation and large-sized premutation alleles could not be examined directly, because of technical limitations. The mother showed a pure premutation-sized CGG repeat tract with no 'AGG gaps', and 29 interspersed CGG repeats with 2 'AGG gaps' (9A9A9 pattern) (Fig. 1f). Mnl-I restriction enzyme digestion analysis, another traditional method of AGG element mapping, confirmed the interruption pattern in the mother (data not shown). Given the above findings, we may infer that the expanded full mutations in the patient, which derived from his mother, also contained no AGG interruptions. This loss of AGG interruptions may affect the repeat instability of the CGG-repeat deletion. In addition, all retrievable deleted proband alleles were transmitted from the maternal grandfather, which was noted in our case.

In summary, we report the first case of a deletion in the sperm of male with fragile X full mutation. Further analyses indicated that the loss of AGG interruptions, a well-known genetic stabiliser, might be associated with germline instability. This finding provides new evidence for CGG-repeat instability in the male germline with full mutation, and updates our knowledge about risk assessments during genetic counselling for males with fragile X full mutation.

Acknowledgements

We are grateful to the patient and his family for their kindness and their dedication to fragile X research. This work was funded in part by grants from the National Key Basic Research Program of China (Grant number: 2012CB944600), National Natural Science Foundation of China (Grant numbers: 81071028, 81172513), National Key Technology R&D Program of China (Grant number: 2012BAI09B05), Program for New Century Excellent Talents in University (Grant number: 7603230006).

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