Supplementary files (X-inactivation)

Procedures of X-chromosome inactivation

The human androgen-receptor gene with a polymorphic CAG repeat and *Hpa* II cleavage sites on exon 1 in X*q*12 is used to identify X-chromosome inactivation (X-inactivation), which represents the transcriptional silencing of the majority of genes on one of the X-chromosomes in mammalian females. *Hpa* II, containing CpG dinucleotides in the recognition site, can digest the DNA when the deoxycytosine residue is unmethylated. The X-inactivation status of each allele is measured by its relative levels of methylation. Genomic DNA was extracted from peripheral blood of two carriers (carrier1/2: III-4/IV-11) and one normal male and female (II-6 and III-11). The *Hpa* II digested/undigested samples were amplified by PCR with primers (forward: 5'-TCCAGAATCTGTTCCAGAGCGTGC-3'; reverse: 5'-GCTGTGAAGGTTGCTGTTCCTCAT-3') under the following conditions: 94℃ for 5 min, 28 cycles of 94℃ for 45 sec, 57.5℃ for 30 sec, 72℃ for 30 sec. The amplification products were analyzed on an ABI 3130 automated sequencer, while the peak height was estimated by GeneScan (Applied Biosystems, USA). The calculation of X-inactivation for each androgen-receptor gene alleles was following the formula: (d1/u1) / [(d1/u1) + (d2/u2)]; d1 = peak height of digested DNA from the 1st allele; u1 = peak height of undigested DNA from the 1st allele; d2 = peak height of digested DNA from the 2nd allele; u2 = peak height of undigested DNA from the 2nd allele. Highly skewed X-inactivation was defined as no less than 80/100 calculated ratio for either one of the androgen-receptor gene alleles in the digested DNA samples.

Results of X-inactivation analysis

The results of X-inactivation assay showed that the allele of X-chromosome in the male control is completely digested, suggesting non-occurrence of methylation at all (Figure A),

while both alleles in the female control were amplified with the ratio 37:63 after digestion with *Hpa* II (Figure B). However, compared with the results in male and female controls, a significant deviation was detected with a skewed X-inactivation in the alleles of carriers 1 (Figure C, 100/0) and carrier 2 (Figure D, 83/17), which was obviously beyond 80:20. Therefore, the two carriers (II-4, IV-11) in the family could be defined as highly skewed X-inactivation.

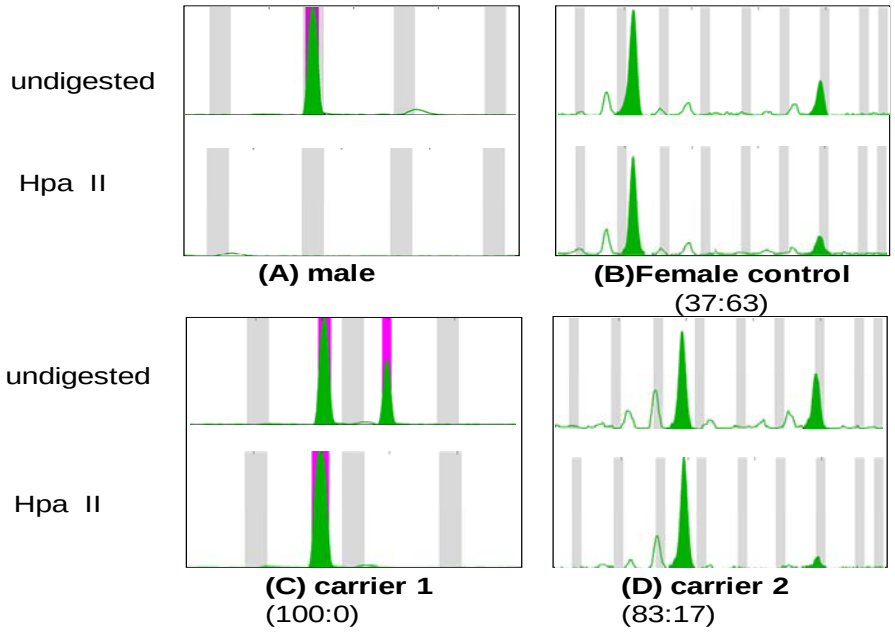

Figure X-inactivation analysis of the carriers in the family. The upper line corresponds to PCR products of undigested DNA; the lower denotes the products of DNA amplification after digestion with *Hpa* II. (A) The allele of X-chromosome in the male control (digested/ unmethylated). (B) The alleles of X-chromosome in the female control (a random X-inactivation pattern with both alleles tending to be amplified equally, 37/63). (C) & (D) The deviated amplification with a skewed X-inactivation in the carrier 1 (100/0) and carrier 2 (83:17).