Occurrence of haemophilia A and B in a Chinese family with mosaicism of the F9 gene mutation in the HB index's maternal grandfather

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Dear Sir,

The haemophiliacs are inherited bleeding disorders caused by low concentrations of specific coagulation factors. The most well known are deficiencies of factor (F) VIII (haemophilia A, HA) and FIX (haemophilia B, HB), both of which show X-linked inheritance. They affect approximately 1 in 5,000 and 1 in 30,000 males, respectively, worldwide (1). So, it is very rare to have both HA and HB patients in one family. To our knowledge, there is only one such case that has been reported so far (2).

In this study, we reported such a pedigree that had both HA and HB (Fig 1). The HA proband was a 34-year-old man and had an abnormal bleeding history from childhood. He was diagnosed as HA at age of three. He had a HA family history, and his two nephews were HA patients as well. Surprisingly, his son also had a history of prolonged bleeding after injury when he was six-month-old and was diagnosed as moderate HB later. So, they requested genetic counselling.

The coagulation studies revealed that the HA proband (II-6) and the HB patient (III-7) had prolonged APTT, whereas PT, TT, Fg were normal. The clotting factor activity assays showed FVIII deficiency in II-6 (FVIII:C 0.3%) and FIX deficiency in III-7 (FIX:C 1.9%).

For genetic analysis, LD-PCR was adopted with some modification to screen the INV22 (3). INV1 was detected using the dual PCR assay as previously described (4). The results showed that INV22 was the causative mutation resulting in the severe deficiency of FVIII in the HA father (II-6). For the HB patient, the coding and boundary sequences of F9 gene were directly sequenced (5). We found that the HB patient had the missense mutation (p.Ser365Cys) in the exon 8 of F9 gene, while his mother (II-7) carried the heterozygous mutation at the same position. As we know, exon 8, the largest exon in F9 gene, is 1.9 KB long coding for almost half of the F9 gene. Half of the reported mutations were found in exon 8 so far (6). Ser365 is in the serine protease domain which comprises the catalytic module containing the highly conserved catalytic triad His221, Asp269 and Ser365 (7). The catalytic serine protease domain of FIXa is responsible for the proteolysis of FX to Fxa. So the mutation of Ser365 may induce structural or functional impairment and patients with mutation at this position usually were severe type according to the FIX:C (<1%). But in this pedigree, the patient’s FIX:C was 1.9% and had moderate phenotype. According to the reference (8), this kind of mutation may be in association with the development of FIX inhibitors. In this pedigree, FIX inhibitor did not develop in the HB patient.

Because the HB patient’s maternal family had no history of HB, we did linkage analysis to ascend the origin of the F9 gene mutation. Six STR sites consisting of DXS8094, DXS1211, DXS1192, DXS102, DXS1227 and DXS8013 were applied to do linkage analysis by multi-fluorrescent PCR method (9). According to the linkage analysis, the HB patient’s causative mutation inherited from his maternal grandfather (I-3). The boy’s maternal aunt (II-8) had the same genotype as her sister (II-7) (Fig 1).

In the extended family, the mutation probably arose in the paternal origin of the index’s mother (10). But in our study, we sequenced the whole F9 gene of the grandfather and did not find causative mutation in his peripheral blood cells. The statistical likelihood was that the spontaneous mutation occurred in the maternal grandfather and mosaicism may exist. We applied ABI PRISM SNaPshot ddNTP Primer Extension kit to confirm and measure the degree of mosaicism of the causative F9 gene mutation. Genomic DNA from blood leukocytes and oral mucosa cells of the HB index’s maternal grandfather was analysed. We first designed a pair of primers (F9F:5’ GTTGACCGAGCCCATGTCTT 3’; F9R: 5’ TGACATACGGGATACCTTGG 3’) and got the 226bp PCR products that would contain the mutant (c.32772T) and wild (c.32772A) allele. Then we used the extension primer (F9F:5’ GAGGTTAGAGATTGATGTCAG 3’) which can extend the PCR products from the 5’ of the relative allele (A/T). During the reaction the ddNTP corresponding to the allele present was incorporated in the example, and the genotype was read as the peak colours, since the ddNTPs were labelled with different fluorescent dyes. Two peaks would be detected in the heterozygote and only one peak could be detected in the patient or the normal.

Our study showed that the maternal grandfather’s oral mucosa cells possessed wild-type as well as mutant F9 genes and the percentage of the mutation was 14.4%. The presence of the mutation in the buccal cells showed that the mutation occurred before the separation of these cells from the blood cells. Since the causative mutation occurred as mosaicism in the grandfather, his descend-
dants could be at risk. So it was important to establish whether any siblings of the index’s mother were affected or carriers. Linkage analysis of HB patient’s maternal aunt showed that she had the same genotype as her mutation-carried sister. But the causative mutation was not found in the aunt’s peripheral blood cells. So we concluded that she was not a carrier. In this case, we presumed that the grandfather was a somatic and gonadal mosaic because he had a mutation-carrier daughter as well as a normal daughter.

Somatic mosaicism may occur during early embryogenesis due to point mutations or small deletions or insertions, as these mutations are thought to originate during mitotic cell divisions. Recent data suggest that mosaicism represent a fairly common event in haemophilia A. In 8/32 families with presumed de novo point mutations, somatic mosaics were found (11). In the early period of embryonic development, three germ layers will differentiate into a variety of proportional tissues. For example, the oral mucosa come from the ectoderm and the blood cells come from the mesoderm. Somatic mosaics appear if mutational event takes place at later stages of embryogenesis and the ratio of the mutated alleles depends on when and where the mutational event occurs. Developmental stage and cell lineage determine the type and degree of mosaicism. Mutations can be detected by over-exposing sequence autoradiographs if it is present in >5% of the cells sampled (12), and Southern blot analysis can only detect approximately 10–20% of the somatic mosaics (13). The significance of mosaic mutations may be underestimated, because they usually remain undetected during routine mutation analysis. In our study, we applied the extension primer PCR method to detect the degree of the mosaics. We assessed the sensitivity of the primer extension PCR method for detecting the mosaics by a mutant/wild DNA mix experiment. Serial mosaic mixture samples were simulated in vitro by mixing decreasing quantities of the mutant (c.32772T) allele DNA (2%, 3%, 5%, 10% and 15%) with complementary amounts of control (c.32772A) DNA. By visual inspection of ethidium bromide-stained analysis results, we es-

Table 1: Detection of serial mosaic mixture samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>&quot;A&quot; peak height</th>
<th>&quot;T&quot; peak height</th>
<th>T/A (practical ratio)</th>
<th>T/A (theoretical ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=2</td>
<td>592</td>
<td>16</td>
<td>0.027027027</td>
<td>0.020408163</td>
</tr>
<tr>
<td>T=3</td>
<td>670</td>
<td>30</td>
<td>0.044776119</td>
<td>0.030927835</td>
</tr>
<tr>
<td>T=5</td>
<td>720</td>
<td>49</td>
<td>0.068055556</td>
<td>0.052631579</td>
</tr>
<tr>
<td>T=10</td>
<td>1983</td>
<td>229</td>
<td>0.115481594</td>
<td>0.111111111</td>
</tr>
<tr>
<td>T=15</td>
<td>671</td>
<td>123</td>
<td>0.183308495</td>
<td>0.176470588</td>
</tr>
</tbody>
</table>

Samples were the serial mosaic mixture simulated in vitro by mixing decreasing quantities of the mutant (c.32772T) allele DNA (2%, 3%, 5%, 10% and 15%) with complementary amounts of control (c.32772A) DNA.

Based on the genetic detection, we concluded that the genetic diagnosis is consistent with the phenotype diagnosis in this family. Occurrence of HA and HB in this family is based on the association of two distinct genetic defects. HA was due to the INV22 while HB resulted from F9 gene mutation originated from the mutation as mosaicism in his maternal grandfather. For sporadic family, we should take into account the mosaicism as it causes a dilemma about the recurrence risk in parents who appear to be non-carriers. The extention primer PCR method is a good approach to detect the mosaicism in haemophilia and can give information which may be valuable in determining the origin of new haemophilia mutations, and in counselling relatives as well.

**Reference**