An aberrant F8 intron 1 inversion with concomitant large duplication and deletion in a Chinese severe hemophilia A patient

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1. Introduction

Hemophilia A (HA, OMIM: 306700) is an X-linked recessive bleeding disorder with a prevalence of 1:5000 new-born male. HA is caused by quantitative or functional defects of the coagulation factor VIII (FVIII) encoded by the F8 gene [1]. The severity of HA is classified into three categories according to the plasma FVIII activity (FVIII:C): severe (FVIII:C <1%), moderate (FVIII:C 1%–5%), and mild (FVIII:C 5–40%) [2]. F8 intron 22 and intron 1 inversion (Inv22 and Inv1) account for ~45% and 1–5% of severe HA cases, respectively. We herein described an aberrant Inv1 with concomitant large duplication and deletion in a Chinese severe HA patient.

2. Methods

DNA sample was extracted from the peripheral blood mononuclear cells (PBMCs) and used for Inv22, Inv1, multiplex ligation-dependent probe amplification (MLPA), and WGS. The int1h-1 is amplified using primers specific for int1h-1 (9F, 9cR) and the primer 2F. Healthy controls show a 1.5-kb fragment and Inv1 patients harbor a 1.0-kb product, while HA carriers have both fragments. The int1h-2 is amplified using primers specific for int1h-2 (2F and 2R) and the primer 9F, yielding a 1.0-kb and a 1.5-kb products from healthy controls and Inv1 patients respectively (Figure 1), while HA carriers have both fragments [4]. Coverage analysis of read depth data from whole-genome sequencing (WGS) was used to analyze the intronic duplication and deletion of the F8 gene. This study was approved by the ethic committee of Tongji Hospital, and informed consent was obtained from the parents.

3. Results and discussion

A 1-year-old boy with severe HA (FVIII:C <0.2%) was admitted, and his parents came for genetic test and counseling for further child. As Inv22 and Inv1 account for near 50% of all severe HA cases, Inv22 and Inv1 were performed for the boy and his mother. Inv22 showed some bands with healthy control (data not shown), however, aberrant patterns of Inv1 were
found in this boy and his mother. The boy had a 1.0-kb band in int1h-1, while his mother had both 1.0-kb and 1.5-kb fragments. For int1h-2, both the boy and his mother carried a 1.0-kb product. They showed aberrant patterns of Inv1 compared with healthy control, Inv1 patient, or Inv1 carrier (Figure 2A).

The int1h-1 is amplified using int1h-1 specific primers (9F, 9cR) and the int1h-2 specific primer 2F, while the int1h-2 is amplified using int1h-2 specific primers (2F and 2R) and the primer 9F. To clearly identify the product of int1h-1 and int1h-2, we amplified int1h-1 and int1h-2 using diverse primer pairs. The int1h-1 was amplified in three PCR tubes including different primers, 9F+9cR+2F (mixed), 9F+9cR (for wild type sequence), and 9cR+2F (for Inv1 sequence). The int1h-2 was amplified in three PCR tubes including different primers, 2F+2R+9F (mixed), 2F+2R (for wild type sequence), and 2R+9F (for Inv1 sequence). The boy showed same pattern of int1h-1 (amplified using 9cR+2F primer pair for Inv1 sequence) with Inv1 patient, but had same pattern of int1h-2 (amplified using 2F+2R primer pair for wild type sequence) with healthy control (Figure 2B). The presence of inversed int1h-1 in the boy suggests the occurrence of Inv1. However, the wild-type int1h-2 deserved further investigation.

To date, aberrant patterns of Inv1 have been reported involving complex duplication/deletion rearrangements near the F8 gene, leading to the presence of zero or two copies of int1h-1/int1h-2 [5,6]. To detect the complex duplication/deletion rearrangements of the F8 gene, MLPA was applied, and no duplication/deletion was found in the exonic regions of the F8 gene in the boy (Figure 2C). However, the intronic regions, 5'-untranslated region (5'-UTR) and 3'-UTR of the F8 gene, were not covered by MLPA due to the lack of probes for these regions.

Previously, we have applied coverage analysis of next-generation sequencing (NGS) data involving the F8 gene to detect large exon deletion in patients of HA, and the results were consistent with MLPA [7]. Moreover, coverage analysis of read depth from whole-exome sequencing (WES) data has been used in the detection of copy-number variations (CNVs) [8]. Compared to WES, whole-genome sequencing (WGS) could detect both exonic regions and intronic sequences. WGS has been also used for CNV analysis [9,10]. Therefore, WGS was performed for the boy on NovaSeq6000 platform by Aegicare Biotech (Shenzhen, China) with a mean coverage of 30X. A large deletion crossing X:154235303-154237171 (hg19) and a large duplication including X:154259274-154376426 (hg19) were identified by the coverage analysis of read depth (Figure 3A and B). The genomic region of int1h-1 amplified using 9F+9cR was located in X:154234156-154236063 and int1h-2 amplified using 2F+2R was located in X:154376000-154377190 respectively (Figure 3C).

The presence of inversed int1h-1 (amplified using 9cR+2F primer pair) in the boy (Figure 2B) confirmed the occurrence of Inv1. The large deletion region (X:154235303-154237171, hg19) included the homologous region of int1h-2 (X:154234382-154235422, hg19) within int1h-1, which might result in the absence of inversed int1h-2 (amplified using 2R+9F primer pair). Moreover, the large duplication region (X:154259274-154376426, hg19) crossed the int1h-2 (X:154376000-154377190, hg19) partially. This duplication might lead to two copies of int1h-2, and one copy was
inversed with int1h-1, while the other copy could be still detected using 2F+2R primer pair showing similar bands with healthy control. Taken together, the genomic rearrangements were caused by an aberrant F8 Inv1 with concomitant a duplication of 117-kb and a deletion of 1.8-kb at Xq28 (Figure 4).

Figure 2. The aberrant patterns of Inv1 in a 1-year-old boy with severe HA. A, The int1h-1 is amplified using int1h-1 specific primers (9F, 9cR) and the int1h-2 specific primer 2F, while the int1h-2 is amplified using int1h-2 specific primers (2F and 2R) and the primer 9F. The boy showed similar int1h-1 band with Inv1 patient, while had similar int1h-2 band with healthy control. His mother had similar int1h-1 bands with Inv1 carrier, while had similar int1h-2 band with healthy control. B, The int1h-1 was amplified in three PCR tubes including different primers, 9F+9cR+2F, 9F+9cR (for wild type sequence), and 9cR+2F (for Inv1 sequence). The int1h-2 was amplified in three PCR tubes including different primers, 2F+2R+9F, 2F+2R (for wild type sequence), and 2R+9F (for Inv1 sequence). The boy showed same pattern of int1h-1 with Inv1 patient, but had same pattern of int1h-2 with healthy control. This result confirmed the presence of inversed int1h-1. C, MLPA was performed to detect duplication and deletion within the exonic regions of the F8 gene. The ratio between 0.75 and 1.25 was considered as normal.

With the exception of classic F8 Inv1, aberrant Inv1 with concomitant duplication/deletion have been found in severe HA patients. Sanna et al. reported aberrant F8 Inv1 with concomitant a 19.32-kb duplication involving a part of the int1h-1 and extending through intron 6, as well as a 41.87-kb extragenic deletion within the telomeric...
position in a severe HA patient from Southern Italy [5]. You et al. reported an aberrant pattern of Inv1 with a duplication of 227.3 kb and a deletion of 2.56 kb within the F8 gene in a Chinese pedigree with severe HA [6].

In conclusion, we reported an aberrant Inv1 with concomitant large duplication and deletion in a severe Chinese HA patient. Moreover, WGS provides rapid genetic diagnosis of hereditary

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**Figure 3.** Coverage analysis of the WGS data. A, A large deletion crossing X:154235303-154237171 (hg19) was found in the boy. B, A large duplication including X:154259274-154376426 (hg19) was identified by the coverage analysis. C, The genomic regions of int1h-1 and int1h-2 amplified using 9F+9cR and 2F+2R respectively. The red dotted line labeled int1h-1 or int1h-2, and the bottom region was the almost identical region.

**Figure 4.** Hypothetical arrangement of the X chromosome in the patient. The deletion occurred in X:154235303-154237171 involving int1h-1. The duplication occurred in X:154259274-154376426 involving int1h-2.
disorders with point mutations, deletions, insertions, and CNVs.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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