Gene Rearrangements and Mutations in Malaysian Patients with Haemophilia A

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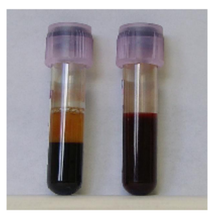
Introduction

Haemophilia A (HA) is an X-linked bleeding disorder caused by mutations in the coagulation factor VIII (FVIII) gene . The FVIII is located on the long arm of chromosome X (Xq28). Consisting of 26 exons, this gene is extremely large (186 kb) and structurally complicated. The molecular defects of FVIII are caused by a broad spectrum of mutations : intron 22 and intron 1 inversions that affect 40-50% and 1-5% of severe HA patients respectively are hot spot mutations (1,2) and the other mutations spread throughout the FVIII. Mutation detection is important for the prediction of inhibitor development and for an accurate diagnosis of female carriers. The aim of this study was to characterise the mutations of FVIII in 100 unrelated Malaysian patients with HA.

Materials and Methods

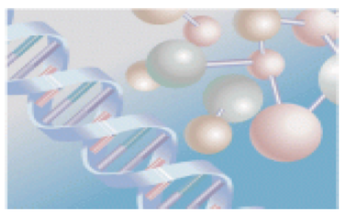
PATIENTS & DNA EXTRACTION

Informed consent

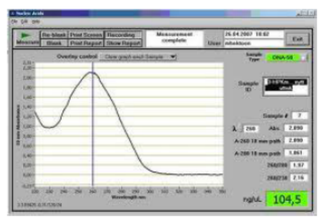


Blood sample collection : 10 ml in EDTA tubes

83 sev, 9 mod, 8 mild HA patients



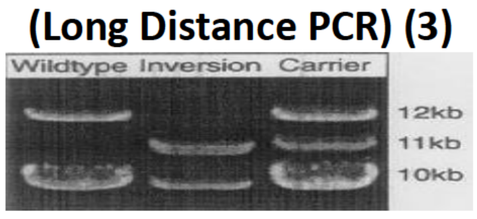
Genomic DNA extraction



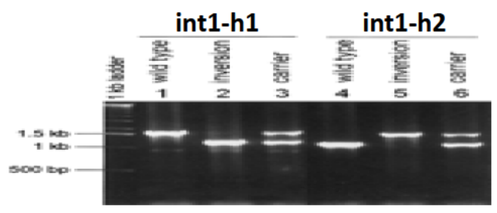
DNA quantification

MOLECULAR DIAGNOSIS OF FVIII MUTATION

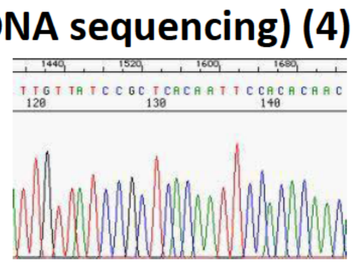
Intron 22 Inversion detection (Long Distance PCR) (3)



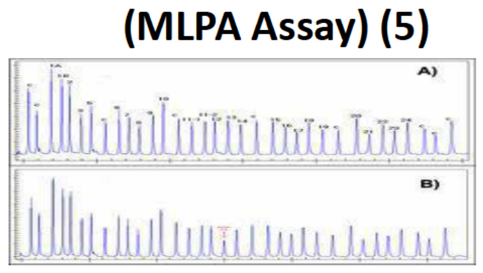
Intron 1 Inversion detection (Conventional PCR) (2)



Other Mutations (DNA sequencing) (4)



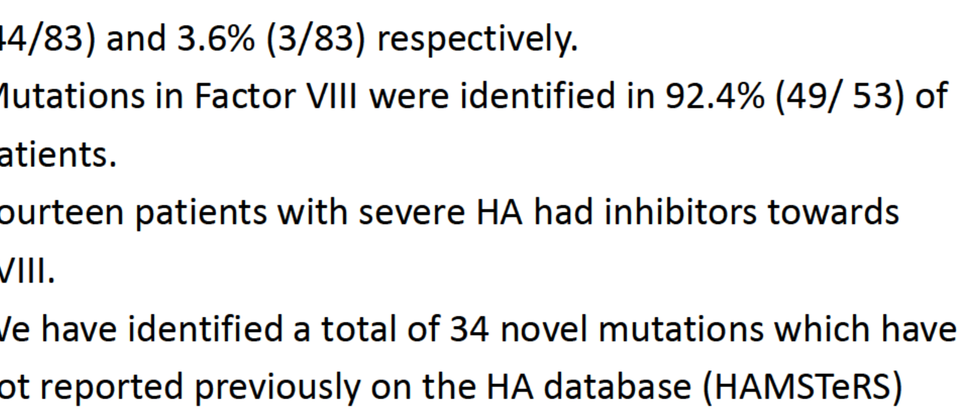
Confirmation of Large Deletions (MLPA Assay) (5)



Results and Discussion

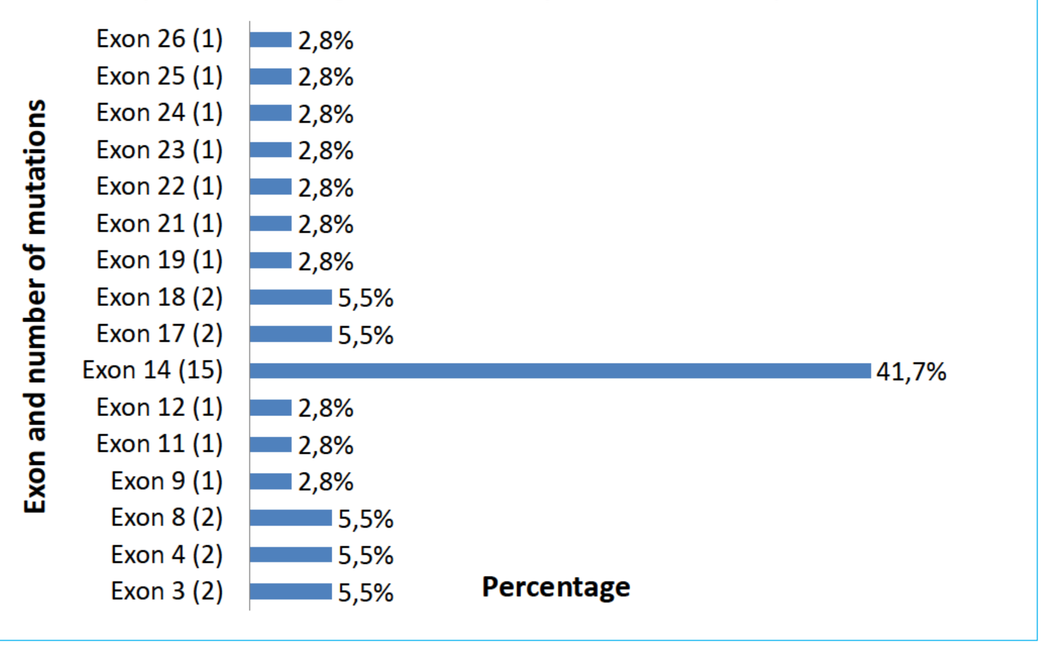
- Intron 22 and intron 1 inversions were detected in 53.0% (44/83) and 3.6% (3/83) respectively.
- Mutations in Factor VIII were identified in 92.4% (49/ 53) of patients.
- Fourteen patients with severe HA had inhibitors towards FVIII.
- We have identified a total of 34 novel mutations which have not reported previously on the HA database (HAMSTeRS) (Fig 1).
- Two patients with multiple mutations in exon 14 were also detected : one with double mutations (a small deletion and a missense mutation) and another with triple mutations (a small deletion and two missense mutations).

Figure 1 : Distribution of the novel mutations in unrelated HA patients



- An insertion/deletion of one nucleotide in a run of 9A at position 3629-3637 (codons 1191-1194) were detected in five patients.
- Most of the mutations (41.7%) were found at the largest exon of FVIII, i.e exon 14 (Fig 2).

Figure 2. Location of FVIII mutations in unrelated HA patients based on exons (not included large deletions and splice site mutations).



Conclusions

The mutation profile among our local HA patients will provide a useful reference database in the detection of carrier status and the diagnosis of HA in Malaysian population.

Acknowledgement

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References

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