Deep intronic variants in the factor VIII gene

INTRODUCTION

Haemophilia A (HA) is the most common X-linked bleeding disorder with an incidence of 1 in 5000 males. Different mutation types in the factor VIII gene (F8) lead to a decrease in coagulation factor VIII activity and variable severities of the patients’ phenotypes. The causative alteration can be detected in the vast majority of HA patients by standard diagnostic screening methods targeting the coding regions of the F8 gene. Still, this approach fails in about 4% of cases. This study intended to analyse the whole F8 gene including all intronic sequences in 16 HA patients by next generation sequencing (NGS) in order to screen for deep intronic variants.

METHODS

Fifteen of the male index patients showed mild and one case a moderate HA phenotype with no abnormality in the coding sequences and splice sites of the F8 gene identified by standard diagnostic techniques. Patient 16 was pre-diagnosed with a duplication of exons 1-22 and a triplication of exons 23-25 of the F8 gene by multiplex ligation-dependent probe amplification (MLPA). Target enrichment for the whole genomic region of F8 was performed with the SureSelectXT system (Agilent) and sequencing on a MiSeq (Illumina). NGS data were analysed with GenSearchNGS (PhenoSystems).

RESULTS

NGS data revealed 23 deep intronic candidate variants in different F8 introns. Three of the single nucleotide variants (SNVs) have been described before as HA causing mutations. Six other variants were recurrent among the 15 cases studied. Several bioinformatic tools were used to score all candidate variants regarding their potential deleteriousness (e.g. by C-scores, Fig. 1) and the creation of de novo splice sites (Fig. 2), also in comparison to already published deep intronic F8 mutations. In each of the patients, at least one SNV or copy number variation (CNV) was predicted to be potentially pathogenic.

The patient with moderate HA additionally showed a deletion of 9.2 kb in intron 1 with both breakpoints located in Alu-type repeats (Fig. 3). The CNVs of patient 16 could be well seen in the NGS data by comparing the coverage of the affected exons, but it wasn’t possible to define exact breakpoints (Fig. 4).

In summary, this NGS approach proved an effective method to analyse the whole F8 gene for potentially pathogenic deep intronic variants and CNVs in a selection of 16 male HA patients. In general, this approach bears the potential to be applied as efficient one-step sequencing method of the complete F8 gene in molecular diagnostics of haemophilia A. Yet, in a diagnostic setting besides in silico prediction further functional studies like mRNA analysis would be required to confirm the causality of deep intronic variants. As this study was pseudonymized, mRNA analyses on patients’ blood samples were not possible. Therefore, in a recent study we successfully tested mini-gene assays to confirm causality of some of the candidate variants.

CONCLUSION

REFERENCES

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