DOI: 10.1111/hae.14428

Haemophilia WILEY

EDITORIAL

Haemophilia diagnostics with modern genomics

In this special issue (https://onlinelibrary.wiley.com/doi/toc/10.1111/(ISSN)1365-2141.modern.genomics.diagnostic.haematology), *Haemophilia* has teamed up with other Wiley haematology journals: The American Journal of Haematology, British Journal of Haematology, European Journal of Haematology, and Haematological Oncology, to bring you a snapshot of the way NGS is applied in the practice of Haematology.

These three selected articles, all recently published in *Haemophilia*, highlight the contribution of modern genomic technology to diagnosis and treatment of haematological diseases.

1 | UNMASKING PREVIOUSLY UNIDENTIFIED PATHOGENIC GENETIC VARIANTS OF HAEMOPHILIA A[1]

Haemophilia A, the most common severe inherited bleeding disorder, is caused by a deficiency and/or dysfunction of coagulation factor VIII (FVIII). The clinical severity of HA depends on the residual factor VIII activity (FVIII:C) and is classified into three levels: severe (FVIII:C < 1 IU/dl), moderate (1 IU/dl ≤ FVIII:C ≤ 5 IU/dl) or mild (5 IU/dl < FVIII:C < 40 IU/dl). The FVIII is encoded by F8 which spans 186 kb of the long arm of the X chromosome (Xq28) and includes 26 exons. More than 3000 different pathogenic F8 variants have been described in all HA phenotypes. Identification of the molecular defect responsible for HA is important for both enhancing haemophilia patient care and identifying female carriers with the possibility of prenatal diagnosis in cases of a male foetus and severe HA. Despite an exhaustive screening, the causative variant is still missing in 2–5% of HA patients. In these patients, a deep intronic variant or a genomic rearrangement disrupting the F8 gene could be causal. Several F8 deep intronic variants have been previously reported to create de novo acceptor splice sites (ASS) or donor splice sites (DSS), increase the strength of a pre-existing cryptic ASS or DSS, promote Alu exonization or reduce F8 RNA expression. Consequently, several groups have developed whole F8 gene sequencing approaches using next-generation sequencing (NGS) in order to identify deep intronic variants and to resolve the mutant genotype in patients in whom conventional genetic approaches failed.

This study aimed to identify F8 deep intronic variants in 15 genetically unresolved French and Canadian HA patients using F8 whole gene sequencing and to characterize these variants using functional analyses. The entire F8 gene was sequenced using an NGS capture method. The F8 mRNA was analysed in order to detect aberrant transcripts. The pathogenic effect of candidate intronic variants was further confirmed using a minigene assay. After bioinformatic analysis, 11 deep intronic variants were identified in 13 patients (eight new variants and three previously reported). Three variants were confirmed to be likely pathogenic with the presence of an aberrant transcript during mRNA analysis and minigene assay.

This comprehensive study is one of very few to combine NGS and mRNA sequencing as well as minigene assay to confirm the pathogenic effect of new deep intronic variants that cause HA through splicing alteration. This work supports that functional analyses are critical to confirm the pathogenic effect of these variants and will be invaluable in the future to study the large number of variants of uncertain significance that may affect splicing that will be found in the human genome.

Although these results are very promising, the challenge will be to implement these strategies in as many genetic centres as possible and to ensure that as many patients as possible can benefit.

2 | THE EAHAD COAGULATION FACTOR GENETIC VARIANT DATABASES: IMPORTANT RESOURCES FOR HAEMOSTASIS CLINICIANS AND RESEARCHERS[2]

Advances in genomic sequencing have facilitated the sequencing of genes associated with disorders of haemostasis. The task of reporting and analysing DNA variants is a major challenge. There are currently two types of repositories for these variants. First, central databases that contain pooled information on variation across the whole genome and locus-specific databases (LSDBs), that collect and display information on sequence variants (mutations) on a gene-by-gene basis and are usually run by a consortium of collaborating researchers with scientific expertise in a particular gene or phenotype.

LSDBs for haemostatic genes were first established with the publication of collated data for variants in F9, F8 and VWF in the early 1990s. The first web-based database for F8 appeared in 1996. Over the years, several additional coagulation factor variant databases have also been developed (F7, F10, F11 and fibrinogen). These resources provided an invaluable resource to the haemostasis community. However, many of these databases were established and maintained by individuals and availability of time and funding had a significant impact on their ability to maintain the database as well as continuity of access to a stable URL.

In response to these concerns, the European Association for Haemophilia and Allied Disorders (EAHAD) initiated in 2011 a Coagulation Factor Variant Database Project with the aim of gathering

together single gene variant databases involved in clinical bleeding disorders that would provide a single web portal to LSDBs for genes in haemostasis. The LSDBs would share a common architecture making navigation of the database(s) easier as well as providing greater support for maintenance of the sites. These new databases described in details in this paper currently present information about the genotype, phenotype (laboratory and clinical) and structural and functional effects of variants described in the genes of factor (F) VII (F7), FVIII (F8), FIX (F9) and von Willebrand factor (VWF).

The analysis of genotype-phenotype relationships in rare diseases such as the bleeding disorders associated with deficiencies in FVII, FVIII, FIX and VWF requires international collaboration and standardized reporting. The establishment of the EAHAD Coagulation Factor Variant Database Project has generated a common database architecture and web-based platform that includes analytical tools to assess the structure/function consequence of variants. This has allowed the implementation of updated variant databases for F7, F8, F9 and VWF. The variant databases represent an invaluable resource for scientists and clinicians in the field of haemostasis.

3 | THE GENETIC CAUSES OF HAEMOPHILIA IN WOMEN AND GIRLS[3]

Haemophilia is not an exclusively male disease. It also affects women and girls who deserve far more attention. The F8 and F9 genes are located on the long arm of the X chromosome making them subject to the unique inheritance pattern of X-linked genes. Males who have a deleterious allele on their single X chromosome exhibit its full effects. Their bleeding phenotype depends on the specific deleterious allele present responsible for complete or partial clotting FVIII or FIX deficiency.

Female haemophilia carriers are expected to have a FVIII or FIX plasma concentration corresponding to half that found in healthy individuals, which is generally adequate for normal haemostasis. However, clotting factor levels may greatly vary in carriers, ranging from very low to the upper limit of normal. A number of variables have been proposed to explain clotting factor deficiency in women and girls with haemophilia (WGH), including co-inheritance of a variant von Willebrand factor allele in carriers of HA, homozygous or compound heterozygous defect alleles of FVIII of FIX genes, structural or numerical aberrations of the X chromosome, extreme lyonization and mutation severity.

WGH are not uncommon. The proportion of females among the unique HA and HB patients attending 135 haemophilia treatment centres in the United States between 2012 and 2020 was found to be 6.7% (1672/25043). Females made up 16% of mild HA patients and 23.7% of HB patients. WGH are obviously at risk, depending on their factor level, of presenting haemorrhagic complications like men with haemophilia (hemarthrosis, haemorrhage from invasive procedures) but also gynaecological and obstetric haemorrhages.

This article provides a very high quality and comprehensive review of the available data concerning haemophilia in women and girls, its complex genetic basis and the diagnostic strategy. This article should contribute to raising awareness of haemophilia in women and girls, an underestimated entity.

Cedric Hermans

Division of Hematology, Cliniques universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium

Correspondence
Professor Cedric Hermans, Division of Hematology, Cliniques universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), 10 avenue Hippocrate, 1200 Brussels, Belgium.
Email: cedric.hermans@uclouvain.be; hermans.cedric@gmail.com

ORCID
Cedric Hermans https://orcid.org/0000-0001-5429-8437

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