Addendum on Genetic Testing to Guideline for the diagnosis and management of the rare coagulation disorders

A United Kingdom Haemophilia Centre Doctors' Organisation Guideline on behalf of the British Society for Haematology

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Introduction

This addendum to the 2014 guideline for the diagnosis and management of the rare coagulation disorders focuses on the use of genetic testing[1]. The rare coagulation factor deficiencies discussed in the guideline are caused by variation in specific genes. Although these genes have all been characterised, testing in clinical practice has previously been limited by the availability of polymerase chain reaction (PCR)-based sequencing. When the original guideline was published, testing was available in a few UK laboratories and was often not accredited. As a result, most patients registered in the National Haemophilia Database with a rare factor deficiency have not been offered genetic diagnosis. In the last decade there have been major advances in genetic testing methodology. High throughput sequencing techniques (HTS, also referred to as Next Generation Sequencing or NGS) were developed during the human genome project and provide access to much greater amounts of DNA sequence than was possible with PCR-based sequencing[2]. This allows for several genes to be analysed in a single test using a panel that contains all of the genes known to be associated with heritable bleeding disorders[3,4]. The Genomics in Thrombosis and Haemostasis Scientific Sub-committee of the International Society on Thrombosis and Haemostasis (ISTH) curates a list of these genes (isth.org/page/GinTH_GeneLists). Annually the sub-committee considers the current state of knowledge of candidate genes and decides whether a causative link with a bleeding disorder has been established requiring the gene to be included in the list, or whether further research into causation is required[5]. In the UK, Genomics England maintains the NHS National Genomic Test Directory and lists the gene panels that are available for testing of patients as part of standard NHS care (https://panelapp.genomicsengland.co.uk/). The R90 panel includes the haemostasis

genes on the ISTH curated list and is designed for testing of patients with heritable bleeding disorders including rare coagulation disorders.

Use of Genetic or Genomic Testing in the Diagnosis of Rare Coagulation Disorders

There are several reasons for identifying the causative variant in patients with a heritable bleeding disorder. In contrast to haemophilia A and B, the factor level often does not correlate well with the severity of the bleeding phenotype in patients with rare factor deficiencies when measured using a clot-based activity assay. This may be because very low levels of factor that are below the detection limit in the assay, are nevertheless sufficient for normal haemostasis. Factor assays are generally unable to distinguish cases with complete absence of production from those with extremely low levels. This is important in conditions such as factor VII deficiency where absence of factor results in a severe phenotype associated with fatal bleeding in the neonatal bleed, whereas a very small level of activity results in a mild, or even asymptomatic, phenotype. In cases with factor activity level below 1 U/dL identification of the causative variant, and whether this would result in a null allele or one with some production of protein, can better predict the bleeding phenotype than a factor assay.

Inhibitor formation following factor replacement in rare coagulation disorders occurs at a much lower rate than in haemophilia A. Knowledge of the causative *F8* variant leads to better estimation of the inhibitor risk than reliance on the factor level only. This is also true of some rare factor deficiencies. In factor XI deficiency the two commonest causative variants in *F11* are a nonsense change (p.Glu135*) and a

missense (p.Phe301Leu)[6]. Homozygosity for p.Glu135* results in a null allele with factor XI:C <1 U/dL whereas homozygosity for the p.Phe301Leu missense variant allows production of a small amount of factor with variable levels of <1 – 15 U/dL. Inhibitor formation has only been described with homozygosity for p.Glu135* and occurs in about 20% of cases (Kamel et al., in press), whereas it does not occur if there is some, albeit undetectable, factor activity[7]. Genetic analysis is required for effectively identifying this risk. There are other phenotypic traits that may also become easier to predict following genetic testing. Dysfibrinogenemia is mostly a bleeding disorder but may occasionally be a rare heritable cause of thrombophilia. Fibrinogen levels are unable to distinguish these phenotypes, and neither is genetic diagnosis at the moment. This is partly because relatively few cases of dysfibrinogenemia have a genetic diagnosis. As this increases, and our understanding of the genotype-phenotype relationship improves, we can anticipate better phenotype prediction. This is a prerequisite for personalisation of treatment in the future and requires universal genetic testing as opposed to selective testing of a few affected individuals.

The mode of inheritance in the rare factor deficiencies is mostly autosomal recessive. This is always the case if a variant results in a null allele. Many heterozygous carriers of a null allele will be asymptomatic with a normal factor level. As the presence of two null alleles in disorders such as factor X deficiency will inevitably be associated with a severe bleeding disorder, sometimes associated with intracranial bleeding in the perinatal period, identification of carriers is essential for counselling prior to or during pregnancy. This requires prior knowledge of the causative variant and allows for a more accurate prediction of the phenotype in the

unborn child paving the way for pre-implantation genetic diagnosis in cases with a severe phenotype.

Alongside these clear advantages to genetic diagnosis, there are also disadvantages and limitations[8]. HTS has greatly improved the efficiency and accessibility of testing for single nucleotide variants. Other types of genetic abnormality, such as copy number variation or gross rearrangements, are less easy to detect by these methods or may not be detected at all. Clinicians need to be aware of the limitations of different HTS platforms, and the sensitivity for different types of genetic variation, when requesting analysis. In genes that are poorly characterised there may be insufficient knowledge to allow classification of a variant as pathogenic or benign. Compared with *F8* and *F9* there is a relatively higher proportion of variants of uncertain significance in genes associated with the rare factor deficiencies. As the analysis technique will often cover multiple genes there is a possibility of incidental findings. Guidance on strategies for dealing with these issues and discussing them in the consent process has recently been published by the UK Haemophilia Centres Doctors' Organisation (UKHCDO) and other organisations [9–11].

Recommendations

- **Genetic analysis should be offered to all patients suspected of having a heritable rare coagulation disorder (1B)**
- **If a high-throughput sequencing (HTS) panel is used, it should include all the genes listed by the International Society on Thrombosis and Haemostasis as causative of coagulation factor deficiencies (1B)**

- **The consent process for genetic testing should discuss the limitations and drawbacks of the methodology used, including the possibility of variants of uncertain significance and incidental findings, and implications for patients and family members (1B)**

Review of the manuscript

Review of the manuscript was performed by the British Society for Haematology (BSH) Haemostasis and Thrombosis Task Force and the advisory committee of the UKHCDO.

Acknowledgements

Additional members of the BSH Haemostasis and Thrombosis task force at the time of writing this addendum were: Will Lester, Julia Anderson, Jayashree Motwani, Karen Breen, Lara Roberts, Sean Platton, Peter Baker, Stella Williams, Khalid Saja and Renu Riat. The author would like to thank them and the BSH guidelines committee for their support in preparing this addendum.

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