Thrombosis & Haemostasis: Research

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Editorial

Added Value of Next-Generation Sequencing for Haemostasis Diagnosis

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Received: October 10, 2017; **Accepted:** October 11, 2017; **Published:** October 20, 2017

Editorial

DNA sequencing technology has made impressive advances in recent years [1]. The Next-Generation Sequencing (NGS) allows for the fast generation of thousands to millions of base pairs of DNA sequences of an individual patient and brings genetic diagnostics into a new era. The benefit of implementing NGS in diagnostics is the introduction of testing many genes at once in a relatively short time and at a relatively low cost, and thereby yielding more molecular diagnoses [2,3].

Several commercial NGS instruments referred to as NGS platforms, have become available. All platforms require some level of DNA pre-processing into a library, a collection of DNA fragments that together represent the genome of an organism suitable for sequencing. Generally, all DNA library protocols involve (fragments of) DNA molecules fused with adapters that contain the necessary elements for immobilization on a solid surface and sequencing [4].

The molecular analysis of *VWF, ADAMTS13* and complement genes are very useful in the diagnosis of VWD and the differential diagnosis of TTP/aHUS. Studying the large-size *VWF* and the several genes implicated in TTP/aHUS using conventional Sanger DNA sequencing in diagnostic laboratories have been at a disadvantage due to its high costs. The advent of NGS is changing this paradigm and greatly improves the molecular analysis of these diseases; massive parallel sequencing has reduced the cost and increased the throughput of DNA sequencing [5-8].

Furthermore, this methodology has already been applied to diseases that the diagnosis is very difficult and almost exclusively made based on well-defined clinical criteria, such as In Hereditary Platelet Disorders (IPDs). The identification of the underlying cause of IPDs is clinically challenging due to the absence of a gold- standard platelet test, and is often based on a clinical presentation and normal values in other hematology assays. As a consequence, a DNA-based approach has a potentially important role in the investigation of these patients. The NGS technologies are allowing the rapid analysis of genes that have been previously implicated in IPDs or that are known to have a key role in platelet regulation, as well as novel genes that have not been previously implicated in platelet dysfunction [9].

The differential diagnosis in patients who have had thrombotic events i.e. the identification of the underlying cause is also often difficult. The prevalence of natural anticoagulants deficiencies (protein C, protein S and antithrombin) is low and probably under diagnosed. The molecular study of respective genes (*PROC*, *PROS1* and *SERPINC1)* increase the diagnostic capacity, allow establishing a genotype/phenotype correlation and to identify patients at venous thrombosis risk. Pathogenic variants were detected in patients with normal plasma levels, confirming the failure to detect qualitative changes by the available functional assays. The molecular study should be considered in patients with a significant personal/family history of thrombosis, and "normal/borderline" levels of these proteins [10]. The contribution of NGS-studies based on known and candidate genes, have been published and these would help to reveal the precise mechanism by which these genes influence the risk of deep vein thrombosis [4,11]. The double heterozygosity in several implicated genes is often the possible explanation of thrombotic phenotype observed in some patients [12].

The expectation that Whole-Exome Sequencing (WES) approaches could identify other genes involved in haemostasis disorders is high. Overall, in recent years there had been a marked increase of new disease gene associations and these results provide insight into study design and analytical strategies, identify novel mechanisms of disease, and reveal the extensive clinical variability of Mendelian phenotypes [13,14]. Indeed, some authors has recently highlighted that genes harbouring both causal variants for Mendelian disorders and risk factors for complex disease traits tend to present higher expression levels than genes associated only with complex disorders [15]. However, these studies still remain under investigation [16].

Conversely, NGS-targeted gene panels are being introduced into clinical practice provide substantial benefits for definitive diagnoses in haematological diseases as demonstrated several recent reports [6-8,12,17,18]. An essential contribution to this is the recently published guidelines for the evaluation and validation of NGS applications for the diagnosis of genetic disorders [16].

The knowledge of all putative variants for every patient permitted a broad overview of the pathogenicity and the combination of alleles that affected each patient. Moreover, this technology has changed the paradigm of routine molecular studies: in the face of the multiple genetic changes found in every patient, the critical challenge became discriminating disease-associated variants from the broader background of variants present in all patients' genomes.

For this, variants must be classified as pathogenic, likely pathogenic, uncertain significance, likely benign or benign, based on the available evidence and according to the practice guidelines for the evaluation of pathogenicity recently published by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [19].

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Citation: Fidalgo T and Ribeiro ML. Added Value of Next-Generation Sequencing for Haemostasis Diagnosis. Thromb Haemost Res. 2017; 1(2): 1007.

This analysis has led to a well-established bioinformatics pipeline according to NGS guidelines [16,19]; and evidences that a clinical-laboratory approach for each patient's genotypic data must be evaluated considering their specific and differential clinical manifestations. For this purpose, its interplay with a detailed clinical data registry and familial studies is crucial. These findings show that NGS is a valuable asset in clinical practice given that a correct diagnosis in haemostasis disorders is essential for determining the most effective treatment for patients with this complex disease.

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