Next Generation DNA sequencing for haemostatic and platelet disorders

Nick Beauchamp, Laura Crookes, Nikolas Niksic & Anne Goodeve
Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, Western Bank, Sheffield, UK.
Email: SDGS@sch.nhs.uk, Web: https://www.sheffieldchildrens.nhs.uk/our-services/sheffield-diagnostic-genetics-service/

Introduction and Objectives

The introduction of next generation DNA sequencing (NGS) is revolutionizing genetic analysis. Many genetics laboratories are moving sequence analysis from Sanger to NGS, with benefits of faster turnaround time,[1-6] and more cost-effective analysis especially where the cause of the patient's symptoms is unclear. For hemostatic and platelet disorders, a gene panel can be designed with several frequently analyzed genes, enabling simultaneous analysis where more than one gene may be responsible for a disorder.

If the possible diagnosis changes, further genes can be analysed by re-running the bioinformatics pipeline.

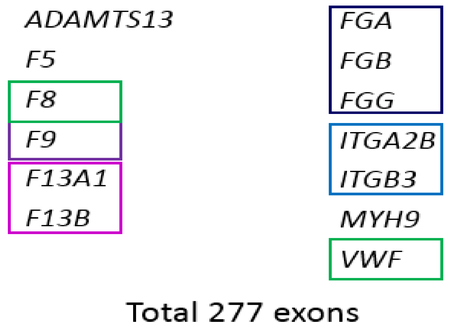

Fig 1. Gene panel. Coloured boxes indicate genes analysed together.

Materials and Methods

A 13 gene panel was designed to include larger genes associated with bleeding/thrombotic disorders and to incorporate disorders where more than one gene may result in the same phenotype.

The panel comprised *ADAMTS13, F5, F8, F9, F13A1, F13B, FGA, FGB, FGG, ITGA2B, ITGB3, MYH9* and *VWF* genes (Fig 1).

NGS analysis was undertaken using Illumina MiSeq or HiSeq. An in-house Genome Analysis Toolkit pipeline was used for data analysis. This removed common single nucleotide variants, leaving those that were previously unseen or likely pathogenic for further assessment.[7]

Results

The panel was introduced in January 2015 following validation which confirmed that all variants previously detected using Sanger sequencing were also seen using NGS.

To date, 71 patients have been analyzed and mutations identified in 50 individuals (70%). Patients have been analyzed for a single gene, or to help to discriminate between disorders for up to 5 genes.

For fibrinogen disorders, FXIII and Glanzmann thrombasthenia, mutations were identified in all seven patients. *F8* mutations were present in 29/36 individuals (81%). 6/10 females without an affected index case available had mutations detected; one was homozygous.

8/14 von Willebrand disease (VWD) patients had mutations identified and 6/10 with reduced FVIII:C levels had mutations identified (5 *F8*, 1 *VWF*).

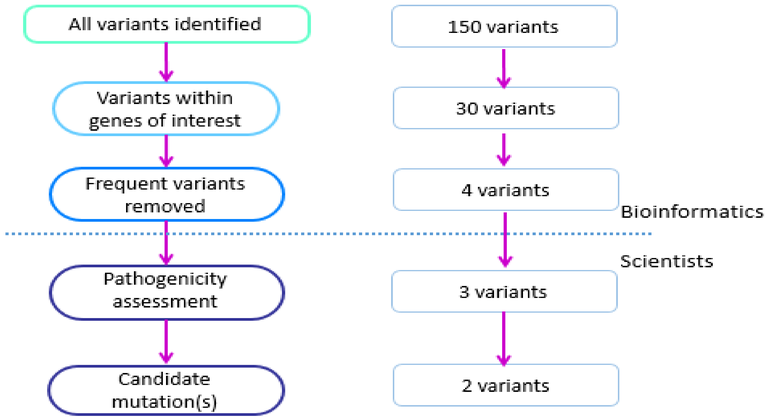

Fig 2. Bioinformatics data pipeline

Gene (with HGVS)	Chromosome	Start bp	End bp	Reference base(s)	Variant base(s)	Zygosity
F8:NM_000132: exon18: c.5954G>A: p.R1985Q	chrX	154132225	154132225	C	T	"hom"
FGA:NM_000508: exon5: c.991A>G: p.T331A	Chr4	155507590	155507590	T	C	het
FGG:NM_000509: exon8: c.901C>T: p.R301C	Chr4	155528085	155528085	G	A	het

Table 1. Sequence variants in a hemophilia A patient (black) and in a dysfibrinogenaemia patient (purple)

Gene(s) analysed	Disorder	No. mutation/ analysed
ADAMTS13	ADAMTS13 deficiency	0/0
F5	FV deficiency	0/0
F8	Hemophilia A	29/36
F8 & F9	Hemophilia A & B	1/1
F8 & VWF	Hemophilia A & 2N VWD	6/10
F8, F9, F13A1, F13B & VWF	Bleeding disorders	0/1
F13A1 & F13B	FXIII deficiency	1/1
FGA, FGB & FGG	Fibrinogen disorders	4/4
ITGA2B & ITGB3	Glanzmann thrombasthenia	2/2
MYH9	MYH9 related disorders	0/4
VWF	von Willebrand disease	8/14
TOTAL	**ALL PATIENTS**	**50/71 (70%)**

Table 2. Pathogenic sequence variants identified in 71 individuals referred for genetic testing.

Conclusions

Gene panels commonly have from 2-100 genes and can help identify/exclude the cause of a disorder considerably more rapidly than sequential Sanger sequencing.

This NGS panel enables simultaneous analysis of genes for hemophilia A and B (family history of the disorder but no living patient), to distinguish hemophilia A and type 2N von VWD where FVIII:C level is reduced and for disorders including those for fibrinogen, Glanzmann thrombasthenia and FXIII deficiency, where more than one gene can cause symptoms. For rare patients with an unknown cause of bleeding, several genes can be simultaneously analyzed.

References

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Anne Goodeve
29-MP-M
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