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Von Willebrand Disease: The Viability of a Technology-Based Diagnostic Modality

Theodore Gutches
Longwood University

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Ted Gutches
Longwood University Department of Biological and Environmental Sciences

Introduction

- Von Willebrand disease (VWD) is an autosomal inherited bleeding disorder.
- The VWF is a multimeric plasma glycoprotein that binds to platelets in order to deliver factor VIII, another essential blood clotting protein, to sites in which vascular injury has occurred.[2,4]
- Three types include: Type 1 (partial deficiency), Type 2 (qualitative deficiency), and Type 3 (complete deficiency).
- Type 1, the mild case, makes up 65% to 80% of cases; type 2, the moderate and qualitatively abnormal case of VWD, makes up 20% to 35% of cases; with type 3, the severe case, affecting 1 in 1 million people.[5]
- Variations in Type 2 include: defects in multimerization (type 2A), spontaneous platelet binding (type 2B), defects in ligand binding with multimers (type 2M) and defects in factor VIII binding (type 2N).
- Next-Generation Sequencing has shown promise in its diagnostic application but has not yet been implemented for small-scale patient diagnostics.[1]

VWF Structure & Function

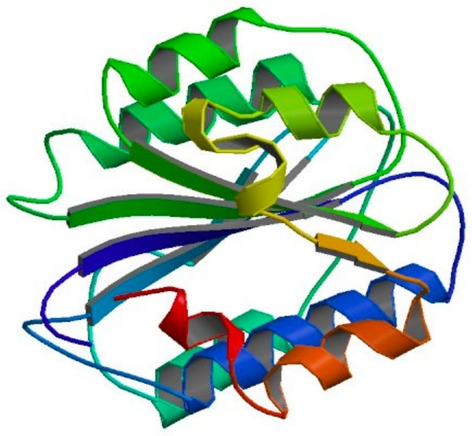

Figure 2: von Willebrand Factor is a multimeric oligomer that exists in multiple domains. Domain A2 is shown and is said to be unprotected from unfolding via disulfide bonds.[3]

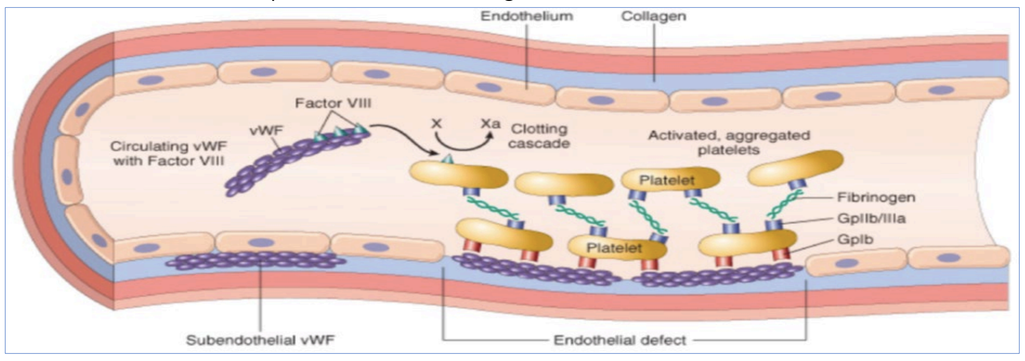

Figure 1: The visualization of von Willebrand factor in the formation of a clot for the coagulation cascade.[4]

Specific Aims

- By first identifying the challenges clinicians face when determining the proper diagnosis of a VWD patient, this study aims to seek an improvement in diagnostic techniques, by implementing the clinical use of genetic testing.
- More specifically, this study aims to improve the total diagnostic time for the intermediate forms of VWD, through the clinical application of Next-Generation Sequencing.

Methodology

Patient Population and Blood Sampling

- Willing patients who have previously been diagnosed with VWD
- ~4mL of blood will be used from each patient

Amplification and Sequencing

- Oligonucleotides will be used for amplification of exons
- Multiplex PCRs will be used for amplification of the VWF
- Each amplicon will be processed and analyzed
- A MiSeq platform will be used for sequence analysis[1]

Sequence Analysis

- NGS will be used for identification of specific variants, further confirmed via Sanger sequencing
- Categorization of the disease according to type will be recorded

Potential Conclusions

- NGS could provide viable results that could advocate for a transition towards a clinically applied system of genetic screening for patients with intermediate forms of VWD
- Identifying new variants among the three types of VWD, through the continued use of gene sequencing for the VWF, could broaden the diagnostic spectrum of VWD, to which clinicians refer.

Potential Pitfalls

- Providing necessary tools in order to reduce costs of small-scale genetic testing for patients in a clinical setting could present limitations in the effort for implementation for this diagnostic modality
- Further specification for warranting such a diagnostic tool would require additional evaluations

References

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