Genetic Influences on the Pharmacokinetic of Factor VIII: Year 1 Progress Report

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Overall Aim: The overall objective of this research proposal is to characterize genetic influences on the pharmacokinetic behavior of intravenously infused factor VIII (FVIII).

Summary of Background and Rationale: The impetus for this research project has been, at least in part, the intense recent interest in prolonging factor VIII half-life through a number of protein engineering strategies. The mean half life for FVIII is 12 hrs, but there is substantial inter-individual variability, with an approximately 5-fold range of circulation survival times. In contrast, the intra-individual handling of FVIII is very stable over time, thus suggesting that each individual has an intrinsic (genetic) capability to clear this protein.

The hypothesis being tested in this project is based on the theory that the FVIII half-life is predominantly influenced by its carrier protein, von Willebrand factor (VWF). Thus, factors that affect VWF clearance should also have a significant impact upon FVIII half-life. There is evidence in the literature to support these proposals.

Summary of Year 1 Progress: This research project received funding in March 2011, and thus we have been now been working on the project for 6 months.

Ethics Approval Process and Clinical Center Participation: The project has been reviewed and approved by the Institutional Review Board at Queen's University and is currently undergoing similar IRB review at the 5 clinical centers that are participating in the study at this time. These are as follows –

1. Hospital for Sick Children, Toronto (Drs Carcao and Blanchette)
2. Hopital St Justine, Montreal (Dr. Georges Rivard)
3. Medical University of Vienna, Austria (Dr. Chistoph Male)
4. University Hospital Leuven, Belgium (Dr. Veerle Labarque)
5. Children's National Medical Center, Washington DC (Dr. Yaser Diab)

We anticipate that the clinical center IRB approvals will be finalized by the end of this calendar year.

Pharmacokinetic Time Points: Formal pharmacokinetic analysis for FVIII is time consuming and practically very challenging, especially in young children. Thus, the standard 9 point analysis as proposed in our original submission would have deterred the enrollment of some patients.

However, subsequent to the approval of this award, in discussions with Sven Bjorkman, we have now been able to revise this schedule to a 5 point sampling schedule as detailed below.

1. Pre-infusion - within 30 mins of infusion.
2. 1 hr post-infusion (+/- 5 mins)
3. 9 hrs post-infusion (+/- 1 hr)
4. 24 hrs post-infusion (+/- 2 hrs)
5. 40 - 48 hrs post-infusion.

This revised blood sampling schedule has now been included in an amended version of our IRB submission. The reduction of post-infusion sampling time points from 9 to 4 has been made possible through the adoption of a population pharmacokinetic model based on studies during the clinical introduction of Advate (manuscript submitted to Blood).

Given the timing of IRB approvals and the organization of patient recruitment, we envisage that the majority of samples to be analyzed in this study will be collected during the 12-18 months between January 2012

and June 2013. In the meantime, we have been collecting important control genetic data that will be required to interpret our study results.

Genetic Analysis of a Control Population: As indicated above, the aim of this study is to correlate variances in FVIII pharmacokinetics with genetic variability at loci involved in determining VWF-FVIII interactions and VWF clearance.

We have recently completed an initial analysis of a control population to evaluate the extent of polymorphism in the FVIII binding region of VWF. The hypothesis being evaluated here is that polymorphic variation within the FVIII binding domain of VWF may influence the carrying capacity of VWF and thus secondarily affect the FVIII half-life.

In these studies, we have analyzed the sequence of the 8 *VWF* gene exons (exons 17-24) that encode the D'/D3 region of VWF known to bind to FVIII. These studies have been performed on 155 individuals recruited at the time of our initial type 1 VWD genetic studies. The study subjects represent a mix of normal individuals, type 1 VWD patients and both affected and unaffected family members of the index cases. We propose that even the data from type 1 VWD index cases is valuable and legitimate for this analysis, and that the only cases that must be excluded are those with known type 2N VWD mutations.

To efficiently evaluate the extent of polymorphic variation in these VWF exons we have implemented a new sequence analysis software strategy. This approach has proven to be very effective and will be extremely helpful as we add increasing amounts of new sequence from our PK study population over the next 2 years.

Location of Polymorphism and Nucleotide #	Variation	Previous Report (SNP reference #)	Frequency	Consequence
Intron 17	T/A	No	3%	Within the intron 17 splicing branch site
Exon 18 2365	A/G	Yes (rs1063856)	10%	Thr789Ala
Exon 18 2385	T/C	Yes (rs1063857)	33%	Silent 795Tyr
Exon 20 2555	G/A	Yes (rs216321)	32%	Arg852Gln
Exon 21 2739	A/C	Yes (rs35191786)	14%	Silent 913Gly
Exon 21 2771	G/A	Yes (rs33978901)	3%	Arg924Gln
Exon 22 2880	G/A	Yes (rs1800380)	41%	Silent 960Arg
Intron 23 3108+85	G/T	Yes	4%	Not at invariant splice site
Intron 23 3109-90	G/C	Yes	15%	Not at invariant splice site
Intron 24 3222+31	C/T	Yes	6%	Not at invariant splice site

This analysis on a control population provides us with the first systematic view of the extent of polymorphism in the FVIII-binding exons of VWF. While there are 4 intronic variants that might differentially influence the splicing efficiency of the two polymorphic alleles, of note, there are 3 missense polymorphisms (Thr789Ala; Arg852Gly; Arg924Gln) that are found in 3-32% of the alleles examined. None of these variants is associated with a type 2N phenotype but more subtle effects on FVIII binding cannot be ruled out.

Most importantly, during this analysis we have found that 45% of the examined subjects are heterozygous for more than 1 of these polymorphic alleles.

Number of SNPs in FVIII Binding Exons	% of Study Subjects
0	7
1	48
2	20
3	13
4	6
5	4
6	0
7	2

Thus, one potential hypothesis that we can test in our PK study group is that an increasing burden of polymorphic variants in the FVIII binding region may influence the FVIII binding capability of VWF. This may, for example, involve an interplay between the coding and non-coding SNPs in providing adequate FVIII binding domains to support normal FVIII survival in plasma.

Year 2 Studies: The plans for Year 2 of this project are as follows

a) Complete IRB approvals in all clinical sites.
b) Initiate patient recruitment at all 5 sites.
c) Complete at least 40 PK studies.
d) Transfer plasma for FVIII and VWF studies to the central lab in Kingston.
e) Complete FVIII and VWF studies on all PK study subjects.
f) Extract DNA for analysis of FVIII binding region variation.
g) Generate information on variant glycan sequences in a control population.