A Genome Wide Association Study (GWAS) to Determine Factors that Contribute to Mucocutaneous Bleeding

Study Objectives and Design

This study, which has been designed to identify genetic loci associated with excessive mucocutaneous bleeding, has three specific objectives. These are:

1. To validate the Condensed MCMDM1-VWD Bleeding Questionnaire for use as a screening tool in tertiary care hemostasis clinics

2. To identify genetic loci that are associated with excessive mucocutaneous bleeding by performing a GWAS on individuals with positive bleeding scores, as defined by the Condensed MCMDM1-VWD Bleeding Questionnaire

3. To ascertain the prevalence of collagen disorders in a population referred for excessive mucocutaneous bleeding.

Subject Recruitment and Consent

All centers are active and recruiting subjects. To date, **196 subjects** have been recruited. All of the recruited subjects have consented to participate in all three parts of the project (bleeding score, GWAS, hypermobility) and >95% have given consent for use of their DNA in the future for other projects. We are extremely pleased about our subjects' willingness to consent to the use of their DNA for future studies and are aware that we are in the process of collecting an extremely valuable "biobank" of well characterized DNA samples.

Results and Discussion

All results and analysis have been categorized based on reason for referral. These include: 1) personal history of excessive mucocutaneous bleeding, 2) a positive family history of a bleeding disorder and 3) other (ie: prolonged aPTT). Numbers of subjects recruited in each category and subject characteristics can be found in Table 1, as can the results of some of the laboratory investigations and bleeding scores. As has been previously seen in studies focused on excessive mucocutaneous bleeding, there is a predominance of female subjects, presumably due to the frequency of menorrhagia as a chief complaint amongst this group. As expected, there is a trend towards higher bleeding scores in those referred for a personal history of excessive mucocutaneous bleeding.

Overall a diagnosis of a bleeding disorder was made in 88/196 subjects. Of the remaining 108 individuals without a diagnosis, 78 of them had positive bleeding scores making the frequency of "bleeders NYD" 78/196 (40%) which is also consistent with previously published data. As can be seen in Table 2, the relative

proportion of individuals with a diagnosed bleeding disorder differs based on the reason for referral, with those referred for a positive family history being significantly more likely to have a diagnosis made, particularly when that diagnosis was hemophilia or being a symptomatic hemophilia carrier. Conversely, those referred for a personal history were more likely to end up in the "bleeders NYD" group.

Problems and Solutions

One issue currently facing the investigators is the speed with which the field of human genomics is evolving. It is possible that a GWAS may no longer be the best approach by the end of the recruiting period. Other strategies for identifying novel genetic loci associated with excessive mucocutaneous bleeding could include an exome sequencing approach and/or whole gene sequencing of candidate genes such as those identified in the CHARGE Study (Cohorts for Heart and Aging Research in Genome Epidemiology). Concurrent with this study, Dr. James and Dr. Lillicrap's research groups have started investigating the role of the CHARGE loci in cases with Type 1 VWD. The preliminary results are encouraging and will be applicable to the analysis of the population being recruited for this study.

Publications and Presentations

An abstract describing preliminary results for Study Objectives #1 and #3 was accepted and presented during the XXIII Congress of the International Society on Thrombosis and Haemostasis in Kyoto, Japan in July 2011 (Jackson S, Poon M-C, Grabell J, Lillicrap D, James PD. The Condensed MCMDM1-VWD Bleeding Questionnaire: Utility as a Diagnostic Tool in the Hematology Clinic. J Thromb Haemost July, 2011, Volume 9 Issue Supplement s2, 251-498, P-TU-459.) The published abstract, and a copy of the poster presented by Dr. Shannon Jackson (acknowledging the support of B-CHERP) is attached to this report. We anticipate that 2 full manuscripts will arise as a result of the study; one addressing Objectives #1 and #3 and the second addressing #2.

Concluding Comments

We are pleased with the progress of the study to date and are confident that with an additional, third year of funding we will accomplish the study objectives. The group of individuals designated as "bleeders NYD" (n=78) represents a potentially highly informative group for future analysis. This group is defined by the absence of a diagnosis, and therefore, the absence of any potential contribution of known genetic loci (as would be seen for example, in cases with von Willebrand disease caused by a pathogenic mutation in the *VWF* gene) and the opportunity to identify and define new relevant genetic loci from this group of subjects is very exciting.

Table 1: Subjects characteristics and laboratory results

	Personal Bleeding/Bruising (n=159)	Family History (n=30)	Other (n=7)	*P* value
Females (%)	**125 (79)**	**27 (90)**	**5 (71)**	**0.403**
Mean Age (range)	**42 (18-90)**	**36 (18-67)**	**36 (18-57)**	**0.074**
Blood Group O (%)	**53/92 (58)**	**13/20 (65)**	**4/6 (66)**	**0.833**
Platelets (x10^9/L), mean (range)	**248 (89-476)**	**237 (146-363)**	**299 (157-776)**	**0.176**
Ferritin (ug/L), mean (range)	**54 (2-442)**	**54 (4-307)**	**47 (6-171)**	**0.963**
PT (sec.), mean (range)	**12.7 (8.7-22.9)**	**14.2 (10.9-30.6)**	**13.7 (13.1-15.4)**	**0.153**
PTT (sec.), mean (range)	**33 (23-76)**	**35 (29-45)**	**36 (31-44)**	**0.341**
VWF:Ag (U/ml), mean (range)	**0.85 (0.02-2.46)**	**0.62 (0.17-1.28)**	**0.65 (0.41-0.92)**	**0.081**
VWF:RCo (U/ml), mean (range)	**0.80 (0.00-3.81)**	**0.49 (0.03-0.99)**	**0.45 (0.25-0.81)**	**0.013**
FVIII:C (U/ml), mean (range)	**0.96 (0.00-3.81)**	**0.71 (0.20-1.35)**	**0.74 (0.17-1.47)**	**0.079**
Median Bleeding Score (range)	**8 (-2-29)**	**6 (-1-22)**	**5 (-2-11)**	**0.086**
Mean Beighton Score (range)	**2 (0-9)**	**2 (0-9)**	**2 (0-3)**	**0.914**
Meets Brighton Criteria (%)	**7 (4)**	**3 (10)**	**0 (0)**	**0.390**

VWF:RCo: personal vs. family history *P*=0.034, personal history vs. other *P*=0.174, family history vs. other *P*=0.976.

Subject characteristics were compared using chi-square tests for gender, blood group and Brighton. The remaining variables were assessed by ANOVA. A Post-Hoc Tukey was used to identify between the group differences.

Table 2: Diagnosed bleeding disorders

	Personal Bleeding/Bruising (n=159)	Family History (n=30)	Other (n=7)	*P* value
Diagnosis	**62**	**23**	**3**	**0.001**
VWD	**30**	**9**	**2**	**0.352**
Hemophilia/ Carrier	**11**	**8**	**1**	**0.005**
PFD/Dense Granule Deficiency	**12**	**1**	**0**	**0.534**
Possible Collagen Disorder	**7**	**3**	**0**	**0.279**
Other	**2**	**2**	**0**	**0.149**
No Diagnosis	**97**	**7**	**4**	**0.001**
Bleeder NYD	**70**	**7**	**1**	**0.024**

PFD = platelet function disorder, NYD = not yet diagnosed, Other = Factor XI deficiency, ITP, Factor VII deficiency
Hemophilia/Carriers: personal history vs. family history *p*=0.004, personal history vs. other *p*=0.417, family history vs. other *p*=0.656.
No Diagnosis: personal history vs. family history *p*<0.001, personal history vs. other *p*=1.000, family history vs. other *p*=0.163.
Bleeder NYD: personal history vs. family history *p*=0.186, personal history vs. other *p*=0.77, **family history vs. other *p*=0.024**

Groups were compared using Pearson Chi Square. Group differences were determined by using a stepwise Fishers Exact Test.