NAME OF PROJECT: International Society on Thrombosis and Haemostasis (ISTH)
Database and DNA Biorepository for Congenital Antithrombin Deficiency – a pilot/feasibility study

- Subcommittee: Pediatric/Neonatal
- Person responsible (Chair / Principal Investigator): Riten Kumar, MD, MSc
- Design: Prospective pilot/feasibility study
- Aim/Objective/Rationale (Needs assessment / Reason)

Specific Aims: The primary objective of this study is to determine the feasibility of establishing an ISTH database and DNA biorepository of congenital AT deficiency at NCH. Our primary (feasibility) and secondary aims are elaborated as follows:

Primary (Feasibility) Aim: We will find that a large scale repository is feasible if the following aims are met:
1. Aim 1a: Enroll 20 pediatric subjects with congenital AT deficiency from 5 participating centers over a period of 1 year.
2. Aim 1b: Complete clinical data collection through REDCap for at least 17/20 enrolled subjects.
3. Aim 1c: Successfully isolate and bank the DNA in the NCH biorepository for at least 17/20 enrolled subjects.

Secondary Aim:
1. Aim 2: Ensure content validity of the REDCap database established for the purpose of this study through an iterative process.

- Methodology (Data expected to collect, sample size and statistical analysis):

Sample Size/ Participating Institutions:
Current published guidelines recommend using a sample size of 10-30 subjects for pilot/feasibility studies. For this pilot study, we have used a convenience sample of 20 subjects. Five participating centers, which have agreed to open this pilot study currently follow 45-48 patients with congenital AT deficiency, and we therefore do not anticipate any issues with enrolling 20 subjects (<50% of patients followed at the participating centers) for the pilot phase of this investigation (table).

<table>
<thead>
<tr>
<th>Name of Center</th>
<th>Number of subjects with congenital AT deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nationwide Children's Hospital</td>
<td>3</td>
</tr>
<tr>
<td>2. The Hospital for Sick Children</td>
<td>25</td>
</tr>
<tr>
<td>3. McMaster Children’s Hospital</td>
<td>0-3</td>
</tr>
<tr>
<td>4. Bleeding and Clotting Disorders Institute</td>
<td>12</td>
</tr>
<tr>
<td>5. IWK Health Centre</td>
<td>5</td>
</tr>
</tbody>
</table>
• Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

Eligibility Criteria:

(1) Pediatric subjects (ages: 0-21 years) with genetically confirmed AT deficiency will be eligible for the pilot study.

(2) Pediatric subjects with venous/arterial thrombo-embolism and suspected AT deficiency will only be considered eligible if they have a minimum of 2 low AT activities measured 3 months apart. First AT activity needs to have been measured at least 3-months after discontinuation of anticoagulation. Age appropriate AT activity levels has been previously identified\textsuperscript{24}.

(3) AT activities may be measured locally using any commercially available assays based on factor-Xa or thrombin inhibition methods.

(4) Subjects found to have acquired risk factors for AT deficiency - liver disease, nephrotic syndrome, asparaginase therapy, heparin therapy, hormonal therapy, pregnancy, congenital heart disease, and acute thrombosis will only be considered eligible if they continue to have a low AT activity (x2), 3 months after resolution of the acquired risk factor.

(5) Subjects with no personal history of thrombosis, but a documented family history of AT deficiency, will be considered eligible, as long as they have a single, low AT activity documented.

(6) Multiple subjects from the same family pedigree tree will be eligible for enrollment into the database/registry.

(7) Written consent/assent for participation in the study and for banking DNA will need to be obtained from legal guardians/participants using local, ethics board approved consent forms.
SSC Subcommittee Project/Collaborative Project

- Expected timeline:

  January 2016 – June 2016
  - Design and content validation of REDCap
  - Research Ethics Board (REB) approval at NCH

  July 2016 – June 2017
  - REB approval at participating centers
  - Subject enrollment

  July 2017 – December 2017
  - Data Analysis
  - Abstract and manuscript preparation

- Expected outcomes (ie. publications):
  - Publication type (SSC Communication, Guidance document or original article):
    - Original articles

- Description of project set/up and management, needed infrastructure and resources (summary):

Methods/ Material:

This study has been designed per published recommendations for conducting pilot/feasibility studies to evaluate recruitment potential, feasibility of international collaborations and coordination of internal processes. We plan to enroll 20 subjects from 5 participating centers over a period of 2-years (July 2016 – June 2018). A data collection instrument previously used in our cohort study will be converted into REDCap to allow us to open the study at multiple centers simultaneously. Collaborating investigators from the 5 participating centers would be able to enter clinical data using REDCap. Blood/DNA specimens will be shipped to NCH for banking at the biorepository. Lastly, the referring physician will have the option of pursuing Sanger sequencing of the SERPINC1 gene at the CLIA certified molecular genetics lab at NCH (cost for this will not be covered by the study). Molecular data with an interpretation will be provided to the referring institution if sequencing is pursued.
Clinical Data Collection and Management

REDCap is a secure web application for building and managing online surveys and databases. The REDCap application allows users to build and manage online databases securely, and is currently in production use or development build-status for more than 200,000 projects across North America. At NCH, we have a dedicated REDCap team that will help build the database and launch it both at NCH and other participating centers. The database will be based on a data collection instrument we previously developed and will undergo content validation by a consensus panel of survey methodologists, hematologists and epidemiologists prior to launch. This will ensure that the database is comprehensive and appropriate and the questions are well defined, clearly understood and presented in a consistent manner. We have already started work on development and validation of the database using divisional funds. Once validated, retrospective clinical data will be collected by a trained research staff at each trial site using REDCap. The REDCap database will be stored in encrypted files on a password protected computer. Completed data can be directly downloaded by the principal investigator into a central trial database maintained at NCH. Data will be updated annually through eCRF, also available through REDCap – thereby providing much needed data on the natural history.

DNA Banking at the NCH Bio-Pathology Center

Participating centers will have the option to bank DNA (from enrolled subjects) at the biopathology center (BPC) at NCH. They can send whole blood (3-10 mL of peripheral blood in an EDTA tube) or isolated DNA to NCH (shipping costs will be covered by study). In case whole blood is sent, manual leukocyte genomic DNA extraction from EDTA-anticoagulated blood will be performed at NCH using the ArchivePure DNA purification kit (5 PRIME GmbH, Hamburg, Germany). The primary objective of the BPC is the long term acquisition/storage of bio-specimens to support research. This center is accredited by the College of American Pathologists Biorepository Accreditation Program (CAP BAP) and is affiliated with multiple international consortiums including the Children’s Oncology Group (COG), Childhood Cancer Survival Study (CCSS), Gynecologic Oncology Group (GOG) and the Cancer Genome Atlas. More than 2 million bio-specimens are currently in storage at the NCH BPC with close to 1,000 specimens being received every day from >500 national and international institutions.

Genetic Analysis for SERPINC1 Mutations (optional)

Collaborating investigators from participating centers will have the option to pursue SERPINC1 Sanger sequencing at the CLIA certified molecular genetics lab at NCH (please see appendix 1 for validation data). The SERPINC1 gene will analyzed by PCR followed by sequencing of both DNA strands of the entire coding region, highly conserved intron–exon splice junctions and 5’ untranslated regions. Again, cost for this will not be covered by the study.

Analysis of novel mutations (if identified while performing SERPINC1 sequencing)

Antithrombin has active and inactive conformations. We will use the 2.75Å crystal structure, PDB 1T1F, chain A, as representative of the active form and the 2.62Å crystal structure, PDB 1E05, chain L, for the inactive form. The hydrogen bonds and solvent accessible surface area
for AT will be estimated using utilities in Pymol with the default parameters (PyMOL Molecular Graphics System, Version 0.99rc6 Schrödinger, LLC). The electrostatic surface area was calculated with the APBS (Adaptive Poisson-Boltzmann Solver)\textsuperscript{25}. Rosetta, a program developed by the Baker protein laboratory\textsuperscript{26}, predicts lowest energy protein structures. The $\Delta\Delta G$ application of Rosetta semi-quantitatively estimates the change in protein free energy due to a single mutation. The resultant energy term is of arbitrary units (reu), but a positive value indicates that the protein has been destabilized by the mutation. Of note, we have successfully used this technique in multiple previous publications\textsuperscript{14,20-22}.

- Possible references: