

HEMOPHILIA INHIBITOR GENETICS STUDY (HIGS) Protocol Summary

Principal Investigator: Erik Berntorp, M.D., Ph.D.,
Department for Hematology and Coagulation Disorders,
Malmö University Hospital, SE-205 02 Malmö, Sweden

Co-Principal Investigator: Jan Astermark, M.D., Ph.D.
Department for Hematology and Coagulation Disorders,
Malmö University Hospital, SE-205 02 Malmö, Sweden

Introduction. The development of inhibitory antibody (inhibitor) to therapeutic replacement clotting factor is one of the most serious complications of hemophilia. Several non-genetic and genetic factors that could influence the risk of inhibitor development in hemophilia have been discussed through the years, but the nature of these factors remains to be fully explored. Recent developments in human genetics plus advances in high throughput microsatellite and SNP genotyping methods have provided tools for the potential detection of host genetic factors of importance for inhibitor response to antigenic challenge with factor VIII or IX during hemophilia treatment.

Objective. To determine genetic factors, other than mutations within the factor VIII gene, that are associated with the development of inhibitors in severe hemophilia A and response to antigen challenge by factor VIII. Secondary objectives include the identification of environmental factors that might increase the risk of inhibitor development, and a characterization of the inhibitory antibodies in terms of epitopes and inhibitor reactivity to various clotting factor concentrates. The amount of circulating factor VIII antigen and thrombin formed in vitro in the presence of by-passing agents will be measured.

Subject Population. This is a multi-center study. Phase I of the investigation involves enrollment of family groups in which two or more full brothers have severe factor VIII deficiency (baseline factor VIII <1%) and one or more have a history of an inhibitor ≥ 1 Bethesda unit (BU). The brothers, both parents, and one or more non-hemophiliac siblings (if available) will be enrolled. Phase II involves enrollment of a family group termed a "triad". A triad is composed of a hemophiliac with severe factor VIII deficiency (baseline factor VIII <1%) and a history of an inhibitor ≥ 1 Bethesda unit (BU) and both of his parents. Phase III involves study of unrelated hemophiliacs for the purpose of confirming the associations identified in Phase I and II.

Study Design. A combination of whole genome scans (Phase I), fine mapping of candidate regions (Phase II) and targeted candidate gene analysis (Phase III) will be used to identify genes that contribute to the development of inhibitors.

Study Procedures. Participation in Phases I and II will require one study visit. Existing clinical data and DNA will be used for the completion of Phase III and no study visit will be required. Collaborative arrangements have been established with the Multicenter Hemophilia Cohort Study (MHCS) and the Hemophilia Growth and Development Study to access the DNA and clinical data of participants in these studies to accomplish the aims of Phase III.

Sample Size. Three hundred family groups (brother pairs and parents) will be enrolled in Phase I, 300 triads in Phase II, and 1400 unrelated hemophiliacs in Phase III. This will provide at least 80% power to detect a twofold, or greater, increase in risk for development of an inhibitor at every Phase.

Statistical Analysis. A genome scan using affected (ASP) and discordant (DSP) pair analysis will be performed on participants in the Phase I study. Phase II will utilize the transmission disequilibrium test (TDT) to detect the linkage of markers and disease gene alleles in the presence of association with the disease phenotype. In Phase III, we will genotype significant markers and candidate genes, including HLA, in unrelated hemophiliacs with and without inhibitors, thus providing an independent confirmation of the results obtained in Phase I and II.

Human Subjects. Prior to enrollment of subjects into this study, the protocol and the informed consent form will be reviewed and approved by the appropriate IRB/IEC. Should amendments to the protocol be required, the amendments will be written by the Principal Investigator and provided to the investigator for submission to the IRB/IEC. Subjects, or their legally authorized representatives, will receive a comprehensive explanation of the study including its purpose, risks, and other elements that are part of obtaining proper informed consent. Subjects will be allowed sufficient time to consider participation in the study after it has been explained to them. By signing the informed consent, subjects (or their legally authorized representatives) agree that they will complete all evaluations required by the study, unless they withdraw voluntarily or are withdrawn from the study for any reason.

Study Organization and Leadership. This is an investigator-initiated study funded through a grant mechanism by Baxter BioScience, Westlake Village, CA. Erik Berntorp, M.D., Ph.D., Malmö University Hospital, Malmö, Sweden, is the Principal Investigator and Jan Astermark, M.D., Ph.D., Malmö University Hospital, Malmö, Sweden, is the Co-Principal Investigator. An Executive Committee (attached) serves as the decision-making and oversight body of the study. Central laboratories are located at the Department for Hematology and Coagulation Disorders, Malmö University Hospital, Malmö, Sweden, the Laboratory of Genomic Diversity, National Cancer Institute—Frederick, MD, USA, and the Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Germany. Study coordination and data management is provided by Rho, Inc., Chapel Hill, NC, USA. Statistical support for the study will be provided by Rho and by the Laboratory of Genomic Diversity, NCI—Frederick, MD, USA.

**HEMOPHILIA INHIBITOR GENETICS STUDY (HIGS)
Executive Committee**

Erik Berntorp, M.D., Ph.D. (Chair)

Department for Hematology and Coagulation Disorders
Malmö University Hospital, Malmö, Sweden

Jan Astermark, M.D., Ph.D. (Co-Chair)

Department for Hematology and Coagulation Disorders
Malmö University Hospital, Malmö, Sweden

Donna DiMichele, M.D.

Comprehensive Hemophilia Diagnostic and Treatment Center
New York Weill Cornell Center, New York, NY, USA

Sharyne Donfield, Ph.D.

Rho, Inc., Chapel Hill, North Carolina, USA

Bruce Ewenstein, M.D., Ph.D.

Baxter BioScience, Westlake Village, CA, USA

Edward Gomperts, M.D.

Childrens Hospital Los Angeles, Los Angeles, CA, USA

George Nelson, Ph.D.

Laboratory of Genomic Diversity
Science Applications International Corporation
National Cancer Institute, Frederick, MD, USA

Johannes Oldenburg, M.D., Ph.D.

Institute of Experimental Haematology and Transfusion Medicine
University Clinic Bonn, Bonn, Germany

Amy Shapiro, M.D.

Indiana Hemophilia and Thrombosis Center
Indianapolis, Indiana, USA

Cheryl Winkler, Ph.D.

Laboratory of Genomic Diversity
Science Applications International Corporation
National Cancer Institute, Frederick, MD, USA