Study Design of SSC Subcommittee Project/Collaborative Project

- Name of the Project:
  Investigation into racial differences in genetic risk factor for venous thromboembolism

- Person responsible (Chair / Principal Investigator): Hiroko Tsuda (Japan)
  (Other Principal Investigators): Steve Kitchen (UK)  
  Elisabetta Castoldi (The Netherlands)  
  Tatsuya Hayashi (Japan)

- Aim / Mandate of the project:
  Factor V Leiden and prothrombin G20210A are well-known hereditary thrombophilias among Caucasians, in contrast, protein S (PS) and protein C (PC) deficiencies are much more prevalent among Asians than non-Asians. PS Tokushima (K155E, K196E in HGVS nomenclature), a PS gene mutation with a phenotype of type II deficiency, and two PC gene mutations with phenotypes of type II deficiency, PROC c.565C>T and PROC c.574_576del, are identified as genetic risk factors for VTE among Japanese and Chinese, respectively. In order to elucidate the racial differences in genetic risk factor for VTE, we build up a global network and investigate worldwide distribution of these three mutations representing type II deficiency.

- Methodology:
  1) Build up a network of professional contacts, primarily consisting of subcommittee members.
     - Asian countries -------- Japan, Collaboration with APSTH
     - European countries ---- UK, The Netherlands, Hungary, etc.
     - USA
  2) Collaborative study to determine the racial differences in the frequencies of the PS and PC gene mutations representing type II deficiency among the general populations and the VTE patients.
     - Recruitment of subjects
       - Period---- between 2013 and 2014
       - Through the use of standardized registry sheets
       - Goal for each participating geographic region
         Healthy individuals: N=500-1,000 (Male : Female = 1 : 1)
         VTE patients : N=300
         Exclusion criteria----under anticoagulation and oral contraceptives, in pregnancy regardless of what age and period after onset of VTE
         Racial groups are divided into 7 groups, white, Hispanic, black, East Asian, South Asian, Other Asian, or Other.
         The study protocol is submitted to the institutional review board in each country according to local requirements and signed informed consent is required from all subjects.

     - Blood (9 volumes) is mixed with 0.109M (or 0.129 M) citrate (1 volume) and centrifuged as soon as possible. Plasma is stored at -40 °C or below until analysis. DNA samples are also prepared and stored at -20 °C or below. If necessary, plasma and DNA and/or buffy coat samples are shipped by cash-on-delivery to Japan.

     - Protein S analysis
       - Total PS assay (total PS activity, total PS antigen, PS specific activity)
Because the total PS assay kit has not been commercially available, frozen plasma samples should be shipped to Shinotest, Co., Kanagawa, Japan by air, where the total PS assay is performed. The WHO 2nd International Standard for Protein S (03/228) is used as a calibrator, and data are expressed as μg PS mL⁻¹. The raw data are returned to participants.

- Conventional PS assays (PS activity, free PS antigen, total PS antigen)
  The WHO 2nd International Standard for Protein S (03/228) is used as a calibrator, and data are expressed as I.U. mL⁻¹.

- Protein C analysis
  - PC assays (PC activity using clotting assay, PC antigen)
    The WHO 2nd International Standard for Protein C (02/342) is used as a calibrator, and data are expressed as I.U. mL⁻¹. Although the PROC c.565C>T variant is detected by both chromogenic and clotting assays, the PROC c.574_576del variant is only by clotting assay.

- Gene analysis
  - Genotyping of the PS and PC gene mutations, PS Tokushima, PROC c.565C>T and PROC c.574_576del, is performed.

- Data analysis
  - The raw data of plasma and gene analyses along with the characteristics of subjects (age, sex, racial group and clinical diagnosis etc.) are gathered to the Chair using the registry sheets, and statistical analysis is performed in collaboration with ISTH SSC members.

- Annual reports on progress will be given at the SSC meetings in 2014 and 2015.
  - The racial differences in the prevalence of PS and PC gene mutations representing type II deficiency among the general populations and the VTE patients.
  - Sensitivity and specificity of the total PS assay and the conventional PS assays for diagnosing type II PS deficiency.

- Year of completion (expected): 2015
  The findings will be published in JTH as an official communication of the SSC.

**Project seeking participants now!**

Interested participants are encouraged to contact Dr. Hiroko Tsuda at tsuda@nakamura-u.ac.jp. The detailed protocol and registry sheets will be available.