Botnia Study – Genetic and metabolic characterization of diabetes

Information for researchers interested in using stored samples and data

Introduction

Diabetes mellitus is a lifelong, incapacitating disease affecting multiple organs. Diabetes is heterogeneous and although it is generally divided into Type 1 and type 2 diabetes, other subtypes exist and more will be identified in the future. About 90 % of diabetic patients have type 2 diabetes (T2D), thereby making T2D to the fastest increasing disease in Finland and worldwide. This epidemic has been ascribed to a collision between genes and the environment. Finland has been in the forefront of genetic research on T2D and many of the key discoveries in the field have emanated from the Botnia study.

The Botnia project was instituted in 1990 in order to investigate and explain these connections at three care centers in Osthrobothnia; Närpes, Korsholm and Malax-Korsnäs. In 1994 the project was expanded to also cover Jakobstad and Vasa, along with other areas in Finland and southern Sweden. Until today, approximately 17 000 people from 1500 families have participated in the Botnia studies. The Botnia Family Study has recruited patients with diabetes and their family members. The non-diabetic spouses married into the families make a control population. The initially non-diabetic participants have been invited for follow-up studies called the Botnia Prospective Study. A population-based arm, PPP-Botnia Study (Prevalence, prediction and prevention of Diabetes) including subjects invited from the population registry was conducted in 2004-2008 with a 6-year follow-up investigation conducted in 2010-2015. Both parts of the Botnia Study are ongoing.

Study aims is to characterize early metabolic disturbances in persons with high risk of developing type 2 diabetes, identify genetic factors which increase the risk of type 2 diabetes and investigate how these genetic factors together with other risk factors lead to the development of diabetes, its clinical phenotype, progression and complications. One aim is also to try to prevent the development of diabetes.

Cohorts

The Botnia Family Study (Recruitment and follow-up ongoing)

The Botnia Family Study was started in 1990 in three health care centers in Western Finland: Närpes, Malax-Korsnäs and Korsholm. All patients with known T2D were invited to the study along with their family members. In 1994 the study was extended to Vasa and Jakobstad and patients with other types of diabetes were included from all centers. At the same time, the study was also extended to other parts of Finland (Mikkeli, Lappeenranta, Pori, Rovaniemi, Joensuu, Posio) and Southern Sweden under the name **SIB Study**. The recruitment for the SIB Study differed from that in Western Finland in that only families in which at least two siblings had T2D were recruited and recruitment was based on advertising.

The initially non-diabetic subjects living in Western Finland or Helsinki area have been invited for follow-up examinations approximately every 3-5 years (**The Botnia Prospective Study**).

Subgroups of the Botnia Family study have also been invited for follow-up studies, e.g. families with monogenic diabetes, families with common or rare variants of interest associated with diabetes, subjects included in GWAS or sequencing studies, GAD-antibody positive patients with diabetes, members from families with both T1D and T2D. Follow-up visits have been done and follow-up information has been gathered through mailed questionnaires combined with laboratory testing in local laboratories with samples sent to Medix Laboratory and to our laboratory (mail investigation).

Participants fill a questionnaire regarding life style, socio-economic factors, family history, disease history and medication, nutrition, and exercise habits. The subjects participate in a 75g oral glucose tolerance test (OGTT) after a 12-hr overnight fast, unless they had known The Botnia Study 3 (5) diabetes and were on glucose-lowering medication, or had fasting plasma glucose >10 mmol/l. Samples for glucose, insulin, free fatty acids and other hormones were drawn at -5, 0, 30, 60 and 120 min. Fasting samples are taken for a wide range of other hormones and metabolites. Diagnosis of diabetes was based on the OGTT or a history of previously known diabetes applying WHO criteria. In uncertain cases, the diagnosis was confirmed from patient records. A sub-group has participated in a combined intravenous glucose tolerance test and euglycaemic hyperinsulinemic clamp. Muscle, fat or skin biopsies are taken from a subgroup.

Since 2002 a sub-study has recruited patients with Type 2 diabetes having relatives with both Type 1 and Type 2 (FHMIX) or only Type 2 diabetes (FHT2) diabetes to evaluate the impact of mixed family history on clinical characteristics and complications (**Botnia MIX Study**). In addition to participants of the Botnia Family Study, new patients were recruited through newspaper advertisements and diabetes nurses around Finland and interviewed by the study physician. Inclusion criteria were: age at diagnosis > 20 years, Type 2 diabetes managed without insulin treatment during the first year after diagnosis and a 1st - 3rd degree relative with Type 1 diabetes and/or Type 2 diabetes. The relatives with Type 1 diabetes were invited to participate. Altogether, 196 unrelated patients with T2D with a relative with T1D were included. As a control group, we included 139 unrelated patients with T2D with family history of type 2 diabetes (FHT2) matched for age, gender, BMI and duration of diabetes with the patients with FHMIX. Subjects without meal-time insulin participated in a 75 g oral glucose tolerance test after an overnight fast. At the second visit within 3 months, patients participated in an i.v. glucagon test followed by an insulin tolerance test for estimation of insulin secretion and sensitivity.

The Prevalence, Prediction and Prevention of Diabetes Botnia (PPPBotnia) study (follow-up ongoing, recruitment of family members ongoing)

The Prevalence, Prediction and Prevention of Diabetes Botnia (PPP-Botnia) study is a population-based study in the Botnia region of Western Finland (1). The study was designed to obtain accurate estimates of prevalence and risk factors for diabetes, IGT and metabolic syndrome in the population and to use this information for prediction and prevention of the disease. The **baseline study** was conducted in 2004-2008 in Närpes-Kristinestad, Jakobstad, Malax-Korsnäs, and Vasa-Korsholm comprising a total population of 135,000 persons. Using the population registry, a random sample of individuals aged 18 to 75 years old (96,000) was selected. In total, 5,208 women and men participated (54.7% of those invited). In the age groups of 18-29 and 60-74 years this represented 10% of the eligible population, and in the age group of 30-59 years it represented 7% of the eligible population.

A **follow-up study** was conducted in 2010-2015, approximately 6 years after the baseline study (median 6.7 yrs). 220 (4.4%) subjects had died since the baseline study, and 3850 (77.2%) of those still living participated. Of them 360 (9.4%) had diabetes, 277 IFG, 183 IGT, 87 both IFG and IGT, and 2856 (74.2%) had normal glucose tolerance. For 87 (2.3%) subjects, glucose tolerance could not be unambiguously verified (missing 2-hour value or unclear diabetes status). The second follow-up started in 2018 and is

ongoing. New 18-29-year-old study subjects that have been randomly selected from the population register, will be invited in 2020.

Trio Study As the parental origin of many genetic variants affects the outcome, in 2015 have started a recruiting of family members of the participants. The study design is the same as for the follow-up study.

PPP Exercise Study (finished)
Consecutive participants from the PPP-Botnia Study who fulfilled the following criteria were invited to the Exercise study: 1) non-diabetic, 30-70 year-old, male or female; 2) poor physical fitness based on a 2 km walking test (fitness index <90 in 2 walking tests); 3) no contraindications for physical training based on a physical examination including ECG. 290 subjects were randomized into two groups, a training group and a prescription-of-exercise group. The basal visit took place between 26.10.2006 - 5.8.2008 and 277 (95.5 %) attended the control visit one year later and 221 (93%) attended the 5-year visit. Muscle biopsies were obtained from 55 participants.

Questionnaires, anthropometric and metabolic measurements, other testing: Body weight, height, waist circumference and blood pressure were measured. The participants filled in 3 questionnaires regarding life style, socio-economic factors, family history, disease history and medication, nutrition, exercise habits, sleep pattern, and depression.

Information on the family history of diabetes (FH+) was based on questionnaire data and defined as known diabetes in at least one first-degree relative. Data on whether participants had a family history of diabetes could be reliably assessed in 89.0% of the male and in 91.1% of the female participants (n=4693).

The subjects participated in a 75 g OGTT after a 12 h overnight fast, unless they had known diabetes and were on glucose-lowering medication, or had fasting plasma glucose >10 mmol/l. Samples for glucose, insulin, free fatty acids and other hormones were drawn at 0, 30 and 120 min. Diagnosis of diabetes was based on the OGTT or a history of previously known diabetes applying WHO criteria. In uncertain cases, the diagnosis was confirmed from patient records. Altogether, 327 participants (6.3%) had diabetes, 354 participants (6.8%) IGT and 416 participants (8.0%) IFG at the baseline visit.

Frequency and intensity of current physical activity and physical activity during the past 12 months was assessed using the validated Kuopio Ischemic Heart Disease Questionnaire. It provides detailed information on common lifestyle, commuting and leisure-time physical activity and enables assessment of total physical activity as metabolic equivalents (MET) h per week (MET×h/week). Based upon leisure-time activity, participants were defined as moderately active, if they performed >30 min physical activity three or more times per week with an intensity resulting in breathlessness and/or sweating.

Physical fitness was assessed by a 2 km walking test, which has been validated up to the age of 65 years and provides an indirect estimate of oxygen uptake. Based upon walking time and heart rate at the end of the test, maximal aerobic capacity (VO2 & max) (ml min−1 kg−1), adjusted for age, sex and BMI was calculated. A prerequisite for participating in the 2 km walking test is the ability to walk briskly achieving a heart rate ≥ 70% of age-related maximal heart rate. Contraindications were activity-related angina or dyspnoea, uncontrolled hypertension, or musculoskeletal symptoms preventing brisk walking. Users of beta-blockers were excluded. Altogether, 1681 participants (40.7%) aged 18 to 65 years performed an adequate walking test.

A frequency-based questionnaire included questions about common dietary components to calculate an index of healthy food habits. Food habits were regarded as healthy if two of the following criteria were fulfilled: (1) daily consumption of vegetables; (2) consumption of low-fat milk; and (3) use of vegetable margarine or no fat on bread.

Ethical considerations

The Botnia Study sample collection has been transferred to THL Biobank in 28 December 2015, following a public notification process in which the biobank transfer was announced in many newspapers on October 2015. The transfer of the Botnia Study to the biobank has been approved by the Coordinating Ethics Committee of Helsinki University Hospital on 15 July 2015. In the future studies an informed consent for THL Biobank will be asked from all new participants.

Botnia Study samples available for biobank research

The following samples are available from more than 15 000 sample donors

- DNA
- RNA (subset)
- Serum
- Plasma
- Urine (subset)
- Fibroblast cell-lines (to be collected in the future, subset)

Botnia Study phenotype and omics data available for biobank research

Below are the details on the **basic data** that is available for Botnia Study participants

- Visit date/s
- Education level
- Occupation classification
- Smoking status
- Classification of glucose tolerance/ diabetes type
- Year of diagnosis of diabetes

Physical examination data

- Weight, height, waist and hip circumference
- Fat %
- Lean body mass
- Blood pressure (systolic, diastolic), duplicate measurements
- Pulse

Biological test results

- Glucose concentrations (fasting or during OGTT)
- Insulin concentrations (fasting or during OGTT)
- C-peptide concentrations (fasting)
- Fasting Lipid concentrations (total cholesterol, LDL, HDL, Triglyceride)

The following omics data are available

- Genome-wide chip data (subset)
- Genotype data imputed to a population-specific reference panel (subset)
- Exomechip data (subset)
- Exome sequencing data (subset)
- Genome sequencing data (subset)

For detailed availability of genome-wide genotypes and sequencing data, see more information in the 'THL Biobank Omics data availability table' at the THL Biobank sample collection page.

Registry data
Information from the Finnish national health registries, such as Care Register for Health Care (HILMO), Cancer Register, Cause-of-Death Register and Drug Imbursement Registers etc., can be linked to sample donors by the separate application process.

Research group

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