**Pharmacogenetics of SGLT2 Inhibitors (SGLT2iPGx)**

**NCT02462421**

**May 16, 2022**

## Clinical Protocol

Based on data in a genotype database, a list of individuals was compiled to be invited to participate in the clinical trial. Individuals with any of four genotypes were eligible to participate: (a) homozygotes for a nonsense mutation in *SLC5A4* (rs62239058); (b) homozygotes for a nonsense mutation in *SLC5A9* (rs850763); (c) homozygotes for a missense variant in *SLC2A*9 (rs1689079); and (d) individuals who were homozygous for the "wild type" major alleles of *SLC5A4*, *SLC5A9*, and *SLC2A9*. A research nurse accompanied by a liaison (a member of the Amish community) made a home visit to invite selected individuals to participate in the study. If they expressed interest, the study was explained in detail and potential participants were invited to sign a document in which they gave informed consent. Thereafter, the research nurse obtained a detailed medical history; measured height, weight and blood pressure; and obtained blood samples for screening laboratory tests (hematocrit, fasting plasma glucose, serum creatinine, serum sodium, plasma TSH, and HbA1c).

The clinical trial included two home visits conducted by a research nurse and an Amish liaison:

1. At the first study visit, the nurse explained the process. Women of reproductive age underwent home testing to exclude the possibility that they were pregnant. The research nurse obtained baseline blood samples and provided supplies needed for the study including a single canagliflozin tablet (300 mg) and supplies necessary for collection of two 24-hour urine samples. The research participant was instructed to take the canagliflozin tablet exactly 24 hours after initiating the first 24-hour urine collection.
2. The second home visit took place two days after the first visit and 24 hours after the participant had taken the canagliflozin tablet. The research nurse obtained blood samples to assess pharmacodynamic responses to canagliflozin and collected the two 24-hour urine collections. The nurses also obtained information to confirm whether the participant followed the instructions and to elicit information about possible adverse events.

*Inclusion/Exclusion Criteria*

Forty individuals were enrolled in this clinical trial between the dates of July 13, 2015 – April 13, 2016. To be eligible to participate in the clinical trial, individuals were required to be of Amish descent, at least 18 years old, and have BMI between 18 – 40 kg/$m^2$. We established the following exclusion criteria:

- Known allergy to canagliflozin
- History of diabetes, random glucose >200 mg/dL, or HbA1c $\geq$6.5%
- Taking any of the following medications: diuretics, antihypertensive medications, uric acid lowering medications, or other medications judged to interfere with interpretation of results obtained in the clinical trial.
- Diagnosis of significant chronic diseases affecting the cardiovascular, gastrointestinal, pulmonary or renal systems or other diseases judged to interfere with interpretation of results obtained in the clinical trial.
- Seizure disorder.
- Pregnancy (self-reported) or breast-feeding within the past three months.
- Estimated glomerular filtration rate < 60 mL/min/1.73 $m^2$
- Hematocrit <35%
- Liver function tests (ALT or AST) greater than two times the upper limit of normal
- TSH outside the normal reference range for the assay.

- Current symptoms of genitourinary infection or two or more genitourinary infections during the prior 12 months.
- History of osteoporosis-associated bone fracture
- History of unhealed foot ulcer

## Statistical Analysis Plan

This study was designed as an open-label clinical trial with four arms based on the participants' genotypes. Based on our power calculations, we aimed to study a total of 110 individuals:

- 20 homozygotes for the nonsense mutation in SLC5A4 (rs62239058; p.E139X)
- 20 homozygotes for the nonsense mutation in SLC5A9 (rs850763; p.E593X)
- 25 homozygotes for the missense variant in SLC2A9 (rs1689079; p.V253I)
- 45 "control" individuals who were homozygous for the major alleles of all three genes.

The clinical trial was terminated early when it became apparent that we would not succeed at meeting our recruitment targets within the available time and budget. Thirty individuals completed the study:

- 4 homozygotes for the nonsense mutation in SLC5A4 (rs62239058; p.E139X)
- 6 homozygotes for the nonsense mutation in SLC5A9 (rs850763; p.E593X)
- 7 homozygotes for the missense variant in SLC2A9 (rs1689079; p.V253I)
- 13 "control" individuals who were homozygous for the major alleles of all three genes.

*Primary Outcomes*

We defined two primary outcomes based on biomarkers for efficacy:

(a) *Glycemic efficacy (drug-induced glucosuria expressed as grams of glucose per gram of creatinine).* The outcome data were based on the second 24-hour urine sample collected after the participants received canagliflozin. (Healthy participants excrete negligible quantities of glucose in urine at baseline prior to receiving an SGLT2 inhibitor.) We analyzed data using unpaired t-tests comparing data from each of the groups of homozygotes for the three genetic variants versus the control group. We did not correct for multiple comparisons in light of the decision to terminate the study early before achieving our recruitment targets. We have reported nominal two-sided p-values.

(b) *Uricosuric efficacy (drug-induced increase in fractional excretion of uric acid).* Fractional excretion of uric acid was calculated as:

[(urinary uric acid) x (serum creatinine)] ÷ [(urinary creatinine) x (serum creatinine)]

The effect of canagliflozin was calculated as the difference between fractional excretion of uric acid after receiving canagliflozin *minus* the fractional excretion of uric acid at baseline. We analyzed data using unpaired t-tests comparing data from each of the groups of homozygotes for the three genetic variants versus the control group. We did not correct for multiple comparisons in light of the decision to terminate the study early before achieving our recruitment targets. We have reported nominal two-sided p-values.

*Secondary Outcomes*

We defined four secondary outcomes based on biomarkers for efficacy. We analyzed data using unpaired t-tests comparing data from each of the groups of homozygotes for the three genetic variants versus the control group. We did not correct for multiple comparisons in light of the decision to terminate the study early before achieving our recruitment targets. We have reported nominal two-sided p-values.

(a) *Natriuresis (drug-induced urinary sodium excretion expressed as percentage increase in sodium excretion in response to canagliflozin).* The outcome data were based on comparisons of urinary sodium excretion in response to canagliflozin divided by urinary sodium excretion of sodium at baseline.

(b) *Serum levels of metabolites (fasting plasma glucose, serum creatinine, and serum uric acid levels expressed as mg/dL)*. The outcome data were calculated as the absolute difference between serum levels after receiving canagliflozin minus serum levels at baseline.