JDRF-ISPAD Research Fellowship

Project: Association of glycemic variability and DNA methylation patterns with early signs of retinal and kidney damage in individuals with type 1 diabetes.

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Scientific background

Type 1 diabetes is one of the most common chronic conditions of children and adults all over the world (1). Its incidence is increasing worldwide (2) with an estimated overall annual rate of up to 3% particularly in the youngest age group (3,4). Its main feature is autoimmune destruction of insulin secreting pancreatic β-cells leading to disturbed glucose regulation and overt hyperglycemia. Consequently, patients with T1D have a lifelong need for insulin replacement therapy. The primary goal in the treatment of T1D is to maintain blood glucose levels as close to normal as possible with the aim of preventing and/or delaying micro and macrovascular complications (e.g. retinopathy, chronic kidney disease, neuropathy, cardiovascular disease and hearing loss), which together represent the major cause of morbidity and mortality in developed societies (5–9).

Current guidelines for adult and pediatric T1D populations suggest hemoglobin A1c (HbA1c), identified decades ago as the primary marker of long-term average glucose control and the 'gold standard' assay that reflects average glycaemia, level should be below 53 mmol/mol (7%) or 58 mmol/mol (7.5%) (10,11). However, other features of diabetic glucose control, which are not reflected by HbA1c, may add to or modify the risk of complications. For example, glycemic variability (GV) has been associated independent from mean glycemia with increased risk for diabetes complications (12,13), negative impact on brain volume and growth (14,15), and markers of cardiovascular morbidity (16) in individuals with type 1 diabetes. Children that got T1D between 1 and 10 years have 4 – 7 times higher risk for premature mortality of any cause and 16 – 93 times higher risk for cardiovascular events and myocardial infarction than matched controls without T1D (17).

Epigenetic patterns with DNA methylation play an important role in regulation of human metabolism. All main epigenetic mechanisms are involved in regulation of gene expression, contributing to the physiological or pathological response to the environmental factors (18). Thus, DNA methylation represents a sophisticated

molecular mechanism for annotating genetic information. Furthermore, the environmental factors themselves can induce epigenetic modifications. In the context of human disease, clinical observations propose that intrauterine growth retardation, low birthweight, or premature birth could have a causal association with the development of hypertension, coronary heart disease, and diabetes later in life. For example, fetuses exposed to maternal diabetes are prone to develop type 2 diabetes as adults more than siblings who were not exposed. These effects could also be mediated by epigenetic mechanisms such as DNA methylation, similar environmental effects could also occur postnatally (19). There are several studies focusing on the role of epigenetic in T1D; however, its role has not been fully understood. DNA methylation alterations may influence the onset and development of T1D through the regulation of the genes involved in autoimmune reactions, β-cell survival and function. Altered methylation of several genes was reported that correlated with impaired kidney function, but not with other long-term diabetic complications.

Methods and research design

The study objectives are to evaluate the correlations between GV, DNA methylations patterns and early signs of diabetic complications in individuals with T1D, to evaluate gender related difference and to validate these observations compared to individuals with long-duration T1D (positive control) and to healthy controls (negative control).

The study adopts a cross-sectional registry-based, case-controlled clinical trial. We will collect data from individuals with T1D for the past (up to) five years and cross-sectional data from positive and negative controls. All eligible subjects will undergo a study assessment including a fasting blood and urine sample, ophthalmological examination, otoscope examination and audiometric testing, peripheral neuropathy evaluation, subclinical atherosclerosis evaluation and DNA-methylation analysis. We aim to include 250 individuals with T1D (case), 100 healthy siblings (negative control), and 100 adult individuals with long-duration T1D (positive control).

Key inclusion criteria (case):

1. Age between 10 and 23 years of age (inclusive)
2. Type 1 diabetes for at least 5 years
3. The subject has been performing ≥ 4 daily SMBG tests for at least the last 12 months;
4. during the last 5 years the subject has attended at least 3 diabetes clinics per year and did the annual routine diabetes laboratory checkup
5. the subject has been in intensive insulin treatment (either multiple daily insulin doses – MDI, or continuous subcutaneous insulin infusion – CSII) for at least the last 12 months
6. the subject or his/her legal guardian assent/consent participating in the study

Additional inclusion criteria (positive control):

1. Type 1 diabetes for more than 20 years, regardless of participant's age

Key Inclusion criteria (negative control)

1. Age between 10 and 23 years of age (inclusive)
2. Subject is a first degree relative of an individual with T1D
3. Non-diabetic apparently healthy control subject
4. the subject or his/her legal guardian assent/consent participating in the study

Key inclusion criteria (case):

5. Physical or psychological disease likely to interfere with normal conduct of the study
6. Untreated coeliac disease or thyroid disease
7. Current treatment with drugs known to interfere with glucose metabolism
8. Subject/caregiver's severe visual impairment
9. Best corrected visual acuity < 0,8 Snellen chart, refractive error > +4,0 Dsph or < -4,0 Dsph
10. Subject/caregiver's severe hearing/vestibular impairment
11. Sickle cell disease, haemoglobinopathy; or has received red blood cell transfusion or erythropoietin within 3 months prior to time of screening
12. Plan to receive red blood cell transfusion or erythropoietin over the course of study participation
13. Pregnancy at enrollment time or within the previous 12 months;
14. The subject is participating in another study of a medical device or drug that could affect glucose measurements or glucose management;
15. The subject has current or recent history of alcohol or drug abuse.

Key exclusion criteria (negative control):

16. Pregnancy
17. Concomitant disease that influence metabolic control (eg. Anemia, impaired hepatic function, history of adrenal insufficiency,.),
18. History of intraocular surgery, intraocular trauma, or any other ocular pathology
19. Best corrected visual acuity < 0,8 Snellen chart, refractive error > +4,0 Dsph or < -4,0 Dsph,
20. Non-diabetes kidney disease
21. History of hearing/vestibular impairment
22. Two or more positive diabetes specific autoantibodies

<u>Primary endpoints</u>:

The primary endpoints are the between group difference (case with high GV variability vs case with low GV variability, male vs. female, case vs. positive and case vs. negative control) in DNA methylation patterns.

<u>Other key endpoints</u>

- DNA methylation patters and glycemic variability.
- Retinal neurodegeneration/vascular changes
- Kidney tubulopathy/nephropathy

- Hearing/vestibular impairment
- Coagulation impairment
- Impaired cellular metabolism
- Subclinical atherosclerosis and cardiovascular risk factors
- Diabetic neuropathy

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Ethical approval

The clinical study protocol was approved by the National Medical Ethics Committee, Ministry of Health Republic of Slovenia on the 28th of May 2019 (attached).

Study progress

Following ethical approval, we have enrolled our first participant in September 2019.
Until now (6th of December), 68 participants have been included: 65 children or adolescents with type 1 diabetes (case), 2 siblings of individuals with type 1 diabetes (negative control) and one adult with long-lasting type 1 diabetes (positive control).

The study is ongoing, and we plan to finish enrollment by the end of 2020.