ZIP8 implications in glucose pathways and kidneys

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1. Abstract

The bivalent metal transporter ZIP8 has a relative low expression in the kidneys compared to the lungs and liver. The SNP rs13107325 where an alanine is changed to a threonine on the 391 position in ZIP8. This occurs in 10% of the European population and is suspected to promote low arterial blood pressure, increased BMI and schizophrenia. To better understand the SNP rs13107325, ZIP8 knock-in mice were generated. The ZIP8KI-mice showed in the glucose tolerance test an increased resistance to elevated blood glucose levels. Muscles were taken to check if the structure or glucose uptake is unusual. Western blot and immunofluorescence showed controversial results so no conclusion can be reached. Further research should be conducted to investigate the differences in blood glucose and muscle trends in ZIP8KI mice. Glucose reabsorption in the kidney should also be examined to explain the weak glucosuria in the mice.

2. Introduction

The SLC superfamily is a group of proteins with a wide spectrum of compounds. Metal-ions, nucleotides, sugars and more can be transported by members of the SLC family. [1] One of them, ZIP8 encoded by the SLC39A8 has the property to transport a huge variety of metal ions like cadmium, zinc, manganese and iron.[2] Various mutation are found in the SLC39A8 gene. One of them, the single nucleotide polymorphism (SNP) rs13107325 is associated with schizophrenia, changes in blood sugar and metal homeostasis.[3] Newer studies revealed that mice which carry the mutation are resistant to elevated blood sugar. To examine this SNP further, ZIP8 knock-in mice (ZIP8KI) are generated with an additional silent mutation at an SspI splice area to check if the bred animals carry the SNP. [4]

By mass, the skeletal muscle is the most abundant tissue in the human body and one of the main regulator of the glucose homeostasis. About 80% of the postprandial glucose in the circulation is taken by the muscles.[5] To take glucose in to the cell, the insulinreceptor has to be activated by insulin. This triggers the Phosphoinositide-3-kinase/proteinkinase B-Pathway (PI3K/Akt-Pathway) and cause in the end the translocation of the GLUT4 storage vesicles (GSV) to the membrane where GLUT4, the glucose transporter 4 is built in to the membrane .[6]

The group of the Glucose Transporters (GLUT) belong also to the SLC-superfamily. GLUT4, encoded by the SLC2A4 gene is the major glucose transporter in muscle cells and adipocytes. Without stimulation, GLUT4 is nearly absent in the membrane. Insulin and physical exercise can trigger the translocation from the GSV or the endosomal pool.[7]

3. Aims and leading questions

The goal of this thesis is to evaluate if the mutation of the SLC39A8 influences the blood glucose regulation induced by the muscles. Structural analysis and protein measurement are used to search for significant changes. The following questions were elaborated:

- Leads the increased glucose uptake to structural differences in the muscle tissue?
- How does ZIP8 affect the expression of GLUT4?
- How is the glucose pathway in muscles affected?

4. Material & methods

The used animals are C57BL/6 mice. Four weeks after birth the animals are genotyped. Homozygous WT and KI are challenged for 45 days with 2% sucrose in the drinking water. Afterwards the animals are sacrificed, the organs and tissue harvested.

For the Western blot, the proteins are extracted as whole tissue lysates with RIPA-buffer. Total protein is measured and the samples are prepared with Laemmli buffer to a total concentration of 10,66µg/µl. After the electrophoresis and transfer,(Figure2) the blot is coated with antibodies and then, the blot is visualized with chemiluminescence.

The histological analysis is done in formalin fixed paraffin embedded tissue. Histological staining's and immunofluorescence were performed. (Figure 2)

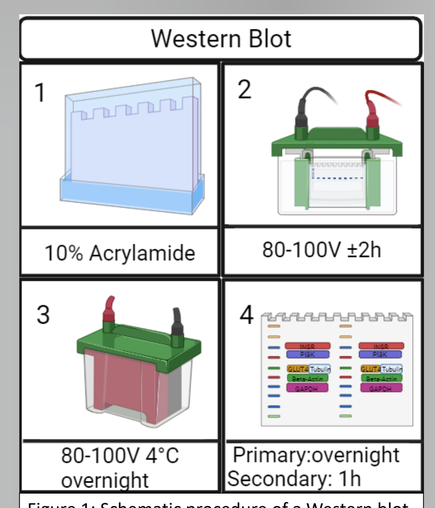

Figure 1: Schematic procedure of a Western blot

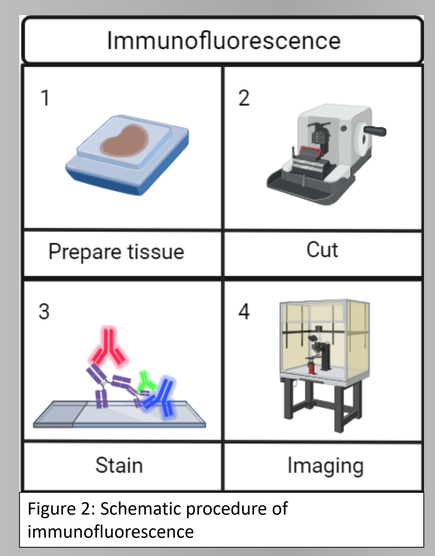

Figure 2: Schematic procedure of immunofluorescence

5. Results

The images of the histological analysis can be found in the thesis. (Chapter 5.1.1) Muscle tissue of ZIP8KI(n=2) and WT(n=2) animals after the high sucrose diet are analysed as whole tissue lysate. The Western blot is loaded with 32µg protein and tubulin is used as housekeeping protein. GLUT4, PI3K and the insulinreceptor (INSR) are the target-proteins. None of the blots showed a significant difference to the control group. (Figure3)

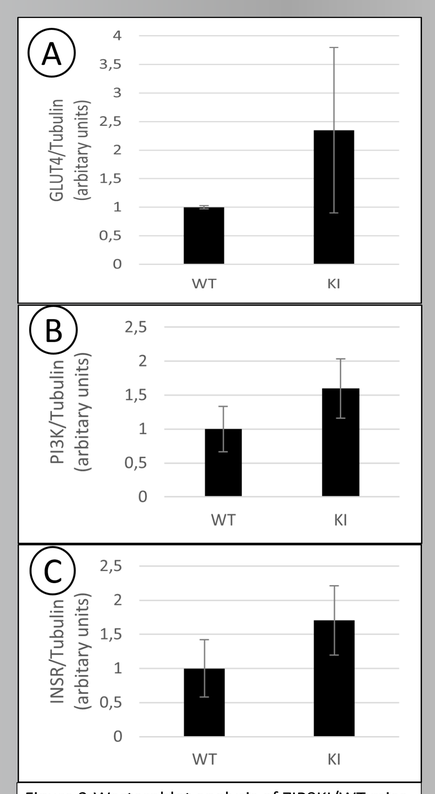

Figure 3:Westernblot analysis of ZIP8KI/WT mice muscles for (A)GLUT4, (B)Pi3K and (C) Insulinreceptor(INSR).(n=2)

6. Discussion

Outcome 1: The different stainings and the immunofluorescence revealed no differences. Therefore, it can be said that the SNP rs13107325 don't change the muscle structure in mice.
Outcome 2: The Western blot for GLUT4 shows no significance but a tendency. The immunofluorescence can't confirm this tendency. Further experiments must be done before this question can be answered.
Outcome 3: INSR, PI3K and GLUT4 showed all no significance. A tendency towards the ZIP8KI was observable. To arrive a conclusion a larger number of samples is needed.
Outlook: More Samples are needed. Then the glycogen in the muscles should be measured. In a second step the renal glucose resorption proteins like SGLT1/2 and GLUT2 should be examined.

References:
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Abbildungen
Figure 1-3: Own Figure (Moser 2023)