**Paired box 6 (PAX6) regulates glucose metabolism via proinsulin processing mediated by prohormone convertase 1/3 (PC1/3)**

**Introduction**

Study investigates how a genetic mutation in the PAX6 gene leads to impaired insulin processing and higher blood sugar levels.

*Note*: PAX6 is the gene that holds the information to build a protein that binds the promoter region of another gene, proprotein convertase 1/3 gene; this promoter region, when activated, leads to the expression and eventual creation of proprotein convertase 1/3 protein. A mutation in the PAX6 gene leads to a fragmented, mutilated protein that can’t bind the promoter region of the proprotein convertase gene, leading to failed activation/promotion of its expression.

Proprotein convertase 1/3 converts an immature form of insulin (pro-insulin) to the mature, functional form of insulin (insulin).

**Methods**

16 participants with this PAX6 mutation.
 - Tested using a DNA screening technique known as PCR, then confirmed the mutation by comparing it to where they believe the mutation is located.

Also used a transgenic mouse model (meaning, the mouse’s DNA has been manipulated to have the mutation) with a partial mutation (heterozygous) to confirm some of the more invasive ideas in the mouse model that represents the human symptoms.
 - The mouse gene is similar to the human gene (both are truncated forms of the full gene)

Drew blood and tested blood for a variety of different things:
- Blood sugar/glucose
- Insulin levels
- adrenocorticotropin hormone
- melanocyte stimulating hormone

Also detected molecular/protein levels.

Finally, they used a luciferase assay in cells, which is an experiment wherein the experimenters can measure the amount a gene is expressed by inserting a luciferase reporter on the other side of a promoter in the DNA. A promoter is a section of the gene that is bound by an activating protein and, when bound, expresses the target gene (luciferase, in this case) – this allows researchers to detect the amount of the expression of that gene by measuring the quantity of luciferase that is expressed. More explained later.

**Results**

*Figure 1*

1B They are showing where the mutation occurs – a switch between the thymine and cystine.
- This mutation leads to, instead of the genetic material/blue print for an amino acid, the formation of a “stop codon”, stopping the gene from being read at that point instead of later on down the gene, where it is supposed to be stopped – this leads to the reading of a shortened, ineffectual protein (a mutation/variant).

1C. This is the genetic representation of the human PAX6 mutation, the similar mouse PAX6 mutation, and the full gene.
- PAX6 is the gene that holds the information to build a protein that binds the promoter region of another gene, proprotein convertase 1/3; this promoter region, when activated, leads to the expression and eventual creation of proprotein convertase 1/3 protein.

*Figure 2*

2A. Fasting blood sugar levels between healthy, PAX6 mutant/variant, and diabetic individuals.
- Only the diabetic individuals show increases in fasting blood sugar levels.

2B. Fasting blood insulin levels.
- Only diabetic individuals have elevated insulin levels.

2C. Given a glucose challenge (set amount of sugar given, after fasting, then blood sugar levels measured) to see how Pax6 mutations impact blood sugar clearance.
- Pax6 mutation individuals have higher blood sugar levels compared to normal individuals.

2D. Wanted to know how age affects blood sugar levels in Pax6 mutant individuals.
- Increased blood sugar levels with increased age compared to younger Pax6 mutation individuals.

2E. Insulin levels measured after a set injection of sugar into the blood stream.
- Pax6 shows lower blood insulin levels at the 30 minute mark.

2F. Injecting insulin and measuring blood sugar levels in the blood stream.
- No difference between Pax6 mutation individuals and normal individuals.

2G. This is showing the amount of pro-insulin (immature insulin) in the blood.
- Pax6 and diabetic individuals have elevated pro-insulin levels vs normal individuals.

2H. This is a measure of the ratio between proinsulin/total insulin – the more proinsulin present, the larger the number (closer to 0.4, in this graph).
- Pax6 mutation leads to greater proinsulin to mature insulin levels.

*Figure 3*

3C. This is a glucose tolerance test (injected sugar) to see what happens to blood sugar (like that done in humans) in mice with the Pax6 mutation at 4 months, 6 months vs normal mice.
- Pax6 mutation mice, at 4 or 6 months, have impaired glucose tolerance (more evident and longer lasting in 6 month Pax6 mutation).

3D. Same thing as 3C, but with the injection of insulin, not sugar/glucose.
- Pax6 mutation mice show same glucose clearance with same amount of insulin added.

3E. Proinsulin/insulin measure in the blood.
- 6 month Pax6 mice show highest proinsulin relative to total, mature insulin.

*Figure 4*

4A. Amount of proprotein convertase protein/enzyme measured (remember: this is the protein that the Pax6 gene helps create as described in the introduction).
- In the Pax6 mutation mice, there is reduced amount of this proprotein convertase molecule/enzyme.

4C. The luciferase assay shows more luciferase expression when the promoter of its gene is bound and told to activate. This one is specific for the Pax6 gene to measure its activity.
- With the full gene, there is increased expression of luciferase with increasing concentrations of Pax6 gene transfected into cells (injecting the gene more times into the genetic code).
- However, with the Pax6 mutant (a mutation in Pax6), there is no increase in luciferase.

*Figure 5*

5A&B. Both of these proteins, ACTH (adrenocorticotropin hormone) and a-MSH (alpha-melanocyte stimulating hormone) are also cleaved by the proprotein covertase – this tests if the effect seen on insulin is also seen in other proteins.
- People with the Pax6 mutation show decreased levels of ACTH and MSH.
- Mice with the Pax6 mutation show decreased ACTH and MSH.