ZIP14 Overexpression in the Beta cells of the Pancreas leads to Diabetes during Iron Overload

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Abstract

Iron overload can result from mutations in genes involved in the regulation of hepcidin, a hormone secreted by hepatocytes when iron levels are high. A common form of iron overload, hereditary hemochromatosis (HH), is due to a single missense mutation in a gene known as HFE (High Fe). Juvenile hemochromatosis, a more severe type of hemochromatosis, results from mutations in the Hjv gene (hemojuvelin). Patients with mutations in the HFE or Hjv genes don't synthetize hepcidin, leading to iron overload, which predominantly affects endocrine tissues. A common comorbidity in patients with hemochromatosis is type 1 diabetes mellitus. It has been postulated that type 1 diabetes develops as from iron loading in pancreatic islets leading to beta cell damage and impaired insulin secretion. Iron deposition in the islets of Langerhans, and damage to pancreatic beta cells, is thought to be a main contributor to the development of diabetes in the context of iron overload. To study iron loading of the pancreas, we used hemojuvelin and transgenic mice selectively overexpressing ZIP14 in the beta cells of the pancreas (*Tg(MIP-Slc39a14); Hjv*$^{-/-}$). Perls' Prussian blue staining revealed that transgenic *Hjv*$^{-/-}$ mice, but not *Hjv*$^{-/-}$ *mice*, accumulated iron in beta cells. Interestingly, by 12 weeks of age male *Tg(MIP-Slc39a14); Hjv*$^{-/-}$ mice develop diabetes. Non-heme iron assay indicated females load less iron in general by 12 weeks, likely due to differential inhibition of Hepcidin by testosterone. When fed an iron overload (FeO) diet, *Tg(MIP-Slc39a14); Hjv*$^{-/-}$ males and females develop early signs of diabetes by ~6 weeks of age suggesting the sex difference between sexes is related to the amount of iron absorbed from the diet. Immunofluorescent stained pancreatic tissue sections stain significantly less for insulin in the transgenic males than the *Hjv*$^{-/-}$ males suggesting impaired insulin secretion. Collectively, our data suggest that overexpression of ZIP14 in the beta cells of the pancreas during iron overload leads to iron loading in the islets, decreased insulin secretory capacity and ultimately a diabetes phenotype. This mouse model represents the first of its kind and will allow us to study the development of diabetes in the context of iron overload.

Hypothesis

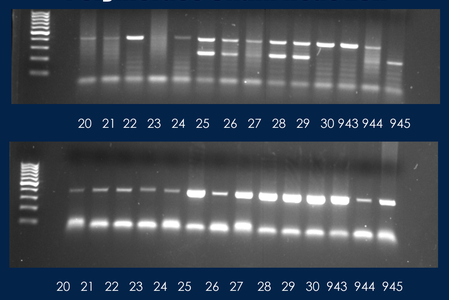
The reason why humans who show Iron overload conditions like patients with Thalassemia and Hemochromatosis develop type 1 diabetes mellitus, is because ZIP14 is expressed in the Beta Cells of the Islets in the pancreas.

Background

- Patients with Hemochromatosis load iron in the beta cells of the islets in the pancreas, leading to impaired insulin production and causing type 1 diabetes mellitus. (Niederal, C. U.S. National Library of Medicine., 1999)
- Unlike humans, mice don't express ZIP 14 in the beta cells of the pancreas, thus they do not develop type 1 diabetes mellitus. (Jenkitkasemwong et al., Cell Metabolism., 2015)
- The aim of the present study is to determine if ZIP14 plays a role in iron loading of the beta cells in the islets of the pancreas. We compared the loading patterns between *Hfe2*$^{-/-}$ and *Hfe2*$^{-/-}$ *; ZIP14 Tg mice.*

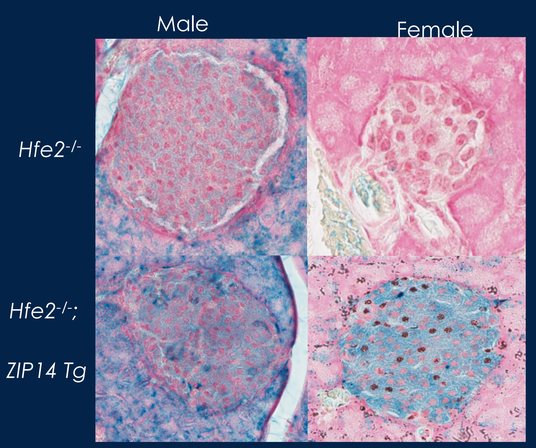
Experimental Data

Polymerase Chain Reaction



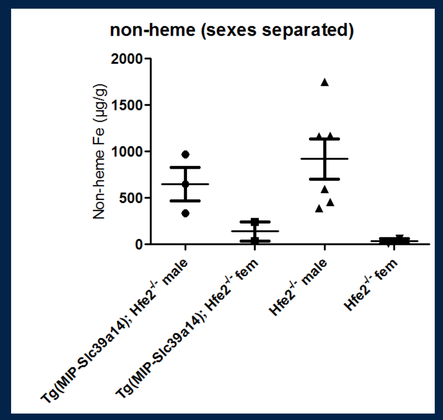
For the top image, double bands represent *Hjv*$^{-/-}$ mice, vs one band which shows Wt. For the bottom image bright band represents the transgenic gene.

Prussian Blue Staining



Hfe2$^{-/-}$ mice load iron in the acinar cells of the pancreas, where the mice with the ZIP 14 Tg gene load iron in the beta cells of the islets. Blue staining is represented by iron.

Non-Heme Iron



Non-Heme iron shows the amount of iron loaded based on the different genotypes measured.

Procedures

- Polymerase Chain Reaction: Determining the genotype of the mice is first priority we are faced with in the lab. We use digested DNA from tail snips along with primers and other reagents. After the solution passed through a thermocycler where it will be heated and cooled through multiple cycles, we run the samples in a gel and read the genotype of the mice by reading the bands given by the experiment, as shown in the labeled image.
- Prussian Blue Staining: One way where we are able to qualitatively show how iron loads across different tissue sections and different genotypes is through Perls. After treating the samples with multiple reagents which will bind to iron, the end product will result in a blue stain where we can identify where iron is stored in the tissue being observed.
- Non-Heme Iron: Another way we distinguish between these tissues and genes is through quantitatively measuring the concentration of iron using a spectrophotometer to read the absorbance of the samples harvested. This experiment allows us to see the differences between genes much more accurately since we its something we can measure.

Conclusions

- Similar to humans, and *Hfe2*$^{-/-}$ *; ZIP14 Tg mice load iron in the beta cells of the islets.*
- ZIP14 is necessary for iron loading in the beta cells of the islets.
- *Male Hfe2*$^{-/-}$ *; ZIP14 Tg mice develop end stage diabetes by around 12 weeks of age, with their female counterparts taking up to 3 times the amount of time to develop it as well.*
- *Inhibition of ZIP14 may help mitigate the excess iron loading of the insulin producing beta cell and delay diabetes development.*

Acknowledgements

- Niederau, C. 1999. "[Diabetes Mellitus in Hemochromatosis]." *Zeitschrift Fur Gastroenterologie*. U.S. National Library of Medicine.
- Jenkitkasemwong S, Wang C, Coffey R, Zhang W, Chan A, Biel T, Kim J, Hojyo S, Fukada T, Knutson MD. SLC39A14 Is Required for the Development of Hepatocellular Iron Overload in Murine Models of Hereditary Hemochromatosis. *Cell Metabolism*. 2015 May 28.