Spring 4-28-2021

The Emerging Role of Pancreatic β-Cell Primary Cilia in Diabetes Mellitus

Emily Blackburn
blackburnemily30@gmail.com

The Emerging Role of Pancreatic β-Cell Primary Cilia in Diabetes Mellitus

Emily Blackburn

Honors Thesis, Molecular and Cell Biology

Thesis Advisor: Dr. Yi Wu

Honors Advisor: Dr. Thane Papke

Abstract

Diabetes mellitus is prevalent in America, affecting more than 10% of the population. The underlying cause of the disease are diverse and can be related to genes, environment and physical inactivity. This review investigates how a minute cellular organelle called primary cilium, found in pancreatic β-cells, affects the development and the function of the pancreas. Gene mutations related to primary cilia can lead to malfunctions or loss of the structure, and cause diseases collectively classified as ciliopathies. Recent studies of two types of ciliopathies, Alstrom's Syndrome and Bardet–Biedl Syndrome, connect the primary cilium defects with insulin secretion in pancreatic β-cells. The overarching goal of this review is to pose clinical questions on how malfunctions within primary cilia development may result in the development of diabetes mellitus in individuals, and to provide insights for treatment options of ciliopathies and thus, diabetes.

Introduction

Diabetes mellitus is a chronic disease afflicting millions of people throughout the world. This disease results from malfunctions in insulin secretion and production, leading to difficulties in glucose metabolism. The inability to breakdown glucose sugars into molecules necessary for cellular respiration and thus energy production can lead to overall decreases in bodily functions. The organ at the center of this disease is the insulin and glucagon producer, the pancreas. Insulin is released when the body is in a fed state in response to glucose. The insulin molecule allows for glucose uptake into cells so it can be stored or broken down for energy production. When the body is in a fasted state, glucagon molecules promote the release of stored glucose for use in cellular respiration. Thus, the pancreas is a primary target for diabetes research and treatment.

Primary cilia are organelles found in many cells throughout the body and has roles in hormone regulation and glucose metabolism using intraflagellar transport. These organelles have sensory functions and act as mechano- and chemoreceptors (Davenport and Yoder 2005). Primary cilia can be found in α and β-cells of the pancreas. These are areas of interest with diabetes because the α-cells produce glucagon, and the β-cells produce insulin (Columbia Surgery 2021). Primary cilia are similar to other cilia in our cells. It is constructed of an axoneme protruding from the basal body of the centrioles in cells and is surrounded by a semipermeable plasma membrane (Davenport and Yoder 2005). Only one primary cilium is present in the cells which utilize primary cilia. Alternatively, motile cilia are found on cells such as the epithelial cells in nostrils and the trachea to help guide mucus into the stomach in order to kill pathogens. The primary cilium is typically immotile, with an axoneme that has a 9 + 0 structure (9 doublets of

microtubules and no singlets) in contrast to that of a motile cilium which has a 9 + 2 structure (Davenport and Yoder 2005). Primary cilia use intraflagellar transport to transport lipids and other molecules between the cilium and the cell body. Complex A and B factors allow for the molecules to be carried in the retrograde and anterograde directions, respectively (Gleeson and Lee 2011). This trait is important because it allows the primary cilia to maintain their function in cellular signaling. The transportation of signaling molecules into the cells allows for cell interpretation of the signals. Primary cilia malfunction/absence has shown irregularities in important developmental pathways such as Wnt, Sonic Hedgehog (Shh), TGF-β, Notch, and fibroblast growth factor (Lodh et al. 2014). If these pathways malfunction there can be severe developmental abnormalities as these molecules are highly important in embryo development.

The lack of or malformation of primary cilia is classified as a group of diseases called ciliopathies. Ciliopathies are often inherited through recessive traits; however, many other genes can affect disease presentations (Gleeson and Lee 2011). Some of the more characteristically distinguishable ciliopathies are caused by single gene mutations, however the exact functions of these mutated genes are still being investigated through comparative genomics (Gleeson and Lee 2011). Diseases relating to ciliopathies have profound effects on the pancreas. Rare ciliopathies relating to the pancreas, such as Bardet–Biedl Syndrome (BBS) and Alstrom's syndrome (ALS), are comorbid with obesity and diabetes.

Primary Cilia in Pancreas Development

As an embryo begins to develop in utero, its organs will begin to develop as well, beginning with the foundation of a neural system. Concurrent with other essential organs,

the embryo's pancreas will begin to develop from the endoderm of the developing fetus. The cells orient themselves in an anterior to posterior orientation using different molecules to distinguish. For example, a concentration gradient is formed by Wnt molecules, with lower concentrations signaling the anterior portion of the embryo. The pancreatic stem cells will begin to differentiate based on location and thus exposure to different molecules. The cells closet the extracellular matrix will become acinar cells with exposure to Wnt, Notch and other hormones. The cells on the interior of the developing pancreas will become either ductal or endocrine cells (Lodh et al. 2014). As shown many of the pathways harmed by ciliopathies are innately important to the development of the pancreas. Thus, it is reasonable that malfunctions of these pathways as a result of malfunctions in primary cilia will have profound effects on pancreatic function.

There has been substantial research on the role of primary cilia in these signaling pathways which is important for providing disease explanation and possible avenues for treatment. For example, primary cilia ensure the proper functioning of the Shh pathway (Lodh et al. 2014). Although the loss of Shh signaling does not impede the formation of the pancreas, some research has shown that the loss of primary cilia function can lead to less differentiation amongst the pancreatic cells (Lodh et al. 2014). Primary cilia also affect the activation of Hh (hedgehog) signaling in the β-cells of adult mice and can result in decreased insulin expression and secretion and decreased glucose sensing (Landsman et al., 2011). Due to the fact that β-cells and other pancreatic cells were unable to differentiate (due to primary cilia malfunction), the β-cells lost their function of insulin

production (Lodh et al. 2014). Unsurprisingly, the dedifferentiation can lead to tumor growth in the pancreas and possibly cancer (Landsman et al., 2011).

Ciliopathies have also been found to affect other signaling molecule pathways such as Wnt, TGF-β, Notch and FGF. There has been evidence of abnormal Wnt regulation in cells lacking primary cilia, but more research needs to be done on the exact mechanisms of this impairment (Lodh et al. 2014). Transforming Growth Factor B (TGF-B) is expressed in various areas of the developing pancreas, contributing to pancreatic cell specification. For the Notch molecules, there is even less understanding. However, it appears that there is some coregulation between the Notch pathways and primary cilia. When Notch is absent, the growth of primary cilia is stunted, and the organelle is much shorter than normal. Meanwhile, primary cilia can regulate the levels of Notch in the pancreas. Notch is also thought to have a role in ciliogenesis (Lodh et al. 2014). Finally, fibroblast growth factor (FGF) also stimulates the growth of primary cilia through ciliogenesis and helps regulate the specification of pancreatic cells. FGF is similar to Notch in that it also regulates the length of primary cilia and is essential for proper functioning. Therefore, coregulation with these molecules as Notch regulates FGF10 and FGF molecules also regulate Shh and Wnt pathways (Lodh et al. 2014).

Primary cilia can also affect the pancreas indirectly. Primary cilia play an additional role during development, particularly in nodal cells. The nodal cells contain the only motile primary cilia in the body. These primary cilia create nodal flow as they move back and forth. This moves around the extracellular fluid allowing for the developmental signaling molecules such as Wnt to be distributed throughout the body and contribute to proper organ placement and growth (diIorio et. al 2014).

Recognizing the interconnectedness of primary cilia in pancreatic development and signaling pathways is important for understanding the severe effects ciliary malfunction or absence can have. In this review the focus is specifically how those ciliopathies can lead to issues with insulin production and secretion which ultimately leads to diabetes mellitus and other diseases.

Ciliopathies Associated with Pancreas Function

The field of proteomics has been imperative to understanding and investigating ciliopathies. This field has established a breadth of understanding on the possible causative diseases of ciliopathies. In important discover by the use of proteomics was the recognition that proteins associated with ciliopathies 'assemble with reproducible stoichiometry' (Gleeson and Lee 2011). The discovery is important in understanding the mechanisms of various diseases and how primary cilia are specifically affected. Understanding the reproducibility of the complexes is essential in comparing multiple patients with the same phenotypic disease presentations. This also means that any potential treatments could be used for a wide variety of patients. Thus, some of the molecular components of ciliopathies are conserved and research can be applied universally.

Ciliopathies are shown to have many symptoms present within the pancreas. For example, mutations in nephronophthisis (NPHP) genes (related to primary cilia) can lead to dysplasia and fibrosis of the liver and the pancreas (Lodh et al. 2014). While nephronophthisis is characteristically a genetic kidney disease, mutations of its genes can profoundly affect primary cilia cells in other organs. This ciliopathy can lead to scarring

and abnormal cell growth within the pancreas, leading to abnormal function. As expected this disease is comorbid with diabetes mellitus

Ciliopathies commonly effect the normal functions of not only the pancreas but also the liver and kidneys. One of the most common ciliopathies is Polycystic Kidney Disease (PKD). PKD is an autosomal dominant disease characterized by renal cyst formation, loss of renal function and abnormalities in the cardiovascular, pancreatic and gastrointestinal systems (Lodh 2019). The causative gene for PKD is IFT88/Polaris which effects proper development of the primary cilia. Without this gene, kidney function is impaired and calcium regulation within the body is abnormal. PKD demonstrates the whole body effects of ciliopathies by presenting with phenotypes such as obesity and elevated blood levels of glucose, insulin and leptin. In PKD, obesity can be attributed hyperphagia. Primary cilia are typically responsible for delivering satiety signals (leptin) without them, the body cannot recognize when it is full (diIorio et. al 2014). Ciliopathies effect multiple organs within the same body and can have effects on the pancreas regardless of the primary attributes of the disease.

Bardet–Biedl syndrome (BBS) and Alstrom's syndrome (ALS), which will be expanded on in relation to insulin regulation and glucose metabolism, are keys to the connection of pancreatic ciliopathies and diabetes. Both of these diseases are characterized by obesity, hypogonadism and renal disease. One major difference between the diseases is that BBS is associated with cognitive deterioration and learning disabilities. ALS is an extremely rare disease, with around 500 cases worldwide ((Volta and Gerdes 2016). ALS patients are shown to have insulin resistance and dilated cardiomyopathy, with around 60% of patients developing cardiac failure (Bush 2011).

For this reason, the rates of onset of diabetes in ALS patients are higher than those in BBS patients and obese patients without ALS (Lodh et al. 2014). BBS is associated with diabetes as well as obesity due to increased insulin sensitivity (Lodh et al. 2014). While the exact causative genes of ciliopathies are poorly understood, it is likely that mutations in ALMS1, BBS1-20, and PKD1 effect ALS, BBS, and PKD, respectively (Lodh 2019). Further exploration on what causes the mutations of these genes and possible treatments for the malfunctions are primary areas of research. Ideally, breakthroughs within the field of ciliopathies could lead to improved management of diabetes and other diseases.

Pancreatic Cysts

One common feature among pancreatic ciliopathies is the presence of pancreatic cysts, primarily forming in ducts. 10% of patients with autosomal dominant polycystic kidney disease develop cysts on their pancreas (Lodh et al. 2014). ADPKD is the most common ciliopathy around the world with more than 200,000 new cases a year in the US alone (Mayo Clinic), demonstrating the association of pancreatic cysts with ciliopathies. Fortunately, in many cases there is not a change in endocrine function as a result of the cysts in most ciliopathies. However, in Alstrom's syndrome, pancreatic cysts are associated with β-cell dysfunction and impairments to glucose metabolism (Lodh et al. 2014). The other ciliopathy, which is highly associated with obesity, BBS, seems to have normally functioning β-cells and glucose metabolism (Lodh et al. 2014).

The exact mechanism by which ciliopathies cause pancreatic cysts is still unknown although research is currently being conducted. There are some hypotheses on the origin of pancreatic cysts. For example, it is highly likely that cysts result from abnormal proliferation of epithelial cells. This mass of cells expands until it is pinched

off to form an isolated cyst. These cysts can become fluid filled as secretions are made within the lumen, leading to expansion of the tissue (Lodh et al. 2014). Acinar cell apoptosis, and duct dilation are closely associated with the presence of pancreatic cysts (Lodh et al. 2014).

Experiments with mouse models have shown that complete lack of primary cilia from pancreatic cells can lead to the production of cysts (Lodh et al. 2014). These results indicate that primary cilia play a role in regulating the cell cycle of pancreatic cells. The primary cilia help to limit cell growth/division to prevent accumulation of a mass of cells. Excessive proliferation of cells can lead to abnormal production of insulin and thus impact glucose metabolism. The development of pancreatic cysts and also been found on pancreases where primary cilia are present but malformed/malfunctioning (Lodh et al. 2014). This indicates the contribution of a wider range of ciliopathies in pancreatic cyst development.

Primary Cilia and Insulin Regulation

While the exact role of pancreatic primary cilia is still being explored, it is hypothesized that the organelle has a role in sensing or regulating ion transport and fluid flow. When primary cilia are absent or malformed, excess fluid may be found in the lumen of the pancreas (Davenport and Yoder 2005). These abnormalities may lead to malfunction of pancreatic exocrine cells as enzyme regulation is affected (Davenport and Yoder 2005). Therefore, -ciliopathies of the pancreas can affect secretion of digestive enzymes such as trypsin and chymotrypsin (to digest proteins), amylase (to digest carbohydrates) and lipase (to digest fats). Collectively these enzymes are often referred to as pancreatic juice (The Pancreas Center). Since ciliopathies appear to have a profound

effect on the exocrine function of the pancreas, one would expect the same to be true for the endocrine function (glucose metabolism via insulin and glucagon). However, research has shown that the endocrine cells of those with primary cilia malfunction appear to be normal but with abnormalities in glucose metabolism (Davenport and Yoder 2005). The effect of ciliary malfunction on organ development has been correlated with abnormalities in energy regulation, obesity and glucose metabolism (Lodh 2019). Thus, developmental impairments of the pancreatic β-cells can lead to a host of other disease phenotypes including diabetes.

Two main ciliopathies have shown malfunction in insulin regulation/secretion as a result of primary cilia malfunction or absence. These diseases are the previously discussed BBS and ALS. The implications of cilia malfunction are especially prevalent in ALS where malfunction of the β-cells resulting in insulin resistance and development of diabetes (Lodh et al. 2014). Due to the fact that primary cilia proteins are associated with differentiation of pancreatic cells, it stands to reason that their maldevelopment could lead to lack of proper β-cells. The lack of proper β-cells would thus result in issues with proper insulin secretion and regulation (Lodh et al. 2014). ALS is caused by a loss-of-function mutation of the ALSM1 gene, meaning a key protein of the primary cilium is either not present or unable to function properly In the pancreas of ALS patients, this mutation prevents β-cells from secreting any insulin, leading to an early onset of diabetes, particularly in childhood. BBS patients show increased sensitivity to insulin with normal glucose management (Lodh 2019). For this reason, the onset of diabetes for ALS patients is typically in early childhood. While BBS patients do not typically develop diabetes in childhood but later in life.

There are several BBS genes associated with the disease, termed the BBSome, which is involved in intraflagellar transport along the primary cilium. Some of these genes have different effects on glucose metabolism. A gene knock-out experiment of BBS4 absence in mice showed a production of malfunctioning β-cells. These cells are inefficient at releasing insulin. Alternatively, loss-of-function with BBS5, BBS7 and BBS9 genes can lead to hypersecretion of insulin. BBS models also showed higher levels of apoptosis amongst the β-cells (Lodh 2019). This could counteract the increased insulin levels and possibly explain the little effect of BBS on glucose management. However, eventually the apoptosis of β-cells will deplete the overall β-cell mass resulting in susceptibility to diabetes.

Not only could the reduction of cells differentiating into pancreatic β-cells promote issues with proper insulin secretion, but the cellular reproduction of existing cells could be inhibited. It has been found that pancreatic β-cells lacking primary cilia are unable to divide, resulting in a lack of β-cells (Lodh 2019). The mechanism by which the pancreas replenishes dead or damaged cells is removed by preventing cellular divisions. Researchers predict that the absence of primary cilia disrupts the signaling mechanism which jumpstarts cell division (Lodh 2019). The ALSM1 gene mutation has been implicated in reducing the ability of β-cells to proliferate (Lodh 2019).

Glucose Homeostasis

Aforementioned, diabetes mellitus is a disease characterized by insulin resistance or lack of insulin production. Without proper levels of insulin in the body, glucose cannot be properly digested and used as an energy source in cellular respiration. Pancreatic β-cells located within the islet of Langerhans are responsible for the secretion of insulin.

The primary cilia located within the pancreatic islet cells (α, β, and δ cells) are essential in regulating hormone secretion. The primary cilia of these cells expand into the luminal space connecting all of the islet cells which allows for easy regulation of cell signaling through the use of G-protein systems and G-coupled receptors (Hughes et. al 2020). A deletion of the β-cell primary cilia in gene knockout mice leads to insulin secretion failure. However, hormone secretions from the α-cells (glucagon) and δ-cells (somatostatin) indicating significant impacts on glucose homeostasis and energy production (Hughes et. al 2020). Thus, ciliopathic effects on the pancreatic β-cells result in defects in glucose homeostasis and regulation of energy metabolism.

The release of insulin from pancreatic β-cells is signaled by an increase in the blood concentration of glucose. More specifically, however, the mechanism behind insulin release involves an influx of calcium ions, as stimulated by the glucose molecules. The release of insulin is in response to pulsatile oscillations of the calcium ions. When β-cells lack primary cilia, the oscillatory/pulsatile calcium behavior does not occur leading to a sustained increase in calcium ion influx (Hughes et. al 2020). Thus, insulin is unable to be properly regulated and secretion is affected, thereby effecting the management of glucose levels in the bloodstream.

Glucose homeostasis is further regulated by Ephrin-type A receptors and Ephrin-type A molecules (EphA/EphrinA) (Volta et. al 2019). These are protein-tyrosine kinase type receptors important in cell signaling and insulin regulation. When these receptors are phosphorylated in β-cells, GTPase activity is suppressed, thereby suppressing insulin secretion. An elevated concentration of glucose leads to influx of the Ephrin ligand which stimulates insulin release (Volta et. al 2019). This demonstrates the balance of regulatory

glucose and insulin mechanisms. An increase in glucose concentrations stimulates the release of insulin, while decreased glucose levels suppress insulin secretion. In pancreatic β-cells lacking primary cilia, the Ephrin receptors, along with other tyrosine kinase receptors are upregulated and hyperphosphorylated (Volta et. al 2019). This results in further suppression of insulin secretion and glucose intolerance within the body. The effect of ciliary impairment on Ephrin receptors is caused by disturbing the recycling of ephrin, resulting in an accumulation of the ligand on the plasma membrane. This results in an inability to dephosphorylate the Ephrin receptor and results in the insulin suppression pathway remaining continuously activated (Volta et. al 2019).

More research needs to be conducted on the exact mechanism by which defects in primary cilia effect glucose homeostasis and insulin regulation. However, it is difficult to determine abnormalities in glucose metabolism caused specifically by a ciliary gene mutation because many other cells and tissues could result in defects. (Davenport and Yoder 2005). Essentially, the issue is that more diseases than those caused by ciliopathies can lead to issues in glucose metabolism and insulin regulation. Not all cases of diabetes mellitus are caused by ciliopathies, many more factors are at play such as familial history, and a host of environmental factors including age, diet and weight.

Diabetes and Ciliopathy

The two most common types of diabetes are diabetes mellitus type 1 and type 2. Type 1 diabetes mellitus typically presents in childhood as the body destroys pancreatic β-cells. This disease presents as a n autoimmune disorder leading to insulin deficiency (Ndisang et. al 2017). To supplement for this insulin deprivation, type 1 diabetics are dependent on external insulin from a young age. The prevalence of this disease is roughly

half a percent, as of 2017, with the rates increasing yearly (Xu et. al 2018). Meanwhile, diabetes type 2 is of national and global health concerns. As of 2017, the rate of type 2 diabetes was approximately 8.5% of adults (Xu et. al 2018). This number has been increasing along with the obesity epidemic as Americans are leading sedentary and unhealthy lifestyles. Type 2 diabetes id characterized by insulin resistance and decreased insulin secretion from pancreatic β-cells (Ndisang et. al 2017). Malfunctions of primary cilia can lead to the development of type 2 diabetes mellitus because of the suppression of insulin secretion and lack of glucose metabolism regulation. Type 2 diabetes can be managed with exercise, diet, monitoring of sugar intake as well as external insulin in severe cases. Diabetes is a major health concern because it can lead to significant disability and early death. In diabetic patients, a multitude of issues can develop from cardiomyopathy to neuropathy.

Obesity is often associated with diabetes while not every diabetic is obese and not every obese person is diabetic. Increased insulin sensitivity or resistance is often associated with obesity while one may not cause the other. An unhealthy diet and lack of exercise certainly contribute to excessive weight gain and obesity but there are genetic components as well. Specifically, four gene loci have been identified which cause ciliopathies and are linked to non-syndromic obesity (Volta and Gerdes 2016). Where non-syndromic refers to loss-of-function mutations effecting hormonal regulation in the hunger area of our brains, the hypothalamus (da Fonseca et. al 2017). Obesity is a phenotype associated with both BBS and ALS. However, because these diseases prove a causal relationship between primary cilia gene mutation and a malfunction in insulin

secretion, it is difficult to discern the obesity connection. The contributions of obesity to in diabetes in these cases are still areas of investigation.

As previously discussed, the malfunction of primary cilia in both BBS and ALS leads to significant effects on the insulin secretory function of primary β-cells. The mutations in primary cilia of BBS, ALS and other ciliopathies substantially reduce the ability to properly respond to elevate blood glucose levels with the release of insulin. Sustained lack of insulin can lead to hyperglycemia, or elevated blood levels of glucose. Hyperglycemia can lead to severe complications if treatment is delayed including, retinopathy, neuropathy, and coronary heart disease (Mouri and Badireddy 2020). Thus, the mutations to primary cilia in pancreatic β-cells affect both insulin regulation and management of glucose homeostasis thereby leading to elevated rates of diabetes mellitus type 2 in ciliopathy patients.

There are dozens of published experimental research articles to explain the association between primary cilia malfunction and type 2 diabetes. In one study, BBS4 gene knock out mice were analyzed to see the connections to obesity, insulin secretion and diabetes. This specific gene was chosen because it mimics the BBS ciliopathy by mutating the genes within the primary cilia. In the mice, impaired regulation of glucose homeostasis as well as suppression of insulin secretion was observed (Gerdes et. al 2014). Young mice showed no signs or morphological abnormalities on the pancreas although some of the islets of Langerhans were larger than normal. This is expected as the mutations in the primary cilia lead to abnormal β-cells in the developing pancreas as signaling pathways are affected. The mutant mice did exhibit slower glucose clearing when compared to the wild type mice indicating early issues in glucose management. At

this point the wild type and mutant mice had similar weights, thus obesity is shown to follow impairments to glucose homeostasis (Gerdes et. al 2014). To investigate the role of primary cilia within the β-cells, short hairpin RNA's were created target BBS4 and Ofd1 (Oral-facial-digital syndrome 1). The deletion of Ofd1 results in a loss of primary cilia, while BBS only impairs the function of the primary cilia (Gerdes et. al 2014).

In this study, it was found that the insulin receptor localizes specifically to the primary cilia in the β-cells. This motion is essential for proper insulin secretion and signaling (Gerdes et. al 2014). The study was able to establish a link between defects in the primary cilia and pre-diabetic conditions in mutant mice. The researchers were further able to prove the link between diabetes and primary cilia dysfunction using diabetic rats. The rats showed non-ciliated β-cells as well as significantly shorter primary cilia when compared to non-diabetic mice (Gerdes et. al 2014). This study established the connections between primary cilia dysfunction/mutation and insulin secretion as well as diabetes.

A similar study focuses on the association of decreased expression of the primary cilia genes to diabetes in both mice and humans. This study examines the effects of primary cilia dysfunction on the proliferation and replication of β-cells. New Zealand Obese mice were utilized in experiments because of the resemblance to obesity and type 2 diabetes in humans (Kluth et. al 2019). The researchers induced β-cell apoptosis and hyperglycemia in these mice to analyze the effects on glucose clearance. The effects of ciliary mutations were examined in the obese mice and islet cells from human tissue (diabetic donors). Through measuring insulin responses in fasted versus fed states of glucose intake, a strong association between lack of primary cilia and decreased

proliferation of the β-cells. The researchers concluded that genetic mutation in the primary cilia of pancreatic β-cells can predetermine diabetes susceptibility (Kluth et. al 2019).

Treatments

As expressed, diabetes is an incurable disease, however it can be managed through medication and lifestyle changes. Those diagnosed with diabetes mellitus need to monitor their diets, especially controlling sugar intake. Exercise and healthy eating can help mitigate some of the phenotypes associated with diabetes and decrease severity. In people at high risk of developing diabetes (pre-diabetics), leading a healthier lifestyle can help prevent disease development. Diabetics who cannot produce insulin (typically type 1 diabetics) are insulin-dependent. These patients will need to carefully monitor their blood glucose levels and administer insulin if levels are too high. It is highly important that blood glucose is monitored in diabetics because hyperglycemia and hypoglycemia can lead to severe complications.

Similarly, there are no cures for ciliopathies due to the genetic component. These diseases can also be managed by the patients but are incurable. There is still plenty of research to be done in regard to treating ciliopathies, but for now, options for treatment of the diseases are limited. Patients with BBS or ALS may need to follow the treatment guidelines for diabetes once insulin secretion and β-cell proliferation is severely impaired. In ALS patients this will occur in early childhood but is later in BBS patients. Currently, next-generation sequencing is used to aid the diagnoses of BBS, a panel is conducted of all the BBS genes within the BBSome (Kenny et. al 2017). For ALS

diagnosis, genetic testing for characteristic mutations of the ALSM1 gene are performed (Paisley and Leeson-Beevers 2016).

Currently, treatments of ciliopathies are becoming more individualized and patient-centered rather than an all encompassing treatment. Due to the specialized nature of primary cilia in ciliopathies, this organelle is a prime target in treatment research. Thus, primary cilium is a target in genetic therapies. Gene therapy consists of editing the mutates gene by splice-correcting and read-through techniques (Kenny et. al 2017). This approach is favored for diseases effecting multiple organ systems and which are affected by a multitude of mutations and environmental factors such as cancer because every patient exhibits a unique 'fingerprint' of the disease presentation. In BBS, many of the presenting phenotypes are treated separately such as diabetes, cardiovascular risk and renal failure risk (Kenny et. al 2017). BBS begins developing in-utero as a developing fetus has already acquired mutations in their primary cilia. Thus, gene therapy techniques could potentially be used to edit the genes in germ-line (sperm/eggs) before the fetus begins to develop (Kenny et. al 2017). However, current research focuses on the editing of somatic cells to avoid controversy and ethical concerns. To edit mutated genes within the body, vectors are uses which have the desired (corrected) gene embedded. These vectors can be entered into organs and fuse with cells to begin editing the genome. There has been success within the retina of the eye in animal models which gives hope for usage in humans. Rod-cone dystrophy is a debilitating phenotype in BBS, with little current treatment. Gene therapy gives hope for treating this condition in the future (Kenny et. al 2017). Research is also being conducted on the potential benefits of using CRISPR/Cas9 gene editing strategies to edit the mutations within ciliopathies.

Additionally, there are no current drug-treatments for ciliopathies, but as scientific technology develops, there could eventually be pharmaceuticals which are able to restore primary cilia presence/function (Kenny et. al 2017).

Many of the issues in generalizing a treatment for BBS is also present in ALS treatment. ALS is an extremely rare disease but even these patients show variability making treatment difficult. Moreover, the multisystem effects and genetic components make treatment options limited as medical technology has not been able to overcome those limitations yet. One major difference in the treatment research between BBS and ALS is dependent on the much larger size of the causative gene of ALS, ALMS1. Due the large size and many effects of the gene it is suspected that gene editing and other gene therapies would be impossible. Researchers are instead focusing on downstream components of the ALSM1 gene for intervention and possible therapeutic treatments (Paisley and Leeson-Beevers 2016). ALS treatments mirror BBS in the fact that they tend to be individualized and conditionally based. For example, patients cardiac and diabetic symptoms will require separate medications and management techniques. For impaired glucose tolerance, Metformin may be prescribed followed by incretin analogs (Paisley and Leeson-Beevers 2016).. However, the multidisciplinary physicians involved in ALS treatment must communicate thoroughly to avoid drug interactions or exacerbation of one condition at the expense of treating another.

New Findings in Ciliopathy Research

As far as current ciliopathy research beyond what has already been discussed, new components to ciliary function and regulation are still being investigated. In addition, new avenues for treatment continue to be explored. It is highly likely that the

primary cilia will continue surprise scientific researchers for decades more. A critical role of primary cilia in glucose homeostasis was discovered very recently, in 2020. In this study, mice were engineered to lack primary cilia in the pancreatic β-cells. This deletion uncovered functions of primary cilia in glucose sensing, calcium influx and communication between the various cell types within the pancreatic islets (Hughes et. al 2020). The glucose sensing triggers an influx of calcium into the cells of the pancreas resulting in the secretion of insulin. The presence of insulin allows for other somatic cells to uptake glucose for usage in homeostasis. Thus, primary cilia are essential for feedback mechanisms in glucose metabolism and for proper utilization of insulin. The study also notes that primary cilia use G-protein coupled receptors for hormone signaling and chemoreception. Moreover, the cells use IFT88 in the intraflagellar transport complex to assemble primary cilia, the loss of this component results in absence of primary cilia. This loss was found to lead to polycystic kidney disease and may result in some of the phenotypes featured in ALS and BBS (Hughes et. al 2020).

Research into he exact mechanisms of primary cilia function and the effects of mutations in primary cilia are still on going. However, even without a complete understanding of the inner workings of primary cilia, researchers are still able to make great headway into uncovering the relationships between diseases of the pancreas and the kidneys to primary cilia malfunction or absence. It was not until as recently as 2006 that researchers connected primary cilia absence to the formation of pancreatic cysts and pancreatitis. The researchers who discovered this link did so by inactivating the *Kif3a* gene which lays a crucial role in formation of primary cilia. These knock-out mice showed severe pancreatic abnormalities such as acinar-to-ductal metaplasia, fibrosis, and

lipomatosis (Cano et. al 2006). The deletion of this gene suggests that pancreatic lesions are caused by absence of the pancreatic primary cilia (Cano et. al 2006). Uncovering this connection of a pancreatic phenotype and primary cilia mutation allows for further researching genetic therapies as treatment.

Current research at Stanford University is investigating G-protein coupled receptors (GPCR) located on the primary cilia (Wu et. al 2020). It is important to note that this paper is still in the peer-review stage and has not been published in journals yet. Theses receptors may help with regulation and secretion of glucagon and insulin from α- and β-cells, respectively. This discovery is instrumental because of the ability of drugs to target GPCR within the body, this avenue could be explored for diabetic treatment. The researchers screened GPCR located on primary cilia within the pancreas and found that those localized to α-, and β-cells do regulate insulin and glucagon secretion using signaling components (Wu et. al 2020). An elevation of cAMP levels within the cells promotes a signaling cascade in conjunction with the GPCR which will then lead to secretion of insulin or glucagon depending on the glucose levels within the body (Wu et. al 2020). It is important to note while this discovery could be monumental in terms of treating ciliopathic effects on insulin secretion and diabetes development, the research needs to first be verified within the scientific community.

Conclusions and Future Directions

Diabetes mellitus is a debilitating disease afflicting over 400 million people worldwide and is especially devastating to the growing elderly population. Primary cilia have been shown in numerous studies to affect insulin secretion and glucose homeostasis in pancreatic islet cells. Mutations of the primary cilium in cells leads to the development

of ciliopathies such as Bardet–Biedl Syndrome and Alstrom's syndrome. Understanding how defects in the primary cilia leads to these ciliopathies and the progression of diabetes is paramount for furthering our understanding of diabetes mellitus as a whole. Further research in this field will enable breakthroughs in treatment for diabetes. By discovering new treatments for diabetes, more people will be able to survive the disease without any severe complications such as neuropathy.

The field of ciliopathies is relatively new and rabidly growing. Most of the key research investigations and references in literature have been done within the last two decades. Clearly there is much more to discovered about the previously thought to be vestigial primary cilia. While ciliopathies affect multiple organ systems, those effecting the pancreas are of particular interest. The α- and β-cells within the pancreas are responsible for the secretion of insulin and glucagon. These hormones are paramount to glucose digestion and usage as an energy resource. Lacking this process would be fatal without medical intervention. More research should be conducted in the field to help not only rare diseases such as BBS and ALS but the hundreds of millions of people worldwide suffering from diabetes and diabetes related complications. Once the function and mechanisms involved with primary cilia signaling in glucose homeostasis is fully understood then this understanding can be applied to medical interventions. For example, could transplants of ciliated pancreatic tissue help improve β-cell proliferation of healthy cells? Could gene therapies be used effectively to treat humans lacking primary cilia? Questions like these need to be addressed in order to improve medical treatment of those lacking ciliopathies.

Moreover, research needs to be done comparing non-ciliopathy related diabetics to diabetics with ciliopathic conditions. This comparison is important in determining if impairments in primary cilia effect the diabetic community or a select few with co-morbid conditions. However, if primary cilia presence enables proper insulin secretion and β-cell proliferation then one could reasonably expect that improvements to primary cilia function in non-ciliopathic diabetics could have positive outcomes. Overall, scientists have just begun to uncover many of the mysteries of primary cilia. Within the next few decades, monumental advances will be made within this field and subsequent medicinal applications.

References

Bush, Andrew. 2011. DIAGNOSIS AND MANAGEMENT OF CILIOPATHIES. *Paediatric Respiratory Reviews*.

Cano DA, Sekine S, Hebrok M. 2006. Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. *Gastroenterology.* 2006 Dec;131(6):1856-69. doi: 10.1053/j.gastro.2006.10.050. Epub 2006 Oct 26. PMID: 17123526.

Columbia Surgery. The Pancreas and Its Functions. The Pancreas Center. Diagnosis, Prevention and Treatment for All Pancreatic Conditions. Accessed: 3/19/2021

da Fonseca ACP, Mastronardi C, Johar A, Arcos-Burgos M, Paz-Filho G. Genetics of non-syndromic childhood obesity and the use of high-throughput DNA sequencing technologies. *J Diabetes Complications.* 2017 Oct;31(10):1549-1561. doi: 10.1016/j.jdiacomp.2017.04.026. Epub 2017 Jun 16. PMID: 28735903.

Davenport, James R., and Bradley K. Yoder. 2005. An Incredible Decade for the Primary Cilium: a Look at a Once-Forgotten Organelle. *American Journal of Physiology-Renal Physiology*, vol. 289, no. 6, doi:10.1152/ajprenal.00118.2005.

diIorio P, Rittenhouse AR, Bortell R, Jurczyk A. Role of cilia in normal pancreas function and in diseased states. *Birth Defects Res C Embryo Today*. 2014 Jun;102(2):126-38.

Gerdes JM, Christou-Savina S, Xiong Y, Moede T, Moruzzi N, Karlsson-Edlund P, Leibiger B, Leibiger IB, Östenson CG, Beales PL, Berggren PO. Ciliary dysfunction impairs beta-cell insulin secretion and promotes development of type 2 diabetes in rodents. Nat Commun. 2014 Nov 6;5:5308. doi: 10.1038/ncomms6308. PMID: 25374274.

Hughes, Jing W. Cho, Jung Hoon. Conway, Hannah E. DiGruccio, Michael R. Ng, Xue Wen. Roseman, Henry F. Abreu, Damien. Uran, Fumihiko. Piston, David W.Piston. 2020.Primary cilia control glucose homeostasis via islet paracrine interactions. *Proceedings of the National Academy of Sciences.* (16) 8912-8923; DOI:10.1073/pnas.2001936117

Kenny J, Forsythe E, Beales P, Bacchelli C. Toward personalized medicine in Bardet-Biedl syndrome. *Per Med.* 2017 Sep;14(5):447-456. doi: 10.2217/pme-2017-0019. Epub 2017 Sep 4. PMID: 29754569.

Kluth O, Stadion M, Gottmann P, Aga H, Jähnert M, Scherneck S, Vogel H, Krus U, Seelig A, Ling C, Gerdes J, Schürmann A. Decreased Expression of Cilia Genes in Pancreatic Islets as a Risk Factor for Type 2 Diabetes in Mice and Humans. Cell

Rep. 2019 Mar 12;26(11):3027-3036.e3. doi: 10.1016/j.celrep.2019.02.056. PMID: 30865891.

Landsman L, Parent A, Hebrok M. 2011. Elevated Hedgehog/Gli signaling causes beta-cell dedifferentiation in mice. *Proc Natl Acad Sci* USA 108:17010–17015

Lee, Ji Eun, and Joseph G Gleeson. 2011. A Systems-Biology Approach to Understanding the Ciliopathy Disorders. *Genome Medicine*, vol. 3, no. 9, p. 59., doi:10.1186/gm275.

Lodh S. 2019. Primary Cilium, An Unsung Hero in Maintaining Functional β-cell Population. *The Yale journal of biology and medicine*, 92(3), 471–480.

Lodh, Sukanya, et al. 2014. Primary Cilia in Pancreatic Development and Disease. *Birth Defects Research Part C: Embryo Today: Reviews*, vol. 102, no. 2, 2014, pp. 139–158., doi:10.1002/bdrc.21063.

Mouri MI, Badireddy M. Hyperglycemia. [Updated 2020 Sep 10]. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430900/

Ndisang JF, Vannacci A, Rastogi S. Insulin Resistance, Type 1 and Type 2 Diabetes, and Related Complications 2017. *J Diabetes Res*. 2017;2017:1478294. doi:10.1155/2017/1478294

R. B. Paisey & K. Leeson-Beevers (2016) Current management of Alström syndrome and recent advances in treatment, *Expert Opinion on Orphan Drugs*, 4:7, 747-759, DOI: 10.1080/21678707.2016.1189322

Volta, F. and Gerdes, J.M. 2017, The role of primary cilia in obesity and diabetes. Ann. *N.Y. Acad. Sci*., 1391: 71-84.

Volta, F., Scerbo, M.J., Seelig, A. et al. 2019. Glucose homeostasis is regulated by pancreatic β-cell cilia via endosomal EphA-processing. *Nat Commun* 10, 5686

Wu, Chien-Ting. Hilgendorf, Keren I. Becacqua, Romina J. Hang, Yan. Demeter, Janos. Kim, Seung K. Jackson, Peter K. 2020. Discovery of ciliary G protein-coupled receptors regulating pancreatic islet insulin and glucagon secretion. *bioRxiv* 2020.10.21.349423; doi: https://doi.org/10.1101/2020.10.21.349423

Xu G, Liu B, Sun Y, et al. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ*. 2018;362:k1497. Published 2018 Sep 4. doi:10.1136/bmj.k1497