Identifying Risk Factors for Cystic Fibrosis Related Diabetes

in

Children and Adolescents

By

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List of Abbreviations

ASL	Airway Surface Liquid
BMI	Body Mass Index
CF	Cystic Fibrosis
CFRD	Cystic Fibrosis Related Diabetes
CFFPR	Cystic Fibrosis Foundation Patient Registry
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
EMRS	Electronic Medical Record System
ERCF	European Epidemiologic Registry of Cystic Fibrosis
FEV1%	Percent Predicted Forced Expiratory Volume
IGT	Impaired Glucose Tolerance
IRB	Institutional Review Board
NGT	Normal Glucose Tolerance
NPH	Neutral Protamine Hagedorn
OGTT	Oral Glucose Tolerance Test
REE	Resting Energy Expenditure
T1DM	Type 1 Diabetes
T2DM	Type 2 Diabetes

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Abstract

Background

As individuals with Cystic Fibrosis (CF) live longer, they can develop complications such as Cystic Fibrosis Related Diabetes (CFRD), the most common complication seen in adults with CF. Those who develop CFRD experience accelerated decline in pulmonary function and higher rates of early mortality than those without CFRD, suggesting that CFRD is a sentinel event in that person's life. Recent evidence suggests that early recognition of CFRD and tight blood glucose control mitigates some negative impacts on health. Limited research describes risk factors that predict the development of CFRD. Proposed risk factors include use of a gastrostomy tube (g-tube) for enteral feeding, low body mass index (BMI) and lower pulmonary function. The goal of this study was to determine clinical characteristics that predict the development of impaired glucose tolerance (IGT) or CFRD among children and adolescents with CF. We anticipated that regular use of a g-tube for enteral feeding, decline in pulmonary function and decline in BMI z-scores would be associated with development of IGT and/or CFRD among children and adolescents with CF.

Methods

A cross-sectional, retrospective chart review of children followed at the Oregon Health & Science University (OHSU) CF outpatient clinic was conducted using the electronic medical record system, Epic, and the Cystic Fibrosis Foundation Patient Registry (CFFPR). Participants born between January 1, 1995

and December 31, 2011, who had at least one oral glucose tolerance test (OGTT) and whose parents consented to their child's participation in the CFFPR were included in this analysis. Those on CFTR modulator therapies and anyone who had undergone a solid organ transplant or who had complex comorbidities were not included. Participants (n=68) were categorized into one of three groups based on glucose tolerance classification at their first OGTT, routinely performed at ~10 years of age, and/or subsequent diagnosis of IGT or CFRD. Normal glucose tolerance (n=25) was indicated by a fasting blood glucose concentration of <100 mg/dl and a 2-h glucose concentration of <140 mg/dl. IGT (n=26) was indicated by a fasting blood glucose concentration of 100-125 mg/dl and/or a 2-h blood glucose concentration of 140-199 mg/dl. CFRD (n=17) was indicated by a fasting blood glucose concentration of >126 mg/dl and/or a 2-h blood glucose concentration of >200mg/dl.

Means and standard deviations, or medians and IQR were calculated for continuous variables. Regression models and hazard ratios were used to establish relationships and determine significant differences between diagnostic groups with regards to time from initial OGTT to either diagnosis of IGT or CFRD.

Results

Time to diagnosis of CFRD after the initial OGTT was significantly less among patients with a g-tube compared to those without (p=0.022). The time to CFRD diagnosis among those with a g-tube was estimated to be 3.1 (95% CI: 1.2-8.4) times sooner than for those without a g-tube.

There were no significant differences between mean weight or height z-scores among diagnostic groups. However, there was a trend towards a significant difference in BMI z-scores between diagnostic groups, such that for every one-unit decrease in BMI z-score, there was an increased risk for diagnosis of IGT or CFRD (p=0.06).

Pulmonary function, as measured by FEV1%, as well as number and severity of exacerbations, were not significantly related to a diagnosis of IGT or CFRD.

Discussion and Conclusion

Our study confirms that the presence of a g-tube for nutrition support is a significant risk factor for developing CFRD. Routine monitoring of blood glucose concentration among patients with CF who have g-tubes should be standard practice to monitor for the emergence of IGT or CFRD so that it can be managed appropriately with insulin if needed. More robust clinical markers to identify individuals with CF who are at increased risk for developing CFRD need to be identified and studied in a larger patient population, to allow earlier diagnosis of IGT and CFRD, and prompt initiation of treatment to mitigate any future complications.

Chapter 1

Introduction

Cystic Fibrosis (CF) is the most common autosomal recessive genetic disorder in Caucasians of northern European descent, with a world-wide prevalence of 1 in 2,500 live births [1]. Prior to the era of universal newborn screening in the early 2000s, children with CF presented with a spectrum of clinical signs that included chronic respiratory infections, pancreatic insufficiency and malnutrition. Most of the morbidity and mortality associated with this progressive disease is attributed to obstructive pulmonary disease, which stems from a cycle of chronic inflammation, infection of the airways, and tissue destruction leading to chronic respiratory failure [2]. Early diagnosis and improved clinical care have led to a significant increase in life expectancy. Today the median predicted survival age of individuals with CF is close to 40 years [3] which represents a dramatic improvement from the 1950s, when children with CF rarely lived long enough to attend elementary school.

As people with CF live longer, they are at risk for developing CFRD, which is the most common complication seen in adults with CF. CFRD shares features common to Type 1 (T1DM) and Type 2 diabetes mellitus (T2DM). However important differences between CFRD and T1DM or T2DM necessitate a unique approach to diagnosis and management. People with CF who develop CFRD experience an accelerated decline in health, including reduced pulmonary function, and a higher risk of premature death than those without CFRD [4].

The development of glucose dysregulation that leads to IGT and CFRD among individuals with CF is likely a result of a dysfunctional transmembrane transport protein, the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). In pancreatic beta cells, dysfunction of the CFTR protein leads to reduced insulin secretion. Although most people with CF develop exocrine pancreatic damage, not all develop glucose intolerance. Glucose intolerance develops insidiously with few or no outward symptoms apparent for years. This silent onset of glucose intolerance necessitates a screening method that can detect abnormal blood glucose regulation in at-risk patients.

CFRD is diagnosed in individuals with CF by fasting hyperglycemia (>126 mg/dL), random hyperglycemia (>200 mg/dl) or an abnormal oral glucose tolerance test (OGTT). Among individuals with CF, hemoglobin A1C (Hgb A1C) often remains within the normal range (<6.5%) in the early stages of the disease and is thus an unreliable indicator of blood glucose regulation. For this reason, Hgb A1C is not used in the diagnosis of CFRD.

There is considerable fluctuation in the degree of glucose intolerance among individuals with CF, which is associated with the degree of insulin deficiency and insulin resistance. Since there are ways to diagnose CFRD once it is established, more attention needs to be placed on early identification of this condition before cellular compromise occurs. Sensitive, predictive criteria that identify individuals with CF who are at risk for developing CFRD need to be established. In addition, appropriate interventions to prevent, delay the onset of, and reduce the severity of this insidious disease need to be developed.

There is limited research describing risk factors that predict the development of CFRD. Proposed risk factors include use of a g-tube for enteral feedings, low body mass index (BMI) and severely compromised pulmonary function. To address this gap, we completed a cross-sectional retrospective electronic medical record review as well as a review of the Cystic Fibrosis Foundation Patient Registry (CFFPR) of individuals with CF, with and without IGT or CFRD, who received ongoing care and follow-up at the Doernbecher Children's Hospital (DCH) in Portland, OR. The goal of this study was to determine clinical characteristics that predict the development of IGT or CFRD among children and adolescents with CF through the following specific aims:

1. Describe clinical characteristics of individuals with CF followed at DCH at their first OGTT

2. Assess whether g-tube feeding, BMI and FEV1% predict the development of IGT and/or CFRD

We hypothesized that among children and adolescents with CF, regular use of a g-tube for enteral feedings, lower pulmonary function and lower BMI z-scores would be associated with development of glucose intolerance and/or CFRD.

Chapter 2

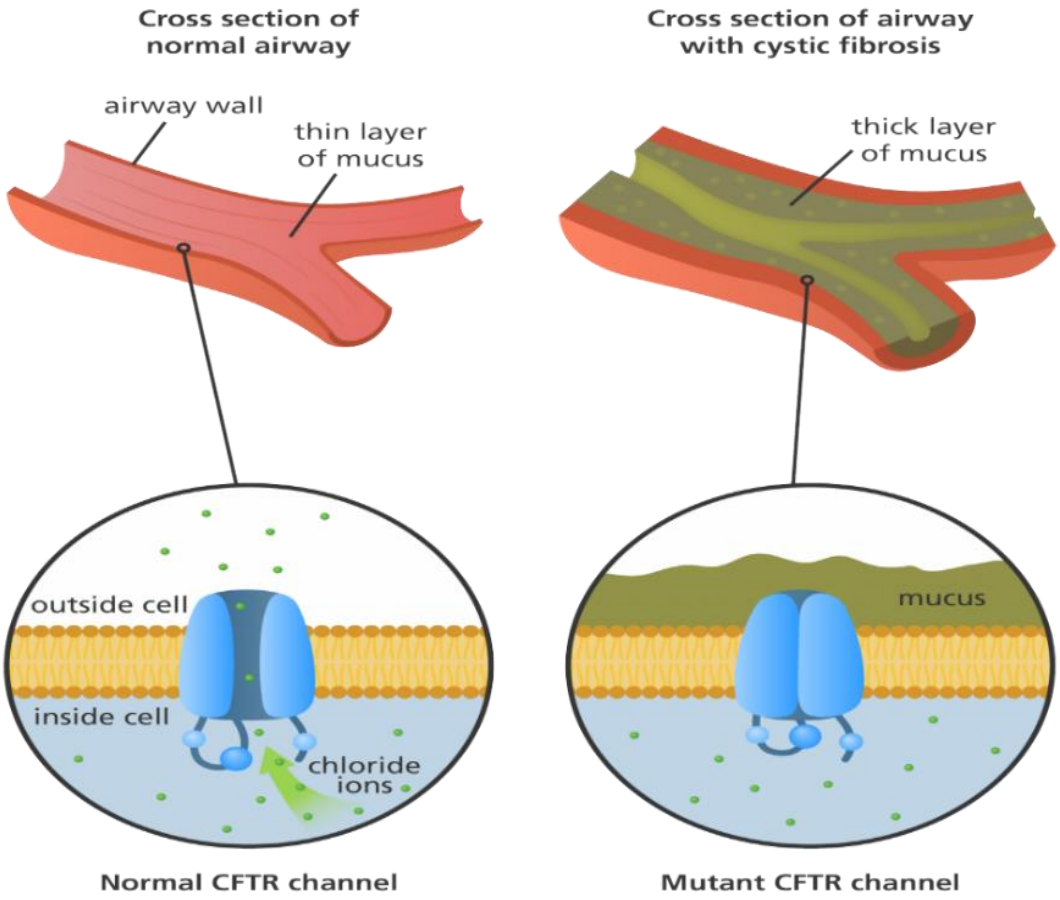
Background

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that affects individuals in nearly every ethnic group, but with higher incidence among Caucasians (about 1 in every 2,500)[2]. There are about 30,000 people in the United States living with CF, and the median lifespan is 40.7 years [5-7]. Currently, all 50 states and the District of Columbia screen newborns for CF. Every state's CF newborn screening program begins by obtaining a blood sample from the baby to measure the concentration of the pancreatic protein, immunoreactive trypsinogen (IRT). In babies who have CF, IRT concentrations tend to be high but IRT concentrations can also be high if a baby is born prematurely or experienced a stressful delivery. To confirm a diagnosis of CF, babies with a high IRT concentration or other associated clinical signs undergo a sweat test to measure chloride concentration in perspiration. A sweat chloride concentration ≥ 60 mmol/l is confirmatory of CF. Genetic testing is also performed to confirm the diagnosis and aid in determining the course of treatment and discussions of prognosis [8].

In humans, a 230-kb gene on chromosome 7 encodes the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein. CFTR is a cyclic AMP regulated transmembrane glycoprotein whose primary role is to transport chloride ions into and out of cells (Figure 1). When the CFTR regulatory domain is phosphorylated and the nucleotide binding domains bind and hydrolyze ATP,

chloride is transported through the protein channel. In sweat glands, CFTR reabsorbs sodium and chloride ions through the ductal lumen. In individuals with CF, mutations of the CFTR gene result in proteins with reduced chloride transport capability. Disruption in the flow of chloride ions and water across apical membranes of epithelial cells leads to altered fluid and electrolyte composition and increased viscosity of luminal secretions. These abnormally viscous secretions are responsible for the progressive obstruction of tubules, ducts, and airways of individuals with CF [9,10].

Figure 1: CFTR Protein Transport of Chloride Ions and Water Across Cellular Membranes in Individuals with and without CF[3]



The Impact of CF in the Lungs

In the lungs, external surfaces of airways are coated with a thin, sodium chloride and antibody-containing film called airway surface liquid (ASL). ASL is in turn coated with a thin layer of mucous. These protective layers trap bacteria and other particles, and cilia, extending from airway epithelial cells into the ASL, move particles out of the lungs in a process called mucociliary clearance. Normally,

CFTR and sodium channels in apical membranes of epithelial cells maintain the volume and viscosity of the ASL, allowing cilia to move freely.

In individuals with CF, chloride transport is impaired and epithelial sodium channels absorb instead of secrete sodium. In combination, these impairments deplete ASL volume and alter its consistency preventing mucociliary clearance. As a result, the mucous layer dehydrates and thickens, airways become obstructed, and bacterial colonization occurs more easily, leading to the chronic inflammation and respiratory infections observed in CF patients [5,6,11].

Pulmonary exacerbations, or episodes of acute worsening of symptoms among individuals with CF such as infections, allergies, and sinusitis or sore throat, are associated with weight loss related to decreased appetite paired with increased energy expenditure from infection[12]. This also contributes to an increased work of breathing, and coughing[12,13]. As a result, resting energy expenditure (REE) may be increased in individuals with CF due to upregulated energy-requiring processes in CFTR regulation[14]. It is thought that disease progression and decreased pulmonary function are inversely related to energy needs among individuals with CF [6,15] .

Under normal circumstances the CFTR protein also binds certain strains of pathogens, such as *Pseudomonas aeruginosa (P. aeruginosa)*, and contributes to the autophagic internalization and subsequent death of the bacterium and epithelial cell. The altered CFTR protein cannot bind pathogens, leaving bacteria free to multiply in the lumen of the airway of individuals with CF increasing their risk for pneumonia and other pulmonary infections [6].

The Impact of CF in the Pancreas

In the pancreas, the CFTR protein is located in the apical membrane of epithelial cells that line proximal pancreatic ducts. When the CFTR protein is not correctly made, as in individuals with CF, pancreatic epithelial cells cannot appropriately secrete chloride and bicarbonate ions. Since these anions are the drawing force for water, the rate of flow of thickened luminal pancreatic secretions decreases, ultimately leading to ductal obstruction and pancreatic dysfunction [7,16].

As pancreatic disease in individuals with CF advances, pancreatic cells, both acinar and islets cells, are replaced with adipose tissue, eventually destroying the pancreas [17]. In addition, mucous blocks the intrahepatic bile ducts, inhibiting appropriate metabolism of fat due to impaired bile salt delivery [18]. Endocrine functions of the pancreas are also impaired in individuals with CF, which leads initially to glucose intolerance, and eventually to CFRD. As pancreatic beta cells are destroyed due to fibrosis and inflammation, insulin insufficiency occurs. Lack of insulin leads not only to impaired glucose uptake by cells, but also to malnutrition. Because insulin is an anabolic hormone, unintentional loss of both lean body mass and adipose tissue may occur without appropriate diagnosis and insulin treatment [19]. Over time, pancreatic destruction and fibrosis lead to amyloid and fat deposition, and the subsequent development of CFRD [20,21].

Development of Cystic Fibrosis Related Diabetes

As the efficacy of pulmonary and nutritional care for individuals with CF has improved, the median age of individuals with CF has dramatically increased. Concurrently, the prevalence of comorbid conditions associated with CF has increased, especially those alterations associated with glucose metabolism. There is an age-related increase in the prevalence of individuals with CF who are at risk for developing impaired glucose tolerance (IGT) and CFRD [22]. CFRD is the leading comorbidity reported in the Cystic Fibrosis Foundation Patient Registry (CFFPR) and more than 30% of individuals with CF develop CFRD by 30 years of age [23]. CFRD is associated with increased premature mortality rates, accelerated decline in lung function, and nutrition-related health issues. The acceleration of pulmonary dysfunction in individuals with CFRD is proportional to the severity of glucose intolerance, but the mechanisms remain unclear. Some proposed contributors to CFRD development include insulin deficiency related to the dysfunctional CFTR protein and its role in destruction of pancreatic cells, malnutrition, acute and chronic infection, elevated energy expenditure, intestinal malabsorption, abnormal intestinal transit time, liver dysfunction, and increased work of breathing [22,24-27].

CFTR protein regulates the function of chloride channels on the apical membrane of epithelial cells and helps regulate trans-epithelial transport of other ions and water. The grouping of mutations into functional categories is as follows:

Class I, no synthesis of CFTR; class II, degradation of CFTR in the endoplasmic reticulum; class III, transport of CFTR to the cell membrane, no appropriate response; class IV, diminished action of CFTR in the cell membrane; and class V, normal but inadequate amounts of CFTR[28]. Genotype may predispose an individual with CF to diabetes via pancreatic dysfunction or may play a more direct role. CFRD results more from decreased beta cell function than decreased insulin sensitivity. The F508 deletion (F508del) is categorized as a Class II mutation and is the most common mutation in CF patients.

The Primary Problem in CFRD

The primary defect in CFRD is severe, but not absolute, insulin deficiency. All individuals with CF, regardless of the presence of CFRD, show evidence of pancreatic beta cell dysfunction. Pancreatic beta cells are responsible for the synthesis, storage and release of insulin into the proximal ducts of the pancreas. The time to peak insulin secretion among individuals with CFRD is typically 90-120 minutes after consuming a standard glucose load as part of an oral glucose tolerance test (OGTT). In comparison, the time to peak insulin secretion among healthy individuals is between 30-60 minutes. The delay in insulin secretion is related to loss of first-phase insulin secretion, which is observed even in individuals with CF with normal glucose tolerance. Insulin deficiency associated with CFRD results in both decreased peripheral glucose uptake and reduced

insulin suppression of hepatic glucose production [29]. This reduced insulin secretion is related to a reduction in beta-cell mass of Langerhans islet cells and dysfunction of the remaining exocrine cells due to the CFTR mutation. Investigators report a significant reduction in the surface area of insulin staining cells in islets of individuals with CFRD compared to both nondiabetic individuals with CF and to individuals without CF [(18)].

Impact of CFRD on Pulmonary Function

The mechanisms underlying pre-diabetic and diabetic effects on lung function in individuals with CF are poorly understood. However, the rate of pulmonary function decline is directly proportional to the severity of glucose intolerance and increased plasma glucose concentrations [30]. Researchers in London hypothesized that airway glucose concentrations would be elevated in individuals with CF when blood glucose concentrations exceeded ≥8 mmol/L, the airway threshold measured by continuous glucose monitoring [13]. Brennan et al concluded that individuals with CF with glucose in nasal secretions had significantly higher blood glucose concentrations than people without glucose in nasal secretions. Elevated glucose concentrations in nasal secretions, in turn, indicates that an active glucose transport process is involved in removal of glucose from the airway lumen.

In a cross-sectional analysis of 7,566 people enrolled in the European Epidemiologic Registry of CF (ERCF), percent predicted forced expiratory volume (FEV1%) was measured to assess airway obstruction [4,11]. Across all ages, FEV1% was lower in individuals with CFRD (52 ± 12%) compared to those with CF but without CFRD (72 ± 10%). Additionally, FEV1% and body weight were lower among individuals with CFRD than those without[4]. Hyperglycemia, which is associated with structural changes in lung tissue, may account for the development of abnormal pulmonary function in people with diabetes. Hyperglycemia may cause pulmonary damage but the mechanism is not purely understood.

Differences between CFRD and Type 1 and Type 2 Diabetes

While CFRD shares features of both Type 1 (T1DM) and Type 2 (T2DM) diabetes, there are important differences which necessitate a unique approach to diagnosis and management among individuals with CF (Table 1) [1]. Type 1 diabetes is characterized by the absence of insulin due to autoimmune destruction of pancreatic beta cells within the islets of Langerhans.[19] Type 2 diabetes is the result of relative insulin deficiency, along with increased insulin resistance, where tissues are unable to respond to insulin. The American Diabetes Association places CFRD in the category of "other specific types" of diseases of the endocrine pancreas [13]. Factors specific to CF that affect glucose metabolism include

respiratory infection and inflammation, increased energy expenditure, malnutrition, glucagon deficiency, and gastrointestinal abnormalities (malabsorption, altered gastric emptying and intestinal mobility, liver disease).

Table 1: Characteristics of Different Types of Diabetes

Characteristic	Type 1 Diabetes	Type 2 Diabetes	Cystic Fibrosis Related Diabetes
Onset	acute	insidious	insidious
Peak age at onset	children and adolescents	adults	young adults
Insulin secretion	eventually absent	decreased	severely decreased but not absent
Insulin sensitivity	normal	severely decreased	somewhat decreased
Treatment	insulin	diet, oral medications, insulin	insulin
Microvascular complications	present	present	present but less severe
Macrovascular complications	present	present	not present
Cause of death	cardiovascular disease	cardiovascular disease	pulmonary disease

Screening/Diagnosis of CFRD

CFRD is often clinically silent and hard to diagnose. As previously discussed, CFRD is associated with weight loss, protein catabolism, decreased lung function, and increased early mortality therefore regular screening for this

condition is important. Oral glucose tolerance tests are considered the “gold standard” to screen for CFRD, as Hgb A1C values are considered unreliable [22]. Individuals are usually divided into glucose tolerance groups based on their fasting and 2-hour blood glucose concentrations as outlined in Table 2[31 32].

According to the Cystic Fibrosis Foundation, annual screening for CFRD should begin at 10 years of age among individuals with CF. An OGTT should be performed in the morning during a period of stable baseline health at least six 6 weeks after any acute exacerbation [2,22,24,33]. Individuals should fast for a minimum of 8 hours and should consume no more than 150 grams (g) of carbohydrate per day during the 3 days preceding the screening. The patient should drink a standard aqueous solution containing 1.75g of glucose per kilogram (kg) of body weight, not to exceed 75g. Blood glucose concentrations are measured immediately before and 2 hours after ingestion of the glucose-containing solution. An OGTT should be performed on two separate occasions before confirming a diagnosis of CFRD. A diagnosis of CFRD is made when either fasting blood glucose or 2-hour post load glucose concentrations are high or both concentrations are high as summarized in Table 2.

Table 2: Diagnostic Criteria for Normal & Impaired Glucose Tolerance and CFRD

	Fasting blood glucose concentration		2-hour post load blood glucose concentration	
Category	mmol/l	mg/dl	mmol/l	mg/dl
Normal glucose tolerance	<5.6	<100	<7.8	<140
Impaired glucose tolerance	>5.6	100-125	7.8-11	140-199
CFRD	>7.0	≥126	≥11.1	≥200

In a prospective Danish study of 191 individuals with CF, two thirds of those with CFRD were asymptomatic and only one third had symptoms of polyuria and polydipsia at the time of the diagnosis [34]. Individuals with CF who develop overt symptoms of hyperglycemia have lower pulmonary function and greater weight loss compared with those identified through OGTT screening but who are asymptomatic [14,16,35]. Therefore, it is important to try to identify individuals with abnormal glucose tolerance before the onset of symptoms.

Management of CFRD

Multiple guidelines have been published concerning the diagnosis and management of CFRD among individuals with CF including those published by the American Diabetes Association, Cystic Fibrosis Foundation, International Society

for Pediatric and Adolescent Diabetes and Pediatric Endocrine society[12]. These organizations recommend that CFRD should be managed by a multidisciplinary team of health professionals with expertise in CF and diabetes [22].

Treatment of CFRD may differ from the treatment of diabetes in the general population. Among people with CFRD, there are additional unique factors to be considered when deciding the optimal therapeutic approach and the time to initiate treatment. Insulin resistance or decreased insulin secretion can lead to tissue level malnutrition with poor glucose uptake leading to circulating hyperglycemia. One of the hallmarks of the onset of CFRD is rapid weight loss. Malnutrition-related weight loss is associated with poor growth, pubertal delay, diminished lung function and early death in CF[22]. CF consensus guidelines stress the importance of consuming a high-calorie, high-fat diet to prevent malnutrition, but the role of diet in managing CFRD has not been extensively investigated[18,22]. Once CFRD is present, use of insulin is the only recommended medical therapy.

Medical Nutrition Therapy for CFRD

Decline in nutritional status has been shown to occur up to four years prior to diagnosis of CFRD and has been correlated with the degree of insulin deficiency at baseline and a corresponding decline in pulmonary function and increase in morbidity and mortality [14]. Malnutrition is associated with poor growth, pubertal delay, diminished lung function and early death in those with CF.

Many individuals presenting with CFRD are at risk for nutritional decline due to elevated resting energy expenditure and malabsorption[5,15,18,22,33,36-38]. Individuals with CFRD should consume the recommended high energy CF diet as described in Table 3. Reduction in carbohydrate intake and avoidance of high glycemic index foods is not recommended for participants with CFRD due to the risk of limiting energy intake and further impairing nutritional status[38].

Table 3: Dietary Management of Different Types of Diabetes

Nutrient category	Type 1 and Type 2 Diabetes	CFRD
Energy	As needed for growth, maintenance	1.2-1.5 times DRI for age; individualized based on weight gain and growth
Fat	Limit saturated fat to <7% of total calories; intake of trans fat should be minimized; consume 2 or more servings per week of fish high in n-3 polyunsaturated fatty acids	No restriction on type of fat. High fat necessary for weight maintenance. Aim for 35-40% total calories
Refined sugars	up to 10% total energy	no restriction
Carbohydrate	Individualized. Monitor carbohydrates to achieve glycemic control; choose from fruits, vegetables, whole grains, and fiber containing foods, legumes, and low-fat milk. Sugar alcohols and nonnutritive sweeteners are safe within USDA consumption guidelines	Individualized. Carbohydrates should be monitored to achieve glycemic control. Artificial sweeteners should be used sparingly due to lower calorie content
Dietary fiber	No quantitative recommendation but encouraged	Encouraged in the well-nourished, but in poorly nourished individuals, it may compromise energy intake
Protein	15-20% of total calories; reduction to 0.8-1.0g/kg with nephropathy	Approximately 1.5-2.0 times the DRI for age
Salt	<2300 mg/day for blood pressure control	Liberal, high salt diet, especially in warm conditions and/or when exercising
Vitamins & minerals	No supplementation necessary unless deficiency noted	Routine supplementation with CF-specific multivitamins and additional fat-soluble vitamins A, D, E, and K
Alcohol	If consumed, limit to a moderate amount; one drink per day for women and two or less for men	Consult with physician because of the higher prevalence of liver disease in CF and possible use of hepatoxic drugs

Table 3. Continued

Nutrient category	Type 1 and Type 2 Diabetes	CFRD
Special Circumstances:		
Gestational Diabetes	Restricted calories/carbohydrate for weight loss and blood glucose control	No calorie or carbohydrate restriction; adequate kcals for weight gain
Impaired glucose tolerance	Weight loss of 5-10% recommended; low-fat diet	No weight loss. Spread carbohydrates throughout the day; consume nutrient-dense beverages

Insulin Therapy for Treatment of Individuals with CFRD

Individuals with CFRD and clinical and/or fasting hyperglycemia should undergo insulin therapy, which is currently the only recommended treatment for this condition[22]. Insulin therapy has been shown to improve lung function, promote weight gain, and reduce frequency of pulmonary exacerbations [39]. There is little evidence regarding the superiority of specific insulin regimens in CFRD, thus, clinical judgement should be used to choose the best regimen for each patient. The CF Foundation recommends multiple daily injections of short-acting insulin before each meal and a small dose of long acting insulin at night[22]. The use of short acting insulin provides flexibility required by the CF diet, allowing the insulin dose to be adjusted to the carbohydrate content of each meal and additional boluses to be given for snacks or night feedings [4]. Little research has been done to determine if a particular type of insulin works better with individuals with CFRD. A study compared Glargine and Neutral Protamine

Hagedorn (NPH) insulin and found significantly greater reduction in fasting plasma glucose of 8±2 mg/dl (p=0.03 with Glargine; however, the study was not blinded and was limited by short duration of 12 weeks [40].

Insulin Therapy for Enteral Feeding of Individuals with CFRD

Diagnosis of CFRD was four times as likely in individuals with CF who were prescribed overnight enteral feeding than those not receiving night time enteral feedings[41]. Those receiving night time feedings had an increased rate of decline in FEV1 which stabilized when they started on insulin [24,42]. Proper insulin administration should be based on the amount of total carbohydrate in the enteral feeding. A combination of Neutral Protamine Hagedorn (NPH) insulin and short acting insulin should be given in a mixed dose at the beginning of the feed[41]. Typically doses of insulin are based on the individual's known carbohydrate ratio and should be given as 70% NPH and 30% short acting insulin. Insulin administered to support night-time enteral feeding should be given in addition to whatever insulin (including basal) the individual is taking throughout the day. Blood glucose goals for individuals receiving continuous enteral feeds should fall between 140-180 mg/dL. Daytime bolus enteral feeds are covered as if the individual were consuming a meal.

Oral Therapeutic Agents to Control Glucose Concentrations in Individuals with CF and CFRD

Use of oral diabetes agents is currently not recommended for individuals with CF and CFRD[22,34,40]. Sulphonylureas enhance insulin secretion by acting on the sulphonylurea receptor in pancreatic beta cells stimulating insulin release, and therefore may be useful in people with CFRD who have residual beta cell function. Concern has been expressed over the use of sulphonylureas in individuals with CFRD due to evidence that it binds to and inhibits CFTR, leading to hypoglycemic issues. Oral agents that increase insulin sensitivity are unlikely to be effective in CFRD, since insulin resistance is not a major etiological factor for this condition[1,43,44].

Proposed risk factors

Compared with other types of diabetes, the risk factors for CFRD are less well characterized; it is not entirely clear which factors differentiate individuals with CF who will, or will not, develop diabetes. Whereas specific genetic factors are associated with an increase prevalence of CFRD[24,45], we do not know whether they do so independently of other identified risk factors. Most observational studies of CFRD were cross-sectional[24,27,45,46] and a few prospective studies exist[47,48]. The most common risk factors identified include sex, increased age, poor pulmonary function, malnutrition, enteral feeding and CFTR genotype.

White et al, after matching 48 adult patients with CFRD to 48 age and sex matched CF controls, observed patients with CFRD were four times as likely to have overnight enteral tube feeding as the controls (p=0.01)[41]. Knudsen et al found no differences when comparing BMI z-scores between patients with and without CFRD (p=0.09)[49]. While De Boer et al concluded in a prospective study of 446 CF patients, that patients with frequent exacerbations were more likely to be female and diabetic and to have poorer baseline lung function[50]. In the current study, we explored the relationships between pulmonary function, BMI z-score and enteral feeding and development of CFRD so that we may be able to modify these risk factors early and reduce risk of diagnosis of CFRD in the future.

Chapter 3

Methods

General Design and Sample Selection

A cross-sectional, retrospective electronic medical record review of existing data of patients followed in the Cystic Fibrosis Clinic at DCH was conducted. This study was reviewed and approved by OHSU Institutional Review Board before data was abstracted from the medical records or the CFFPR.

Children and adolescents born between January 1, 1995 and December 31, 2011 who were followed at the OHSU CF Center were included in this study. Other inclusion and exclusion criterion are listed in Table 4.

Table 4: Inclusion/Exclusion Criteria

Inclusion Criteria	• Patient with CF born between January 1, 1995- December 31, 2011 • Followed at the OHSU CF Center • History of having had an OGTT • Parental consent in CFFPR
Exclusion Criteria	• No history of having had an OGTT • Treated with lumacaftor/ivacaftor • History of any organ transplant

Data Collection

Demographic (sex, ethnicity, genotype, age at CF diagnosis, age at diagnosis of IGT or CFRD, and presence of a g-tube at initial OGTT), anthropometric (weight, height, BMI), pulmonary function (FEV1% and number and severity of exacerbations), and OGTT data were collected. Anthropometric data was obtained through the CFFPR to make sure data was consistent and included measurements from all medical facilities through which the patient received care.

Participants were divided into one of three groups based on glucose tolerance status assessed at their initial OGTT as defined in Table 2 (normal glucose tolerance, IGT, or CFRD), or a subsequent diagnosis of IGT or CFRD. If a patient was ever diagnosed with CFRD, either at their initial OGTT or at a subsequent OGTT assessment, they were classified as CFRD. Patients who were initially classified as IGT and then developed CFRD were allocated to the CFRD group. Patients who were initially classified as CFRD but whose condition improved to IGT were also allocated to the CFRD group.

Determination of Glucose Intolerance

Individuals with CF complete an OGTT each year, starting at about 10 years of age, as part of the standard of care at DCH unless already diagnosed with

CFRD. The OGTT process includes drawing a fasting blood sample to determine the fasting blood glucose concentration. A glucose solution is then administered based on the individual's weight per manufacturer's instructions. Individuals who weigh less than 42kg are provided with a measured volume of a standard glucose-containing beverage that provides 1.75 grams of glucose per kilogram (kg) of body weight; those who weigh 42kg or more are provided with a measured volume of the standard glucose containing beverage that provides 75g of glucose (GlucoCrush™ Cardinal Health, Dublin, Ohio). OGTT results and glucose tolerance classification were standardized based on ADA guidelines established in 2014 (see Table 2). Glucose tolerance classification was established by the pediatric endocrinologist who follows all patients in the DCH CF clinic.

Calculations and Statistical Analysis

Characteristics of the study sample including demographic, anthropometric, glucose tolerance, and pulmonary function data were summarized using descriptive statistics. Z-scores for anthropometric data were calculated using the World Health Organization (WHO) reference data based on the child's sex and age. Means and standard deviations, or medians and interquartile range (IQR), were calculated for continuous variables. Regression models and hazard ratios were used to establish relationships and determine significant differences between groups with regards to time from initial OGTT to

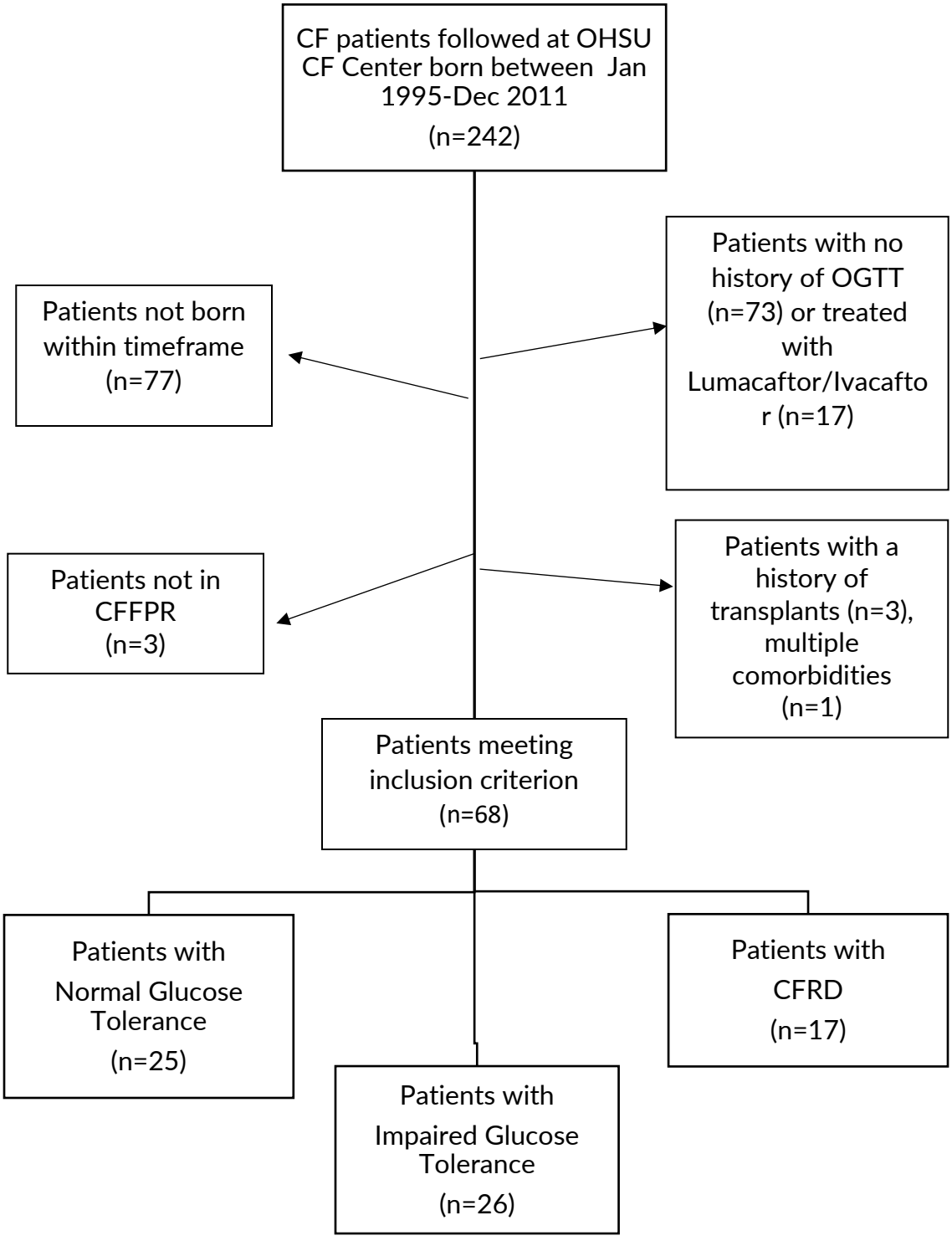
either IGT or CFRD diagnosis. Kaplan-Meier curves and accompanying estimates of cumulative incidence of either IGT or CFRD were computed and compared between groups using the Peto-Peto test. Kaplan Meier curves were also used to estimate the time required for a given proportion of the sample to reach a diagnosis of IGT or CFRD. Categorical variables were summarized using frequencies and percentages and compared between groups using chi-square tests or Fisher's exact test. Stata (StataCorp, College Station, Texas) was used for all analyses and differences between groups were considered significant at $p \leq 0.05$.

Chapter 4

Results

Medical records of 242 pediatric patients followed in the OHSU CF Clinic were available for review. Medical records of 174 patients were excluded from analysis for the following reasons: the patient was not born between January 1, 1995 and December 31, 2011 (n=77); the patient had no record of OGTT (n= 73) or was treated with Lumacaftor/Ivacaftor (n=17); the patient was not included in the CFFPR (n=3); the patient had a history of organ transplant (n=3); and a patient was excluded due to comorbid acute lymphoblastic leukemia who required frequent doses of steroid (n=1). After applying the pre-established exclusion criterion, 68 patients remained eligible for inclusion in this study. These 68 patients were categorized into normal glucose tolerance (NGT, n=25) impaired glucose tolerance (IGT, n=26) or CFRD (n=17) diagnostic groups based on their initial OGTT results or subsequent diagnosis of IGT or CFRD. A summary of included and excluded patients is presented in Figure 2.

Figure 2: Distribution of CF sample into Normal Glucose Tolerance (NGT), Impaired Glucose Tolerance (IGT) or CFRD diagnostic groups



Patient Characteristics

Characteristics of patients with normal or impaired glucose tolerance or CFRD are illustrated in Table 6. The total study sample consisted of 31 males (46%) and 37 females (54%). Based on a sample size of 54 (7 patients had missing data for date of diagnosis of CF), the mean age at diagnosis of CF was 3.5 ± 4.5 years. The majority of patients, 63 of the 68 (93%), identified as non-Hispanic white and 11 (16%) had meconium ileus at birth. Of the 68, 26 (38%) were homozygous for F508del, 33 (49%) were heterozygous for F508del, 8 (12%) had other mutations, and 1 patient had no genetic mutation data. The average age at initial OGTT was 11 ± 2.4 years; 21% had a g-tube to support enteral feeding at this time. The average age of patients diagnosed with IGT was 11 ± 2.6 years. The average age of patients diagnosed with CFRD was 11.2 ± 2.9 years. Of those in the IGT group, 62% were heterozygous for F508del. Of those in the CFRD group, 47% were heterozygous for F508del. There were no significantly different characteristics among these variables when comparing diagnostic groups.

Table 5: Characteristics of Patients with Normal Glucose Tolerance, Impaired Glucose Tolerance and CFRD

	Overall (n=68)	Normal Glucose Tolerance (n=25)	Impaired Glucose Tolerance (n=26)	Cystic Fibrosis Related Diabetes (n=17)	p-value
Sex					0.38
Male	31 (46)	14 (56)	11 (42)	6 (35)	
Female	37 (54)	11 (44)	15 (58)	11 (65)	
Ethnicity					0.17
Non-Hispanic	63 (93)	21 (84)	25 (96)	17 (100)	
Other	5 (7)	4 (16)	1 (4)	0 (0)	
Genetic Mutation					0.33 [a]
F508del/F508del	26 (38)	10 (40)	9 (35)	7 (41)	
F508del/Other	33 (49)	9 (36)	16 (62)	8 (47)	
Other/Other	8 (12)	5 (20)	1 (4)	2 (12)	
Not determined	1 (1)	1 (4)			
Use of g-tube at initial OGTT					0.38
No	54 (79)	22 (88)	20 (77)	12 (71)	
Yes	14 (21)	3 (12)	6 (23)	5 (29)	
Years of age, mean (SD)					
At diagnosis of CF	3.5 (4.5) [b]				
At initial OGTT	11.0 (2.4)	10.9 (2.0)	11.0 (2.6)	11.2 (2.9)	0.90
At diagnosis of		10.9 (2.0)	12.4 (2.8)	13.0 (3.3)	

Values are n (%) unless otherwise specified; [a} based on n=67 (excludes unknown group), [b] based on n=54; p-value is comparing significance between diagnostic groups

Anthropometric Measurements

Anthropometric data of the NGT, IGT, and CFRD groups are shown in Table 7. The NGT group had the highest average weight and height z-scores at initial OGTT of 0.17 ± 1.01 and -0.09 ± 0.82, respectively. The average BMI z-score was highest in the IGT group with a value of 0.40 ± 0.69. At diagnosis of IGT or CFRD, the CFRD group had lower overall height, weight and BMI z-scores than the IGT group. However, none of these values were significantly different when comparing between normal glucose tolerance, IGT or CFRD groups.

Table 6: Weight, Height and BMI Z-scores of the Normal Glucose Tolerance, Impaired Glucose Tolerance and CFRD groups

	Overall (n=68)	Normal Glucose Tolerance (n=25)	Impaired Glucose Tolerance (n=26)	Cystic Fibrosis Related Diabetes (n=17)	p-value
At Initial OGTT					
Weight	-0.03 ± 0.94	0.17 ± 1.01	0.03 ± 0.84	-0.43 ± 0.94	0.12
Height	-0.28 ± 0.90	-0.09 ± 0.82	-0.39 ± 0.94	-0.40 ± 0.97	0.41
BMI	0.23 ± 0.87	0.34 ± 0.94	0.40 ± 0.69	-0.20 ± 0.90	0.06
At diagnosis					
Weight			0.9 ± 0.82	-0.48 ± 1.00 [a]	
Height			-0.49 ± 0.99	-0.49 ± 1.12 [a]	
BMI			0.47 ± 0.65	-0.25 ± 0.97 [a]	

Values are mean ± SD; [a] based on n=16; p-value is comparing significance between diagnostic groups

Pulmonary Function and Exacerbations

Pulmonary indices for the normal glucose tolerance, IGT, and CFRD groups are summarized in Table 8. At initial OGTT, mean FEV1% of the entire cohort was 91.4 ± 21.1%. The normal glucose tolerance group had the highest mean FEV1% of 97.0 ± 23.3% while the IGT and CFRD groups had similar FEV1% values of 87.2 ± 18.4% and 88.1 ± 20 respectively. At IGT or CFRD diagnosis, the CFRD group had a slightly lower mean FEV1% of 84.8 ± 22.8% versus 87.5 ± 18.6% at IGT diagnosis. Values were not significantly different between groups.

Analysis of the number and severity of lifetime exacerbations showed that the mean number of total exacerbations was highest in the IGT group, 13.6 ± 11.5. The trend for the IGT group having the highest number of lifetime exacerbations was consistent when analyzing exacerbations based on severity, however means were not significantly different between diagnostic groups.

Table 7: Pulmonary Function (FEV1%) and Number & Severity of Lifetime Exacerbations among Patients

	Overall	Normal Glucose Tolerance	Impaired Glucose Tolerance	Cystic Fibrosis Related Diabetes	p-value
FEV1% at					
Initial OGTT	91.4 ± 21.1	97.0 ± 23.3	87.2 ± 18.4	88.1 ± 20.4	0.29
n	51	21	20	10	
Diagnosis			87.5 ± 18.6	84.8 ± 22.8	0.71
n			24	12	
Follow-up time, yrs.					0.10
n	67	25	25	17	
Median	14.8	12.1	14.7	17.1	
IQR	11.6-18.2	11.3-16.3	12.0-17.4	12.9-19.4	
Lifetime Exacerbations					
Total	10.7 ± 10.2	7.4 ± 7.1	13.6 ± 11.5	11.2 ± 11.1	0.16
Mean per 10 yrs.		5.8	9.4	6.8	
Mild	6.2 ± 4.9	5.0 ± 4.3	7.5 ± 5.7	6.1 ± 4.4	0.35
Mean per 10 yrs.		3.9	5.3	3.9	
Moderate	1.2 ± 2.0	0.8 ± 1.3	1.6 ± 2.8	0.9 ± 1.6	0.36
Mean per 10 yrs.		0.6	1.1	0.5	
Severe	3.1 ± 4.9	1.5 ± 2.2	4.2 ± 5.2	4.1 ± 6.7	0.11
Mean per 10 yrs.		1.2	2.8	2.4	

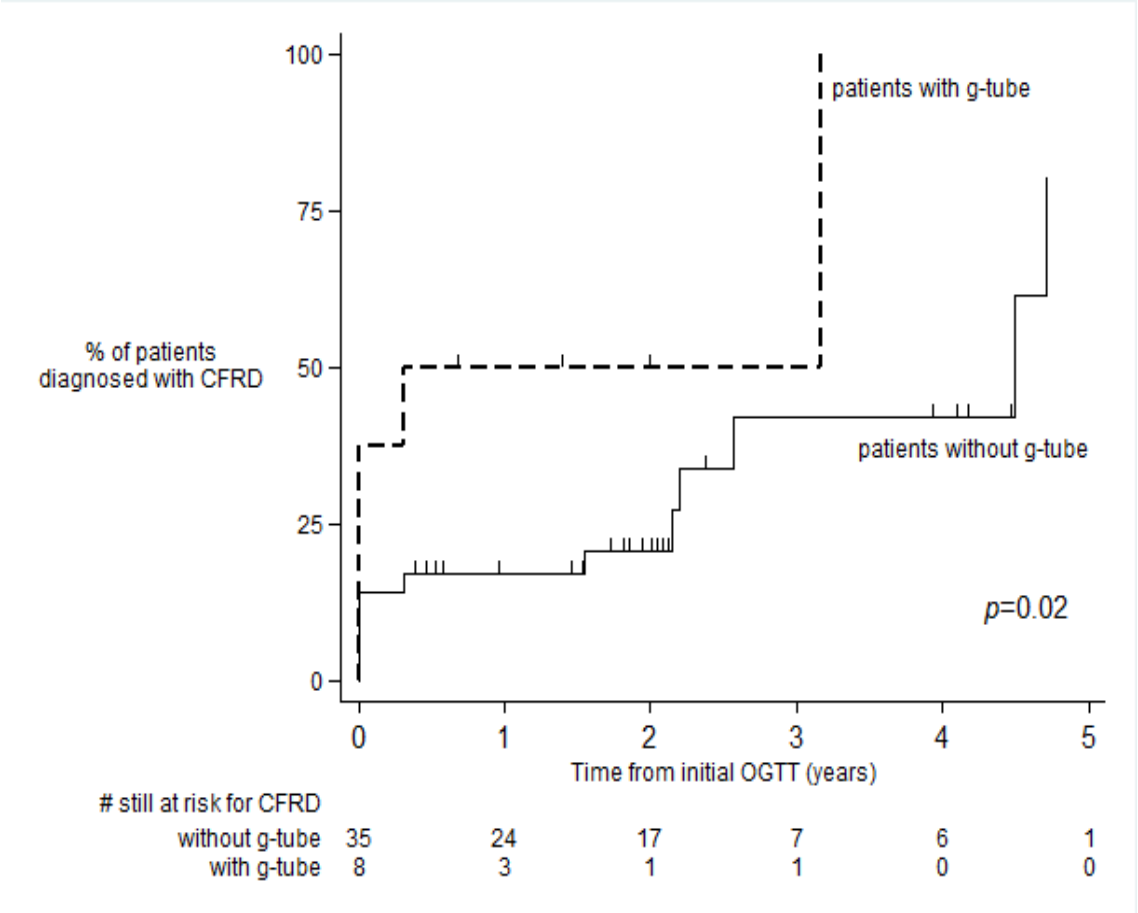
Values represent mean ± SD; FEV1% is the percent forced expiratory volume of the total vital lung capacity which a patient can expire in the first second of forced expiration; IQR= inter quartile range; p-value is comparing significance between diagnostic groups

Relationships between Sex, Genotype and Use of Gastrostomy-tube for Enteral Feedings and CFRD Diagnosis

Survival analyses were conducted to determine the relationships between sex, genotype and use of a g-tube for enteral feedings and time to diagnosis of

CFRD. Figure 3 shows the time from initial OGTT to diagnosis of CFRD among patients with and without a g-tube in the NGT (n=25) and CFRD (n=17) groups. At the time that patients completed their initial OGTT, 14% (95% CI: 6-31%) of those without a gastrostomy-tube were diagnosed with CFRD while 37.5% (95% CI: 14-77%) of those with a g-tube were diagnosed with CFRD. The median time after the initial OGTT to diagnosis of CFRD was 4.50 years among patients without a g-tube and 0.31 years among patients with a g-tube. The time to diagnosis of CFRD after initial OGTT was significantly different (p=0.022) when comparing patients with or without a g-tube. The rate of CFRD diagnosis among those with a g-tube was estimated to be 3.1 (95% CI: 1.2-8.4) times higher than the rate of CFRD diagnosis for those without a g-tube.

Figure 3: Time from Initial OGTT to Diagnosis of CFRD among Patients with or without a Gastrostomy-tube

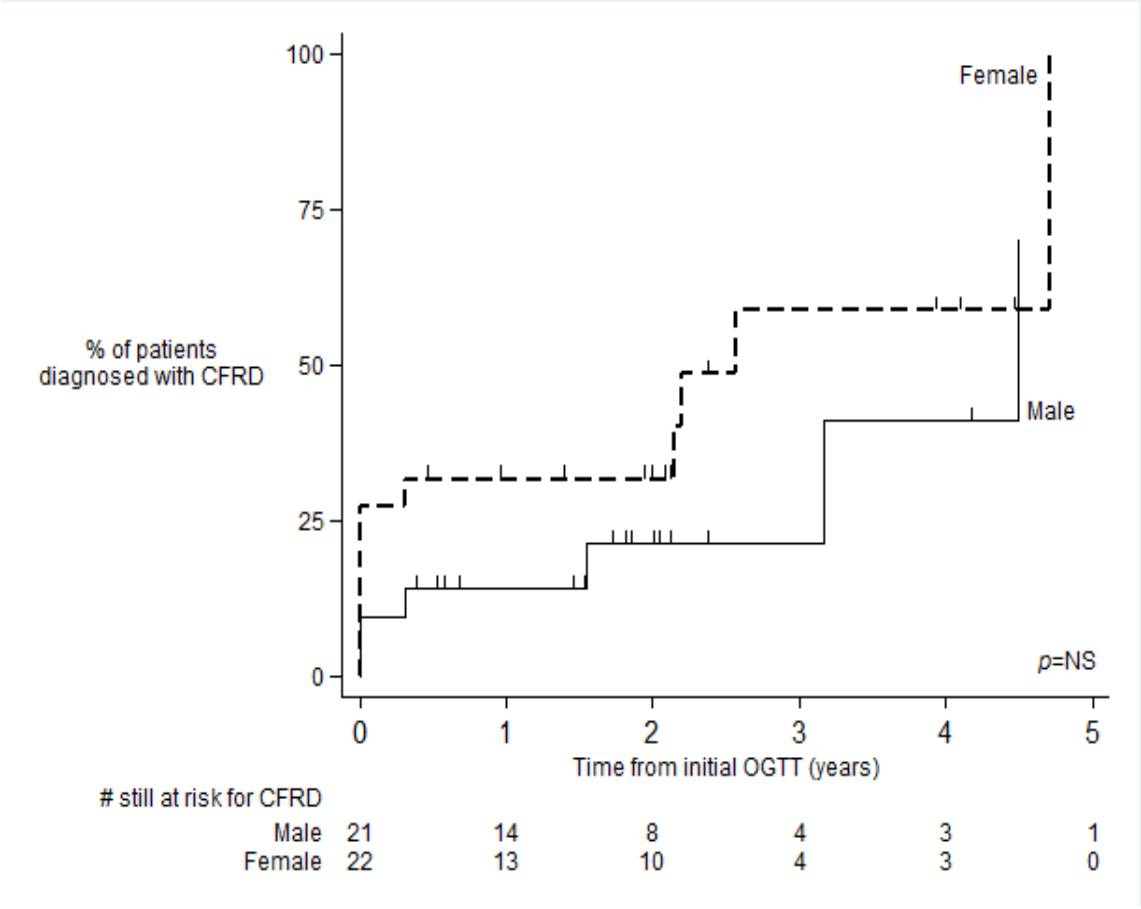


Time 0 is the initial OGTT. Tic marks on the two curves represent time of last contact for normal glucose tolerance patients still at risk of developing CFRD and who do not have any follow-up data past the indicated time of censoring. Patients with a diagnosis of IGT are excluded from this analysis as they represent a separate diagnosis. Data presented below the figure shows the number of patients still at risk of being diagnosed with CFRD at each given year.

Figure 4 shows the survival analysis of the relationship between time from initial OGTT to diagnosis of CFRD for male and female patients. Females had a higher prevalence of CFRD (27%, 95%CI: 13-51%) at initial OGTT than males (10%, 95%CI: 2.5-33%). Median time after initial OGTT to diagnosis of CFRD is estimated to be 4.49 years among males and 2.57 years among females. The time

after initial OGTT when a patient was diagnosed with CFRD between males and females was not significantly different (p=0.19).

Figure 4: Time from Initial OGTT to Diagnosis of CFRD by Sex of Patient

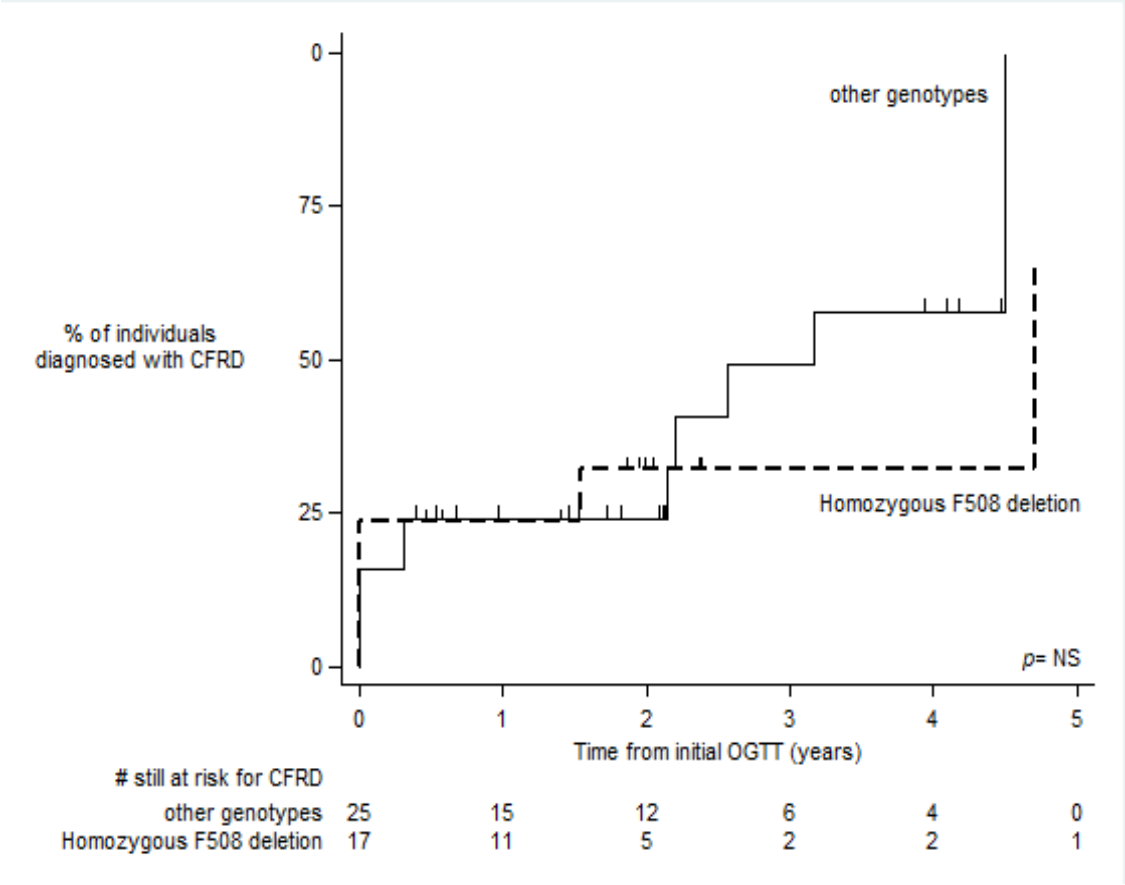


Time 0 is the initial OGTT. Tic marks on each curve represent patients with normal glucose tolerance who remain at risk of developing CFRD in the future but do not have any follow-up data past the indicated point in time. Patients with a diagnosis of IGT are excluded from this analysis. Data presented below the figure shows the number of patients still at risk of being diagnosed with CFRD at each given year.

Figure 5 shows the time from initial OGTT to a diagnosis of CFRD for patients with different CF genotypes. At initial OGTT, 16% (95%CI: 6.3-37.2%) of patients with “other genotypes” were diagnosed with CFRD while 23.5% (95%CI: 9.6-51.2%) of patients homozygous for F508del were diagnosed with CFRD.

Differences between the group with “other genotypes” compared to those who were homozygous for F508del was not significantly different. The median time after initial OGTT until patients with “other genotype” were diagnosed with CFRD was 3.17 years, while patients homozygous for F508del had a median time from initial OGTT to CFRD diagnosis of 4.71 years. The rate of CFRD diagnosis for those with “other genotype” was estimated to be 1.38 times the rate of a CFRD diagnosis was for those with a homozygous F508del genotype (95% CI: 0.45-4.17 times as much; p= 0.57).

Figure 5: Time from Initial OGTT to Diagnosis of CFRD by Genotype of the Patient



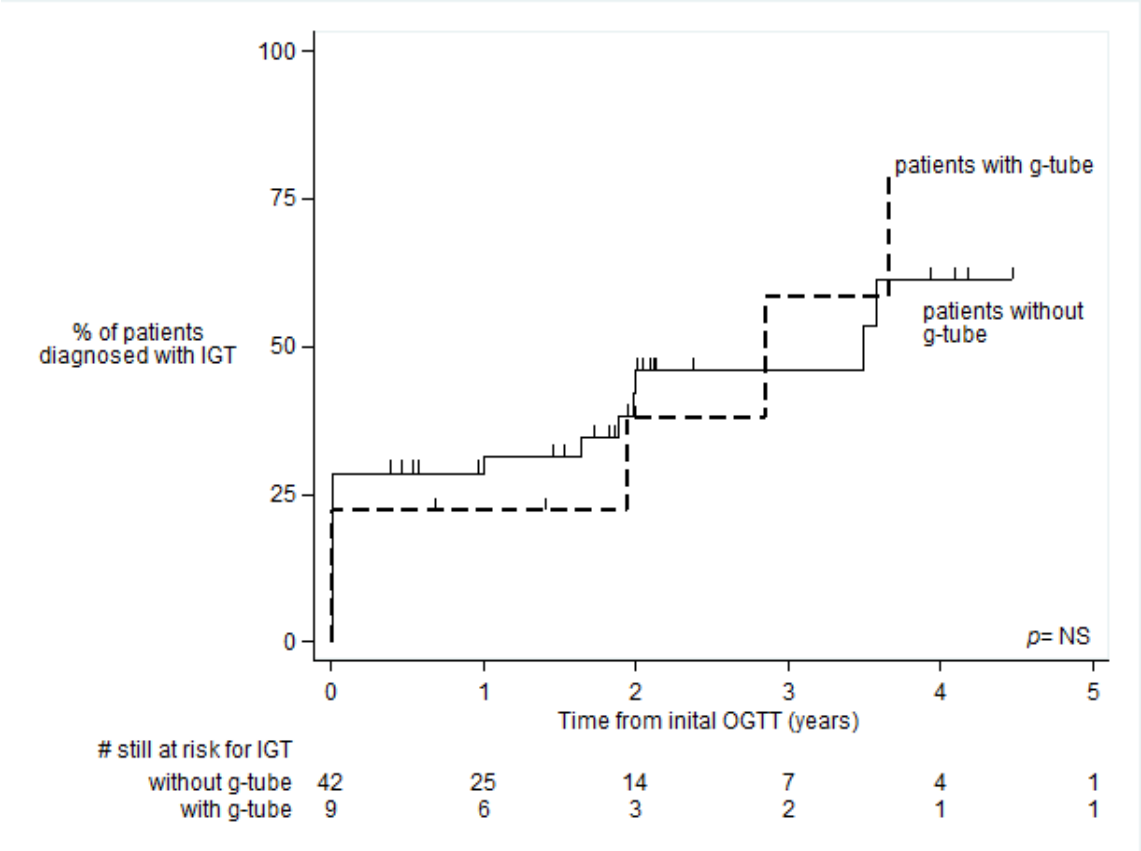
Initial OGTT is shown at Time 0. Tic marks on each curve represent patients with normal glucose tolerance that remain at risk of developing CFRD in the future, but who do not have any follow-up data past the indicated point in time. Patients with a diagnosis of IGT are excluded from this analysis. Data presented below the figure shows the number of patients that remain at risk of being diagnosed with CFRD at each given year.

Relationships to IGT Diagnosis:

Survival analyses were also performed to determine relationships between the sex, genotype and g-tube and diagnosis of IGT. Figure 6 shows time from initial OGTT to diagnosis of IGT among patients with and without a g-tube. Unlike the CFRD figures, there is an increased diagnosis of IGT at initial OGTT among

patients without a g-tube than those with a g-tube. At the time that patients completed their initial OGTT, 29% (95%CI: 17-45%) of those without a g-tube were diagnosed with IGT while 22% (95%CI: 6-64%) of patients with a g-tube were diagnosed with IGT. Median time after initial OGTT to diagnosis of IGT was estimated to be 3.49 years for those without a g-tube and 2.86 years for those with a g-tube. Rates of IGT diagnosis over time were not significantly different between the groups.

Figure 6: Time from Initial OGTT to Diagnosis of IGT among Patients with or without Gastrostomy-tube

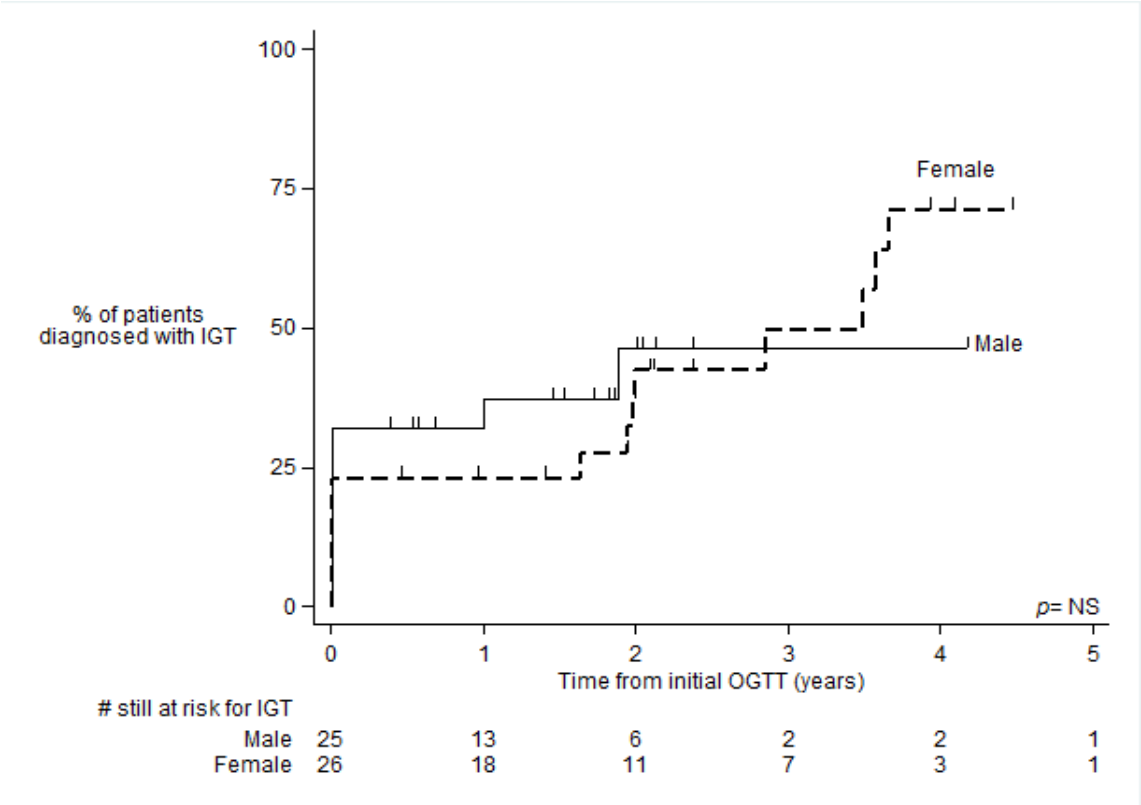


Time 0 is the initial OGTT. Tic marks on the two curves represent time of last contact for normal glucose tolerance patients still at risk of developing IGT and who do not have any follow-up data past the indicated time of censoring. Patients with a diagnosis of CFRD are excluded from this analysis as they represent a separate diagnosis. Data presented below the figure shows the number of patients still at risk of being diagnosed with IGT at each given year.

Figure 7 shows time from initial OGTT to diagnosis of IGT for male and female patients. Males in the IGT group, unlike those in the CFRD group, showed a slightly increased incidence of IGT at initial OGTT (32%, 95%CI: 17-53%) compared to females (23%, 95% CI: 11-44%). Median time after initial OGTT to diagnosis of IGT was estimated to be 5.55 years among males and 3.49 years

among females. Rates of IGT diagnosis are not significantly different between the sexes.

Figure 7: Time from Initial OGTT to Diagnosis of IGT by Sex of Patient

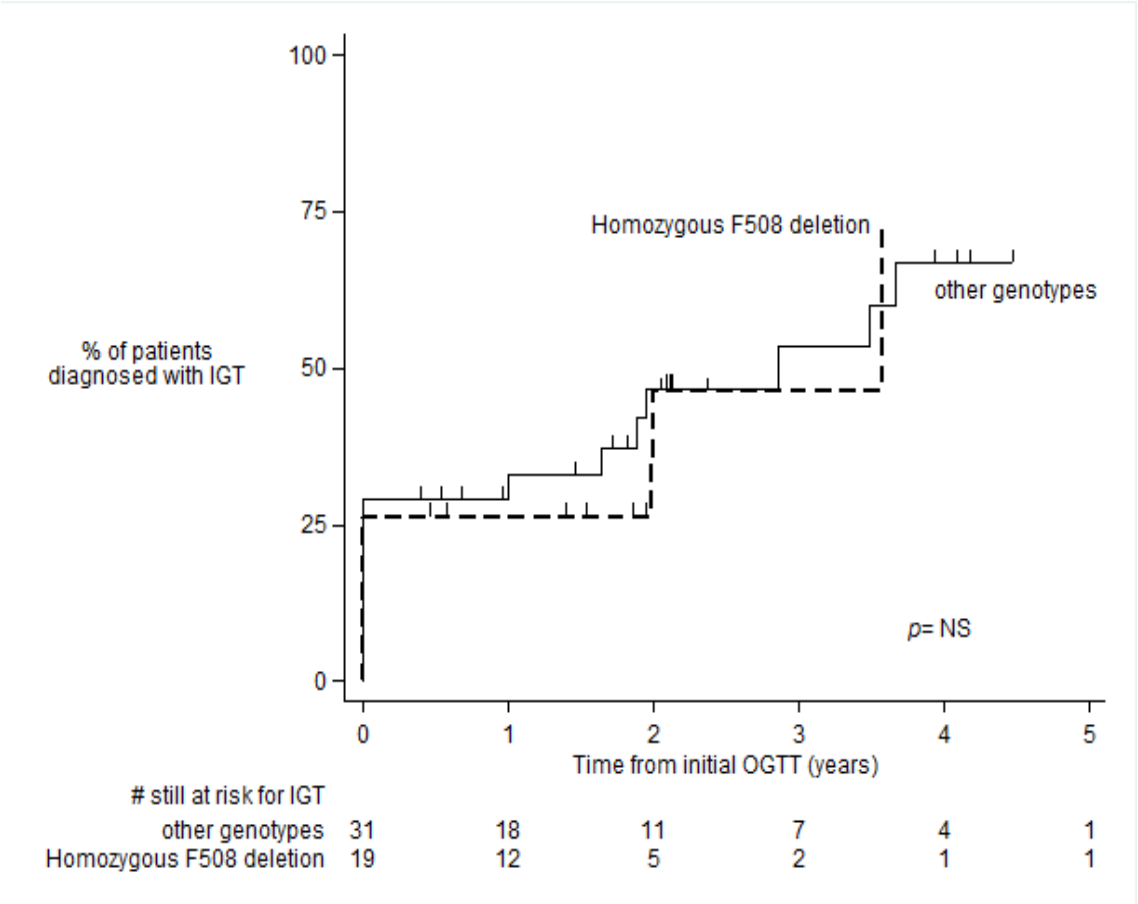


Time 0 is the initial OGTT. Tic marks on each curve represent patients with normal glucose tolerance who remain at risk of developing IGT in the future but do not have any follow-up data past the indicated point in time. Patients with a diagnosis of CFRD are excluded from this analysis. Data presented below the figure shows the number of patients still at risk of being diagnosed with IGT at each given year.

Figure 8 shows time from initial OGTT to diagnosis of IGT for patients with different genotypes. At the time patients completed their initial OGTT, 29% (95% CI: 16-48%) of those with "other genotypes" were diagnosed with IGT while 26% (95%CI: 12-52%) of those homozygous for F508del were diagnosed with IGT. Half (50%) of the patients with "other genotype" received an IGT diagnosis after

2.9 years beyond their initial OGTT while it took 3.6 years for 50% of patients with homozygous F508del to receive their diagnosis of IGT after the first OGTT.

Figure 8: Time from Initial OGTT to Diagnosis of IGT by Genotype of the Patient



Initial OGTT is shown at Time 0. Tic marks on each curve represent patients with normal glucose tolerance that remain at risk of developing IGT in the future, but who do not have any follow-up data past the indicated point in time. Patients with a diagnosis of CFRD are excluded from this analysis. Data presented below the figure shows the number of patients that remain at risk of being diagnosed with IGT at each given year.

Conclusions

The first aim of this study was to describe clinical characteristics of individuals with CF followed at the OHSU CF Center at their initial OGTT. Sixty-eight patients were included in this cross-sectional analysis: 54% were female,

93% were non-Hispanic white, 49% had at least one F508 deletion and 21% had a g-tube at initial OGTT.

Anthropometric measurements were obtained from the CFFPR for consistency. Mean height and weight z-scores were slightly negative and mean BMI z-scores were slightly positive. There were no significant differences in mean z-scores between diagnostic groups. There was a trend towards a significant difference in BMI z-scores between diagnostic groups ($p=0.06$).

Pulmonary function of the patients at initial OGTT showed no differences across diagnostic groups in FEV1%. Nor was there a difference in number/severity of exacerbations. Overall, FEV1% was 91.4 ± 21.1% for the cohort. Patients experienced an average of 10.7 exacerbations during their lifetime.

The second aim of the study was to assess whether g-tube feeding, BMI z-score and FEV1% predict the development of IGT and/or CFRD. We hypothesized regular use of g-tube for enteral feedings, lower pulmonary function and lower BMI z-scores would be associated with development of IGT and/or CFRD.

Time to diagnosis of CFRD after the initial OGTT was significantly less among patients with a g-tube compared to those without ($p=0.022$). The time to CFRD diagnosis among those with a g-tube was estimated to be 3.1 (95% CI: 1.2-8.4) times sooner than for those without a g-tube.

There were no significant differences between mean weight and height z-scores among diagnostic groups. However, there was a trend towards significance

in BMI z-scores between diagnostic groups, such that for every one-unit decrease in BMI z-score, there was an increased risk for diagnosis of IGT or CFRD (p=0.06).

Pulmonary function, as measured by FEV1%, as well as number and severity of exacerbations, were not significantly related to a diagnosis of IGT or CFRD.

Chapter 5

Discussion

As individuals with CF live longer, they are at increased risk for developing CFRD, the most common complication seen in adults with CF. There is limited research describing risk factors that predict the development of CFRD, which limits our ability to intervene early to delay or prevent the onset of this insidious disease. In this study, we explored whether sex; genetic mutation; g-tube placement; weight, height and BMI z-scores; and pulmonary function predicted the diagnosis of IGT or CFRD.

A higher frequency and an earlier onset of diabetes among females were repeatedly reported[24,25,49,51-54], while one study found no difference among sexess[55]. In our study, 54% of the cohort were female; of those diagnosed with CFRD, 65% of them were female. In 2013, Scheuing et al investigated the occurrence of CFRD depending on age, gender and nutritional status among CF patients screened between 2001 and 2010[54]. Similar to our study, they used Kaplan Meier curves and proportional-hazards ratios to look at sex and CFRD diagnosis. Females had a 3.5-fold higher risk for CFRD (p=0.02) and a significantly earlier diagnosis of CFRD compared to males (p=0.004). This study however, showed a higher proportion of CFRD diagnosis in females later in life, something our study did not analyze. Adler et al used a population-based longitudinal study of 50 CF clinics in the United Kingdom to identify potential risk factors of CFRD[51]. In a multivariate model, they found female patients were ~60% more

likely to develop CFRD than male patients, and each year of age was associated with a 2-3% increase in risk. Neither study could explain the etiology of this sex difference. Therefore, the relationship between sex and diagnosis of CFRD needs to be further studied.

The CFTR F508 deletion is found in up to 70% of Caucasian CF patients[56]. Some studies report CFRD is related to pancreatic exocrine-endocrine insufficiency, which correlates with CFTR mutations[47,53]. In our study, 41% of patients diagnosed with CFRD and 35% of patients diagnosed with IGT were homozygous for F508del. However, we observed a much higher prevalence of heterogeneity in our IGT and CFRD diagnostic groups, 47% and 52%, respectively. Preumont et al determined the clinical phenotype of the adolescent/adult patient with CF according to heterozygosity and homozygosity for F508 deletion and analyzed the characteristics according to glucose tolerance status[57]. In this study, 40% of patients were homozygous for F508del and 24% were heterozygous (p=0.24). The prevalence of homozygous F508del, after allocating patients into diagnostic groups, was 51% in normal glucose tolerance group and 68% in the combined IGT/CFRD groups (p=0.20). In a prospective study that had a similar incidence of CFRD compared to our study, patients homozygous for F508del were more likely to develop CFRD than those with other genotypes[47]. Research relating diagnosis of CFRD and homozygosity for F508del is abundant[24,49,51-53,57]. Our small sample size could be the reason why we saw less homozygosity than others.

Many reports have proposed that CFRD is associated with enteral tube feeding, with an incidence of CFRD among those with g-tubes ranging from 5%-50% after one to two-years of follow-up[58-60].After analysis, we saw a significant difference comparing diagnosis of CFRD among those with and without a g-tube (p=0.022). The prevalence of enteral tube feeding at initial OGTT was 35.7% in our study, consistent with previous research. White et al established whether intensive nutritional intervention prevents pre-diabetic nutritional decline in an adult population with CFRD[41]. After matching 48 adult patients with CFRD to 48 age- and sex-matched CF controls, patients with CFRD were four times as likely to have g-tubes as the controls (p=0.01).

We observed no differences in mean height, weight and BMI z-scores between CFRD, IGT or normal glucose tolerance groups. However, we did observe a trend towards significance with lower mean BMI z-score among those diagnosed with CFRD and IGT compared to those with normal glucose tolerance (p=0.06). Our study was consistent with other epidemiologic studies, which showed mixed results[58-60]. Knudsen et al investigated the incidence of CFRD among adolescents over time to identify characteristics associated with early diabetes onset[49]. In 161 CF patients, they found no significant differences when comparing BMI z-score between patients with and without CFRD (p=0.09).

CFRD adds an additional treatment burden to CF patients, who already take many medications, as well as daily airway clearance treatments, to maintain lung function and adequate nutrition. A decrease in FEV1% and an increase in the number of exacerbations are characteristics of CFRD. In our study, the IGT group

had a higher number of exacerbations as well as a lower FEV1% than the CFRD group when adjusted for a 10-year follow-up period. However, neither of these values were significantly different among groups. De Boer et al concluded in a prospective study of 446 CF patients that patients with frequent exacerbations were more likely to be female and diabetic and to have poorer baseline lung function[50]. Meerkerk et al reported a median frequency of exacerbations of 0 among patients without CFRD and 3 among those with CFRD within a 6-year follow up period[61].

Study Strengths

This study design had many strengths, including a comprehensive medical record review to describe the patient cohort in detail and to thoroughly explore possible confounding factors that might impact our conclusions. Excluding patients who used lumacaftor/ivacaftor and those who had transplants or multiple comorbidities reduced outside factors that could positively or negatively impact the development of IGT or CFRD. Anthropometric data was taken from the CFFPR database, which helped eliminate reliance on, or inclusion of, measurements obtained from non-CF clinic visits, where measurements may be performed with less precision. Having a single pediatric endocrinologist review all OGTT results and classify patients into normal glucose tolerance, IGT, or CFRD diagnostic groups according to the 2014 ADA guidelines reduced group allocation

bias. Completing this analysis in a young pediatric/adolescent sample allows us to identify risk factors before the onset of other comorbidities.

Study Limitations

One limitation of this research was our relatively small sample size. The small sample size was due in part to missing OGTT data. Routine OGTT testing at 10 years of age did not become standard of care in the OHSU CF clinic until 2015, which is why many CF patients were excluded from this study. A second limitation is that diagnoses of IGT and CFRD were based on the results from a single OGTT. The current clinical care guidelines recommend a second confirmatory OGTT be performed prior to making a diagnosis of IGT or CFRD in individuals with CF. We did not require a second confirmatory OGTT for allocation to diagnostic groups for ease of analysis, therefore our rates of IGT and CFRD may be higher than those reported in other studies. A third limitation to this study is that the timing of initial OGTT could not be easily ascertained (e.g. whether it was done when the patient was healthy or if it was done during or near an exacerbation). The OHSU CF Center is the only accredited CF center in Oregon and serves a geographically diverse patient population. In efforts to increase OGTT screening, OGTT is sometimes obtained at the end of a hospital admission for a CF pulmonary exacerbation, not after the recommended six weeks removed from an exacerbation. This is a pragmatic, real-world implementation of recommended guidelines and could artificially increase the

number of patients diagnosed with IGT or CFRD. Finally, a fourth limitation to our study is that the OHSU CF Center is a top-10 center for BMI. This means that this patient cohort has higher BMI compared to other centers, which limits generalizability. It could also explain why we found a trend towards significance in BMI z-scores and CFRD diagnosis instead of a statistically significant relationship. Finally, FEV1% is known to be a late marker of CF lung disease and is most often normal in the age group in question. A more sensitive marker of lung disease, such as lung clearance index, might be able to detect a difference between groups if one exists.

Conclusions

Our study confirms that the presence of a g-tube for nutrition support is a significant risk factor for developing CFRD. Routine monitoring of blood glucose concentrations during g-tube feeds should be standard practice to monitor for the emergence of IGT or CFRD so that it can be managed appropriately with insulin if needed. There was a trend towards significance in lower BMI z-score and diagnosis of CFRD, consistent with other studies. This reinforces the need to screen for IGT and CFRD in patients whose BMI is declining. In our study, pulmonary function, genotype, and sex were not associated with the development of IGT or CFRD. More robust clinical markers to identify individuals with CF who are at increased risk for developing CFRD need to be studied in a larger patient

population, as this would potentially allow for earlier diagnosis of IGT and CFRD, as well as more prompt initiation of treatment to mitigate complications of hyperglycemia, particularly frequent pulmonary exacerbations and rapid decline in lung function.

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