Chapter One

Introduction

1.1 Background to the Study

Diabetes mellitus (DM) is a metabolic disorder that is characterised by persistent hyperglycemia. This condition occurs as a result of changes in the metabolism of carbohydrates, proteins, and lipids, which are caused by absolute or relative insulin insufficiency and organ system failure[1]. People with diabetes may experience hyperglycemia, which can have a negative effect on their quality of life, the well-being of their families, and society as a whole. Type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus (GDM), and other diabetes variants can be classified depending on their aetiology[1]. Type 1 diabetes is caused by an autoimmune reaction to proteins in pancreatic islet cells. Type 2 diabetes, on the other hand, is caused by a combination of environmental and genetic variables, including obesity, excessive caloric consumption, physical inactivity, stress, and ageing[2]. The wide range of pancreatic diseases makes it difficult to explain the cause of selective β-cell death in the islet in type 1 diabetes mellitus.

When overt hyperglycemia first occurs, you may observe pseudoatrophic islets that have cells that secrete pancreatic polypeptide, somatostatin, and glycogen, as well as normal islets and islets that have both β-cells and invading lymphocytes and monocytes[2]. When pancreas cells die due to an autoimmune response, the body does not produce enough insulin. This leads to the metabolic issues that are associated with type 1 diabetes. The main pathophysiological features of type 2 diabetes are decreased insulin production and increased insulin resistance. The degradation of pancreatic cell function progresses significantly over

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time, even though ageing, obesity, insufficient energy intake, alcohol consumption, smoking, and other factors are all unique risk factors in the development of type 2 diabetes mellitus. Gestational diabetes mellitus (GDM) is a serious complication of pregnancy that is diagnosed in women who do not have a prior diagnosis of diabetes but who do have chronic hyperglycemia throughout pregnancy. Hyperglycemia is usually caused by a decrease in glucose tolerance that occurs when the beta cells of the pancreas are not functioning properly and insulin resistance is ongoing. Excess weight, obesity, advanced mother age, and a hereditary sensitivity to diabetes comprise risk factors for gestational diabetes mellitus (GDM)[4]. Gestational diabetes mellitus can result in problems after birth, macrosomia, type 2 diabetes, and cardiovascular disease in the mother.

It is believed that genetic, epigenetic, and environmental variables have a role in the development of gestational diabetes mellitus (GDM), and the underlying mechanisms are complex and change over a long period of time. In general, pancreatic β-cells are unable to make up for a long-term lack of fuel, which leads to insulin resistance, hyperglycemia, and an increased supply of glucose to the growing foetus. In addition, evidence indicates that placental factors, gluconeogenesis, oxidative stress, low-grade chronic inflammation, and adipose expandability are all factors that contribute to the development of gestational diabetes mellitus (GDM)[5]. There are many causes of type 1 and type 2 diabetes (T2D), with genetic predisposition being one of the most important[6]. Neonatal diabetes mellitus (NDM) and maturity-onset diabetes of the young (MODY) are two prevalent types of monogenic diabetes. These types of diabetes are usually caused by mutations in genes that encode transcription factors or other proteins that are important in the development or function of the endocrine pancreas. Mutations in many monogenic diabetes genes may induce NDM and

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MODY. Neonatal diabetes mellitus (NDM) is a disorder that can be diagnosed within the first six months of life. It can be classified as either permanent (permanent NDM [PNDM]) or temporary (transient NDM [TNDM]). The transient form usually goes away during infancy but often comes back later in life[6]. Neonatal diabetes mellitus (NDM) is not commonly associated with high-risk human leukocyte antigen (HLA) haplotypes or the development of islet autoimmune antibodies, unlike typical type 1 diabetes. This is especially true when mutations occur in the signal transducer and activator of transcription gene, which is related to NDM. Transcription 3 has recently been associated with unusual cases of type 1 diabetes and autoimmune illnesses that develop early in life[7]. MODY is a type of diabetes that is caused by mutations in numerous islet-related genes.

It is an autosomal dominant, monogenic form of diabetes that usually appears before the age of 25. MODY 7 and 8 are the two main types of MODY. They are caused by mutations in glucokinase (GCK-MODY or MODY2) and hepatocyte nuclear factor 1 homeobox A (HNF1A-MODY or MODY3)[8]. The organs that exhibit *HNF1A* expression are the kidney, stomach, intestine, liver, pancreas, and spleen. A mutation could have an impact on the function of HNF-1A in a number of organs. MODY3 is characterised by a severe deficit in the release of insulin from pancreatic β-cells, but the clinical manifestations might differ from family to family. People with MODY3 may develop a variety of diabetes-related issues, such as microvascular abnormalities that damage the kidneys and retina. Although one-third of people may eventually need insulin therapy, the majority of them can be sufficiently managed for long periods of time with dietary changes and sulfonylurea treatment[9].

The *HNF1A* gene is responsible for controlling liver-specific genes, including those that are involved in glucose metabolism, transport, and insulin synthesis. Type 2 diabetes is

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frequently associated with mutations in the *HNF1A* gene[9]. The *HNF1A* protein is made up of three domains: the dimerisation domain, the DNA-binding domain, and the transactivation domain. Some mutations in the dimerisation or DNA-binding domains do not have an effect on the normal allele, while other mutations have dominant negative consequences[10]. The I27L, A98V, and S487N polymorphisms are common variants of the *HNF1A* gene that have been found in both T2D and non-diabetic groups. *HNF1A* (MIM*142410) is located on chromosome 12q24.2 and consists of 10 exons. This gene encodes a transcription factor that is essential for the expression of at least 222 liver-specific genes that are critical for glucose synthesis and storage, as well as lipid metabolism, which includes cholesterol and lipoprotein synthesis[11].

The cause of maturity onset diabetes of the young type 312 was found to be mutations in the *HNF1A* gene. This kind of diabetes is marked by severe hyperglycemia due to insufficient insulin secretion by beta cells. It is inherited in an autosomal dominant manner and usually appears before the age of 25[12]. The *HNF1A* gene may have variations that can show different levels of severity. These variations may lead to monogenic diabetes, increase the risk of type 2 diabetes mellitus, or be benign[11]. In a Latino population, the low-frequency polymorphism p.Glu508Lys was linked to type 2 diabetes mellitus[13]. The study's cohort of patients with type 2 diabetes mellitus did not show any differences in clinical phenotype between those who carried the HNF1A p.Glu508Lys mutation and those who did not. Compared to older obese control patients, young, thin people with type 2 diabetes mellitus had three times as many low-frequency nonsynonymous mutations in several MODY genes[14]. Furthermore, another study investigated whether the functional classification of the uncommon coding *HNF1A* variations that were discovered might be used to predict the risk of type 2 diabetes mellitus in

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the general population. A group of single nucleotide polymorphisms (SNPs) that reduced HNF-1A transactivation to below 60% in comparison to the wild type protein was shown to be related with type 2 diabetes mellitus[15]. According to genome-wide association study (GWAS) meta-analyses, the *HNF1A* gene has a risk mutation (rs7957187) that is linked to late-onset type 2 diabetes mellitus (T2DM). A recent study has found that the minor A variation of *HNF1A* rs1183910 is associated with a higher prevalence of type 2 diabetes mellitus (T2DM) [16].

Additionally, it has been suggested that other common variants of *HNF1A*, including I27L (rs1169288), A98V (rs1800574), and S487N (rs2464196), may be linked to lower insulin secretion or an increased risk of T2DM in people of normal weight who have lower insulin resistance than their obese counterparts[17]. MODY is a condition that often affects people under the age of 25. It is caused by heterozygous mutations in a number of transcription factors that control the growth and maturation of pancreatic β-cells. In addition, mutations in enzymes that are connected to β-cell glucose sensing can result in early-onset diabetes. However, it is challenging to determine the global prevalence of this condition because MODY is often misdiagnosed as type 1 or type 2 diabetes mellitus. In addition, the occurrence of the disease varies among different populations because it is uncommon. In addition, the prevalence of MODY varies by country and ethnicity, and certain populations show a higher incidence of the condition[18]. This variation could be related to differences in the number of places that offer genetic testing. This study must analyse the *HNF1A* gene in diabetic patients because *HNF1A* encodes the transcription factor hepatocyte nuclear factor 1 alpha, which is predominantly associated to the aetiology of symptomatic maturity-onset diabetes of the young (MODY)[19]. In addition, study shows that a frequent mutation (G319S,

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located in exon 4) has a major impact on the development of T2D in the Oji-Cree community of Canada, with a minor allele frequency of 8.7%[19]. Additional research suggests that *HNF1A* mutations across the allele frequency spectrum are related with a higher risk of developing Type 2 Diabetes (T2D), since frequent variants in this gene are linked to multifactorial T2D. This emphasises the need of identifying *HNF1A* in individuals with diabetes. It is important to identify rare *HNF1A* mutations that have significant functional repercussions since this may affect how pre-diabetic mutation carriers are treated and which anti-diabetic medications are chosen.

In recent years, there has been a considerable increase in the number of people with diabetes mellitus, as well as a change in the demographics of the disease's epidemiology. Diabetes mellitus is increasingly affecting populations that were previously unaffected or only slightly impacted by the disease, resulting in a considerable increase in the number of cases[4]. This presents a major challenge to health financing by both governments and non-governmental organisations. According to the latest recent prevalence figure from the International Diabetes Federation (IDF), 425 million people around the world have diabetes mellitus, and more than half of these people have not been diagnosed. This number is significantly impacted by the growing economies of Africa and Asia. In Nigeria, the prevalence of diabetes mellitus among people aged 20 to 69 years is documented at 1.7%. As diabetes mellitus becomes more common, the consequences that come with it are growing increasingly severe. Nigeria has the largest population in Africa, with an estimated 200 million people. Of these, around 79 million are adults between the ages of 20 and 79[4]. Diabetes is found in one-third of instances in rural settings, while the other two-thirds are found in metropolitan areas. In Nigeria, there are almost two million instances of diabetes

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that have not been treated, were an estimated 105,091 deaths caused by diabetes in 2013. [4]. Nigeria has the highest number of diabetes cases in Africa, followed by South Africa with 2.6 million cases, Ethiopia with 1.9 million cases, and Tanzania with 1.7 million cases. The estimated number of people with diabetes globally in 2013 was 382 million, with a large share (175 million) undiagnosed. This resulted in an overall prevalence rate of roughly 8.3%. More than half of the deaths caused by diabetes occur in people who are less than sixty years old[5].

According to research conducted in Nigeria, as many as 73% of diabetes patients do not check their own blood glucose levels. According to a recent study, the prevalence of hypertension and peripheral neuropathy in Nigeria was above 50%. The prevalence of retinal issues was 35%, cataracts was 25%, cardiovascular disease was 5%, foot ulcers was 16%, and nephropathy was 3%. Surveys showed that glycaemic control and other goals, such as lipids, glycated haemoglobin (HbA1c), blood pressure, and education, did not meet expectations. The increasing incidence of diabetes has resulted in a rise in the number of amputations, cerebrovascular diseases, cardiovascular problems, and renal disorders in groups that were previously not acknowledged as having these serious health issues.

1.2 Statement of the Problem

Although there has been significant progress in understanding the genetic basis of diabetes, the molecular alterations and functional implications of *HNF1A* gene mutations in people with diabetes are still not well understood. A complete understanding of how mutations in the *HNF1A* gene contribute to the development and progression of diabetes can lead to more accurate diagnoses, personalised treatment plans, and the discovery of new treatment targets. As a result, it is essential to conduct research on the relationship between diabetes and

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mutations in the *HNF1A* gene at the molecular level in order to enhance patient care and treatment. This research should clarify the specific genetic changes, their effects on gene expression and protein functionality, and their clinical implications.

1.3 Rationale

The research will contribute to understanding the molecular pathways and biological processes impacted by *HNF1A* gene mutations by examining the specific genetic changes and their possible functional effects on blood glucose levels. This information may help to better understand the pathophysiology of diabetes and may help to identify new treatment targets.

Justification: Understanding the fundamental mechanisms underlying the onset, progression and severity of diabetes can be improved by looking at the molecular characterization of the *HNF1A* gene in diabetic individuals. Additionally, because diabetes is a heterogeneous condition, a precise diagnosis and categorization are essential for choosing the right course of action. Diagnosing MODY3, a specific subtype of diabetes associated with *HNF1A* mutations, can be made easier by identifying *HNF1A* gene mutations in diabetic patients. It's critical to distinguish MODY3 from other types of diabetes since it has unique clinical characteristics and treatment consequences. A trustworthy diagnostic method for identifying people with MODY3 can be found in the molecular characterization of the *HNF1A* gene, which ensures precise classification and individualized management.

Also, the molecular characterization of the *HNF1A* gene in diabetic patients is justified given its potential to advance our knowledge of the pathogenesis of diabetes, allow precise diagnosis and grouping, direct specific therapy modalities, ease genetic guidance, and reveal

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novel therapeutic targets. The outcomes and management of patients with diabetes may be improved as a result of this research.

1.4 Aim and Objectives of the Study

The aim of the study is to identify and characterize *HNF1A* among diabetic patients in Lagos, Nigeria.

The study objectives are to:

i. identify non-genetic risk factors associated with DM among the study participants
ii. detect and analyze the *HNF1A* gene type of variants among the diabetic patient compared with the control (non-diabetic patient)
iii. determine the influence of age and gender on *HNF1A* gene types
iv. assess the frequency and prevalence of *HNF1A* gene variants in the study population.

1.5 Research Questions

i. what are the non-genetic factors associated with DM in the study population?
ii. what are the *HNF1A* gene variants associated with DM?
iii. which age and gender has influence on *HNF1A* gene variation?
iv. what is the prevalence and pattern of *HNF1A* gene variants in the study population?

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1.6 Significance of the Study

This study seeks to provide a thorough understanding of the molecular characterization of the *HNF1A* gene in diabetic patients and its clinical implications. By addressing these research objectives, testing the hypotheses, and responding to the research questions, the study hopes to improve diabetes diagnosis, treatment, and management. This is because understanding the biochemical changes brought on by diabetes-related *HNF1A* gene mutations may point to new treatment targets. The identification of these pathways and molecular mechanisms may make it possible to develop specialized therapeutics to alter the main pathways and molecular mechanisms impacted by *HNF1A* mutations. This could result in the creation of better, more targeted treatments for people with diabetes, including those with MODY3.

1.7 Scope of the Study

This is hospital-based study on diabetic population attending Federal Medical Centre, Lagos, among diabetes patients in Lagos State, Nigeria.

Study Design: The is a cross-sectional, experimental, analytical study.

1.8 Limitation of the Study

The study may be limited by a sizable sample size due to the limited availability of funding, as it did not receive any form of external funding. It is also important to bear in mind that if the sample size is small, it may limit the statistical power and the ability to draw robust conclusions and generalizability of findings. In this study adequate sample size calculation has been made to address the concern. Likewise, it is important to also bear in mind that diabetes could occur as a heterogeneous and complex mechanism which may involve *HNF1A* gene mutations among study participants and various external factors, such as lifestyle, diet,

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and environmental factors, which can complicate the interpretation of results. Coexisting medical disorders or medication use are examples of confounding factors that may influence the results of the study. To ensure that the study's findings are properly evaluated and used, it is necessary to be aware of and acknowledge these limitations.

1.9 Operational Definition of the Study

This work relates to a methodical study aiming at identifying the molecular characteristics of the HNF1A gene in Lagos State diabetes patients. This category searches in the HNF1A gene genetic variants, mutations, or polymorphisms linked to diabetes susceptibility, progression, or therapy response.

Important Characteristics:

1. **Molecular characterization**: The work consists in molecular analysis of the HNF1A gene applied with DNA sequencing, genotyping, and bioinformatic techniques.

2. **Diabetic patients**: This study focuses on diabetic patients residing in Lagos State, aiming to identify the genetic factors contributing to diabetes within this group.

3. **HNF1A gene**: Investigations into HNF1A are ongoing, as its function is crucial for the operation of pancreatic beta cells and glucose metabolism.

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Chapter Two

Literature Review

2.1 Conceptual Review

2.1.1 Concept of Diabetes Mellitus

Diabetes mellitus (DM) is a group of metabolic diseases that are commonly marked by chronic hyperglycemia. This hyperglycemia can be caused by deficiencies in insulin synthesis, insulin function, or both. There is a hereditary disorder known as glucokinase insufficiency that causes irreversible neonatal diabetes. This illness is characterised by a failure in the glucose-insulin signaling system. The key differentiating feature of this disease is the degree of hyperglycemia that increases the risk of microvascular consequences (retinopathy, nephropathy, and neuropathy). This sickness is fundamentally described by the degree of hyperglycemia that elevates the risk of these consequences.

There is a correlation between it and a decrease in life expectancy, a loss in quality of life, an increased risk of macro vascular complications (such as peripheral vascular disease, ischaemic heart disease, and stroke), and a significant morbidity coming from certain diabetes-related microvascular disorders. Each and every one of these elements contributes to a decline in the quality of life. There are a number of pathogenetic factors that can be responsible for the developing of diabetes. The processes that contribute to insulin insufficiency and those that cause insulin resistance are both included in this category. Both of these mechanisms are responsible for the reduction of beta cells in the pancreas. Insensitivity to insulin or insufficiency of insulin leads to insufficient insulin action on target tissues, which in turn leads to metabolic abnormalities in carbohydrates, lipids, and proteins.

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There are a number of symptoms that are associated with diabetes mellitus, including blurred vision, increased thirst, polyuria, and weight loss. Frequently, the symptoms are either hardly noticeable or altogether missing[4]. There were 285 million persons around the world who were diagnosed with diabetes in the year 2010. According to projections, this number will increase to 439 million adults by the year 2030, which will account for 7.70% of the total population of the world. Here are the two basic types of diabetes mellitus that are most commonly seen[2]: The major cause of type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is the absence of insulin synthesis by pancreatic beta cells.

This condition is often referred to interchangeably as diabetes. On the other hand, the fundamental cause of type 2 diabetes, also known as non-insulin-dependent diabetes mellitus (NIDDM), is a decreased sensitivity of target tissues to insulin. Chronically elevated blood glucose levels and varying degrees of malfunction in the absorption and utilisation of carbohydrates, proteins, and lipids are the hallmarks of diabetes mellitus (DM), a metabolic illness that is marked by consistently elevated blood glucose levels. There is a possibility that diabetes mellitus is one of the oldest diseases that humans have ever encountered. In the beginning, it was mentioned in a document that was created in Egypt roughly three thousand years ago[3]. 1936 was the year that diabetes mellitus (DM) was separated into two distinct types: type 1 and type 2. 1988 was the year that marked the beginning of the recognition of type 2 diabetes mellitus (DM) as a component of metabolic syndrome. The condition known as diabetes mellitus (DM) can have a number of different causes and aetiologies; nevertheless, along the course of the disease, all of these disorders frequently exhibit abnormalities in either insulin sensitivity or insulin secretion, or both. The development of type 2 diabetes is determined by a complex combination of risk factors that include

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environmental variables, genetic factors, and risks associated with behaviour. That is, three, four, and six. There are a number of factors that have been related to diabetes, including genetic flaws, various disorders, and the hormonal milieu during pregnancy, and some drugs[4]. On account of its persistent clinical consequences and impacts on cardiac function, renal failure, retinal degeneration and blindness, diabetic ketoacidosis or hypoglycemia, and infection, diabetes mellitus has the potential to significantly raise morbidity and death rates[5]. The condition known as diabetes mellitus (DM) and the comorbidities that are linked with it can result in a variety of outcomes, including the failure of organ systems and chronic damage. Vascular abnormalities are caused by the interaction of low-grade inflammation, advanced glycation end products as a result of oxidative stress, and neovascularisation of the vasa vasorum.

These abnormalities, when combined with vascular illnesses, can lead to complications[6]. It is possible that diabetic peripheral nephropathy and neuropathy will become common sequelae of diabetes if the risk factors, which include hypertension, dyslipidaemia, and hyperglycemia, are not controlled well[6]. As a result of the risk variables not being adequately addressed, this consequence occurs. There is a possibility of neuropathy developing in thirty-three point five percent of the population. Since the beginning of time, it has been known that those who have diabetes that is dependent on insulin have a much increased risk of acquiring atherosclerosis. The metabolic condition known as diabetes mellitus (DM), which is marked by various metabolic abnormalities, is frequently associated with a significant disease burden in developing nations like Nigeria. The level of awareness regarding diabetes mellitus (DM) in Nigeria was insufficient at the beginning of the 1990s. In the past, people in Nigeria believed that diabetes mellitus was caused by "curses" or "hexes," and the diagnosis of the

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condition was determined by analysing the glucose levels in the blood or urine. Individuals who have diabetes mellitus (DM) are currently able to easily obtain and take oral hypoglycemic medicines; however, insulin is not a viable alternative at this time. Because the national health insurance system is inadequate, the majority of persons who have diabetes are responsible for bearing the financial burden of their own care. Because individuals are responsible for paying for their own diabetes management, the "out of pocket" expense is a consequence of this commitment. Those who are employed at minimum wage and use insulin would be required to allocate 29 percent of their monthly salary to the purchase of the prescription[7]. Individuals who have diabetes mellitus (DM) regularly make use of complementary and alternative treatments, which are widely considered to be an essential component of the management of DM. There are a number of indications and symptoms that are associated with diabetes mellitus, the most prevalent of which are polydipsia, polyuria, vision impairment, and weight loss. An insufficient amount of insulin activity on the tissues that are responsible for regulating glucose, lipid, and protein metabolism might lead to irregularities in these particular metabolic processes[8].

2.1.2 Classification of Diabetes Mellitus

In 1980, the World Health Organisation (WHO) produced the Second Report, which was the first classification that was universally acknowledged around the world. Type I diabetes and type II diabetes are the two basic kinds of diabetes mellitus that are distinguished from one, respectively. The prevalence of both types of diabetes is high. Additionally, it was discovered that there were other forms of diabetes present in addition to gestational diabetes. In 1985, when it was first published, Diabetes Mellitus: Report of a WHO Study Group was met with widespread praise, and it continues to be extensively utilised even now. In light of the fact

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that patients are classified according to medicine rather than pathology, it was proposed that the phrases "insulin-dependent diabetes mellitus" and "non-insulin-dependent diabetes mellitus" be removed from the vocabulary used in the medical field. In order to differentiate between the primary form of diabetes that is caused by inadequacies in insulin production and the cases that are primarily caused by the death of pancreatic islet beta-cells, the classifications Type I and Type II were formally utilised[10]. There is a possibility that the most important aspect of the new diabetes classification aims is the goal of combining etiological perspectives on diabetes care. Type 1 diabetes, type 2 diabetes, other specialised kinds of diabetes, and gestational diabetes are the four basic categories that are used to classify diabetes mellitus.

The outmoded and imprecise categories insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetic mellitus (NIDDM), which were defined by the World Health Organisation in 1980 and 1985, respectively, regarding diabetes mellitus, have been superseded by the four classifications of diabetes mellitus[9]. The Hyperglycemia is a symptom that is associated with a number of chronic metabolic disorders, including those that are related to diabetes. A significant number of diabetics do not fall into a single group, and the characteristics that are detected throughout the diagnosis process are crucial in defining the kind of diabetes that they contain. It is possible for a woman who has been diagnosed with gestational diabetes mellitus (GDM) to continue to have elevated blood glucose levels after delivery, which could increase the likelihood that she would be diagnosed with type 2 diabetes[10]. There is a widespread and pressing health risk that is affecting people all over the same world. Multiple variables, including but not limited to: increased development, age-related dietary changes, decreased physical activity, and other harmful lifestyle and

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behavioural patterns, have contributed to the worsening of the problem. Multiple factors have contributed to the problem. There are numerous lesser kinds of glucose intolerance, with impaired glucose tolerance being the most recognised one[11]. Diabetes mellitus and many other forms of glucose intolerance are today affecting nearly every population on the planet. In the absence of suitable preventative and control measures being put into place, epidemiological research indicate that it is anticipated that this tendency will continue moving forward. The rise in blood sugar levels can be attributed to a number of different sources, including genetic and acquired influences. Attention should be paid to this matter. Therefore, either the amount of insulin that is produced is decreased, or the insulin's ability to keep euglycemia stable is significantly diminished.

The fact that hyperglycemia is so common suggests that its clinical expression and progression are likely to vary from person to person, dependent on a variety of factors, including those that are inherited and those that are environmental. Throughout the course of history, both type 1 and type 2 diabetes have been subjected to analysis and research. Diabetes mellitus during pregnancy is a disorder that often goes away after the delivery of the baby[11]. The presence of complete insulin insufficiency and a significant incidence of autoantibodies that assault pancreatic islet beta cells are the defining characteristics of type 1 diabetes. One of the most important contributors to type 2 diabetes is insulin resistance. Due to the fact that it is useful in practice, this classification continues to be valuable for the treating physician, despite the fact that it appears to be straightforward in contemporary culture. Due to the fact that patients are in need of a definitive response regarding their insulin requirements and the progression of their condition, this situation has come about. At the moment, diabetes manifests itself in a variety of distinct forms. Examples of these

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illnesses include latent autoimmune diabetes in adults (also known as LADA) and maturity-onset diabetes of the young (also known as MODY). The condition known as monogenic diabetes, also known as MODY, is caused by anomalies in genes that are related with the formation of the pancreas, the activity of beta cells, or glucose recognition. A subtype of type 1 diabetes, LADA is essentially a subtype of diabetes. In diabetics, the LADA11 virus affects ten percent of the general population[11].

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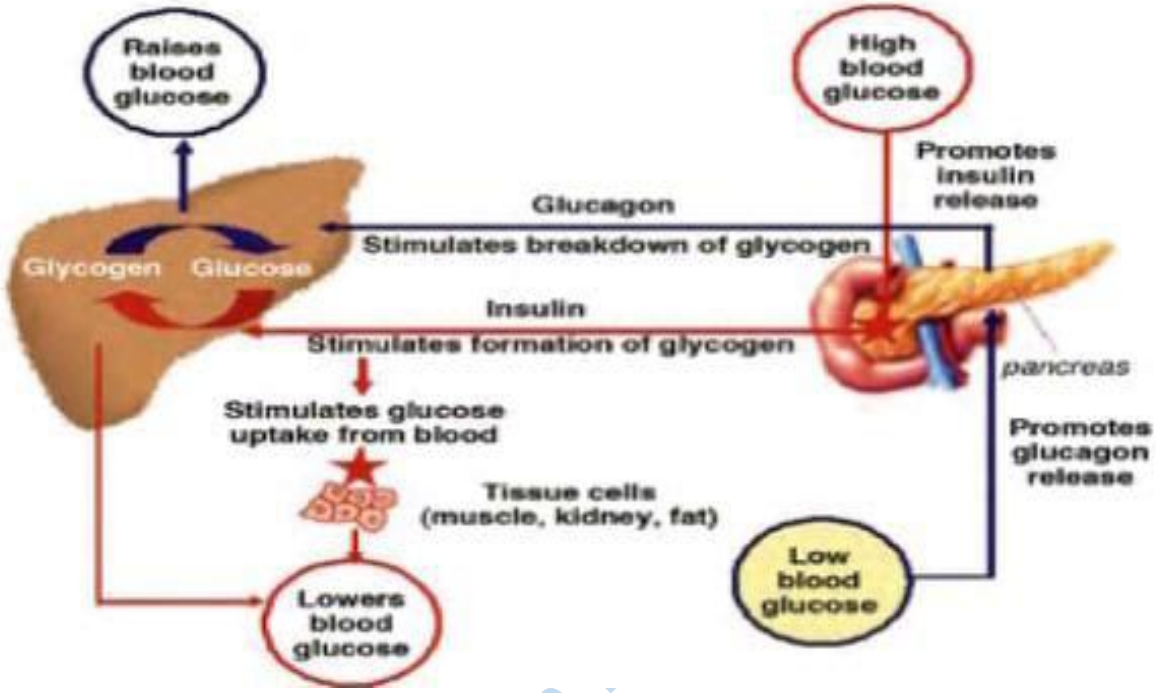


Figure 2.1 Homeostasis of Glucose[2]

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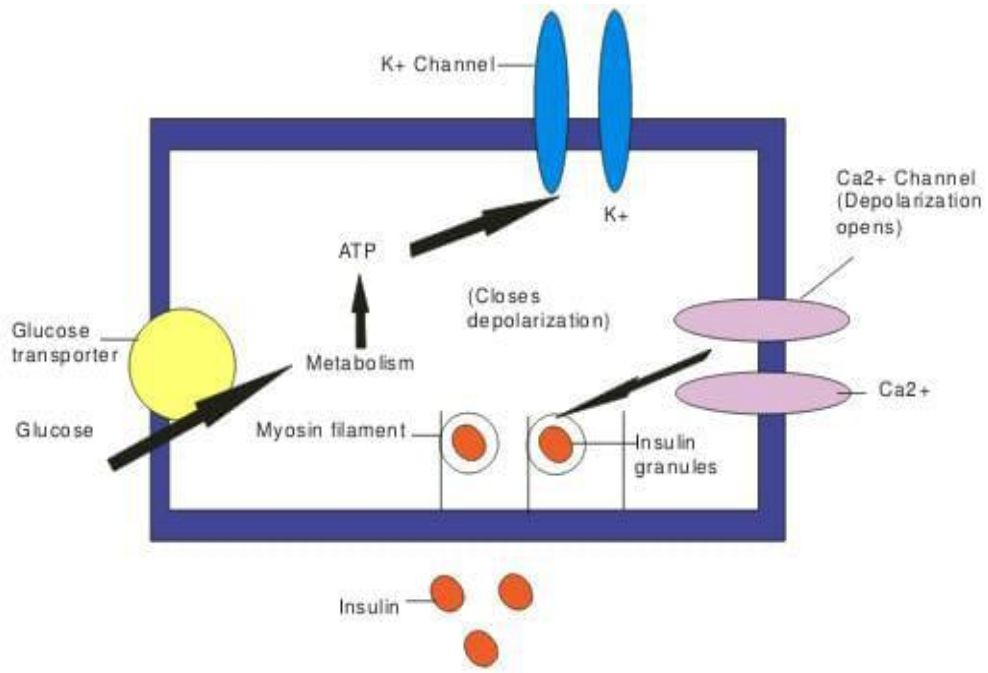


Figure 2.2 Control of insulin release from the pancreatic ß-cells by glucose[2]

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Individuals who suffer with type 1 diabetes mellitus, also referred to as juvenile diabetes, have a complete lack of insulin in their bodies. This illness is characterised by an autoimmune mechanism that causes beta cells to be destroyed, which ultimately leads to an inadequate amount of insulin[12]. There is a high probability that individuals who have type 1 diabetes have antibodies that target glutamic acid decarboxylase, islet cells, or insulin. The existence of these antibodies is evidence that the autoimmune processes that would ultimately result in the death of beta cells are already in the process of being established[12]. Individuals with type 1 diabetes must consistently undergo insulin therapy to maintain their blood glucose levels within the normal range.

The majority of people who are diagnosed with type 1 diabetes, also known as juvenile diabetes, are children and adolescents. However, adults may also be affected by this condition on occasion[13]. In type 1 diabetes, the pancreatic beta cells that are responsible for insulin production are attacked and destroyed by the immune system. This is the only characteristic of type 1 diabetes. The result is that the body either produces an insufficient amount of insulin or generates none at all[13] in total there is no truth to the notion that diabetes can be caused by consuming a lot of sugar.

Those who have type 1 diabetes are afflicted with the condition because their immune system destroys the beta cells that are responsible for making insulin. Diseases that are caused by mutations or anomalies in a single gene are the root cause of monogenic diabetes, which is a rare form of diabetes. The term "monogenic diabetes" refers to a multitude of distinct forms. Newborn diabetes mellitus (NDM) and maturity-onset diabetes of the young (MODY) are the two primary kinds of monogenic diabetes[14].

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Both of these conditions are distinguished by their similarities. In total, it is more likely that non-autoimmune type 1 diabetes mellitus (NDM) is the cause of diabetes that manifests itself before the age of six months, in contrast to autoimmune type 1 diabetes mellitus (T1DM). The early onset of mild hyperglycemia within the context of autosomal-dominant genetic disorders is what distinguishes the MODY group from other groups. Insulin resistance is not the primary problem; rather, the basic issue is malfunction in beta cells. A total of eight different genes have been identified as being associated with MODY as a consequence of mutations. In addition to the demographic of young people who are typically diagnosed with type 1 diabetes, there is also a community of elderly adults who endure a slower progression of the disease. During the latter years of their lives, they may exhibit symptoms of type 2 diabetic mellitus (T2DM). However, tests of the anti-glutamic acid decarboxylase (GAD) antibody will demonstrate autoimmune activity, which will ultimately lead to insulin dependence. Latent autoimmune diabetes in adults (often abbreviated as LADA) is the name given to this condition[14].

Children all around the world are affected by type 1 diabetes, which is a condition that is both chronic and dangerous. It has been calculated that the mortality risk in prosperous regions is eight to ten times higher than the danger in nations that are economically disadvantaged. When it comes to wealthy nations, the majority of incidents either result in death within a short period of time or go unnoticed by medical workers unless they are extensively reviewed or recorded[14]. The incidence of type 1 diabetes is said to exhibit a sixtyfold internal gradient during epidemic periods, according to a hypothesis that has been put up. In order to accurately establish the effects of diabetes on healthcare and the economy, it is necessary to do research and analysis on the incidence, mortality, morbidity, and

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healthcare related with type 1 diabetes all over the world[15]. One reason for this is because diabetes is a disorder that almost everyone has at some point in their lives. Furthermore, it is vital to design and carry out training programs in diabetes epidemiology on both a national and a worldwide scale in order to prevent and address the serious consequences of diabetes and the impacts that are linked with it. Approximately five to ten percent of all instances of diabetes are type I diabetes; however, the prevalence of this form of diabetes is quickly increasing all over the world, which has major short-term and long-term repercussions. According to the World Health Organisation Consultation Report, type I diabetes is defined as the death of beta cells in the pancreas, which might potentially lead to diabetes mellitus. The phrase "insulin is vital for survival" is appropriate in this circumstance because it helps to prevent the beginning of ketoacidosis, coma, and death. Interactions between genetic predisposition and environmental circumstances can either stimulate or inhibit the development of type 1 diabetes.

These interactions are complicated and involve a number of different components[15]. This interaction ultimately leads to the death of pancreatic β-cells, which ultimately leads to an insufficient amount of insulin being produced. The HLA genotypes DQ and DR are principally accountable for the transmission of the disease among individuals through genetic means. On the other hand, these alterations may either provide protection against the disease or increase an individual's susceptibility to the start of the disease. When it comes to beginning β-cell death and changing immune function, environmental elements, such as viruses, chemicals, or nutrients, often play a crucial role. This is in addition to the hereditary predisposition that is generally present. The T-cell-mediated immune system is stimulated as a result of this process, which results in inflammation and the formation of autoantibodies

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such as IAA, GAD, and IA2. They may present itself many years prior to the clinical manifestations of type 1 diabetes as well. In children, the clinical presentation is often acute, and it is characterised by severe sensations of thirst, polyuria, and a loss in weight. Adults who have residual β-cell function may not have any symptoms for a considerable amount of time; however, children frequently exhibit acute symptoms[16]. The management of type I diabetes involves continuing care, which includes the administration of insulin, monitoring of blood glucose levels, the planning of diets, and the determination of potential complications. Due to the many different components that make up this treatment, it is best successfully delivered within a healthcare team that is comprised of multiple disciplines. The principal causes of morbidity and death that are associated with type I diabetes are the sequelae, which include diseases that affect both the macrovascular and microvascular regions of the body[15].

The etiological factors that are contributing to the increased incidence of type 1 diabetes in children have not been thoroughly researched and documented. Despite this, there has been a significant increase in the number of cases of type 1 diabetes among children around the world towards the end of the 20th century. There have been consistent shifts in both the geographical and temporal contexts of the progression of type 1 diabetes[16]. There is a possibility that the identification of the elements that contribute to the occurrence of type 1 diabetes will lead to the development of treatments for the disorder. Through the utilisation of chemicals that are produced from 1, 3, 4-thiadiazine, there have been initiatives put into place to treat the abnormalities that are inherently connected with diabetes. An increase in nsulin levels as well as a decrease in blood glucose and HbA1c have been observed in rats as a consequence of these measures[15]. Therapeutic treatments for diabetes are administered by the use of chemicals that impede the pathogenetic route that has been developed.

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The large growth in disease burden in Europe and North America, in conjunction with their significant influence on the trajectory of global health, has resulted in type 1 diabetes being increasingly accepted as a non-communicable condition that is the subject of widespread discussion on a global scale[3].

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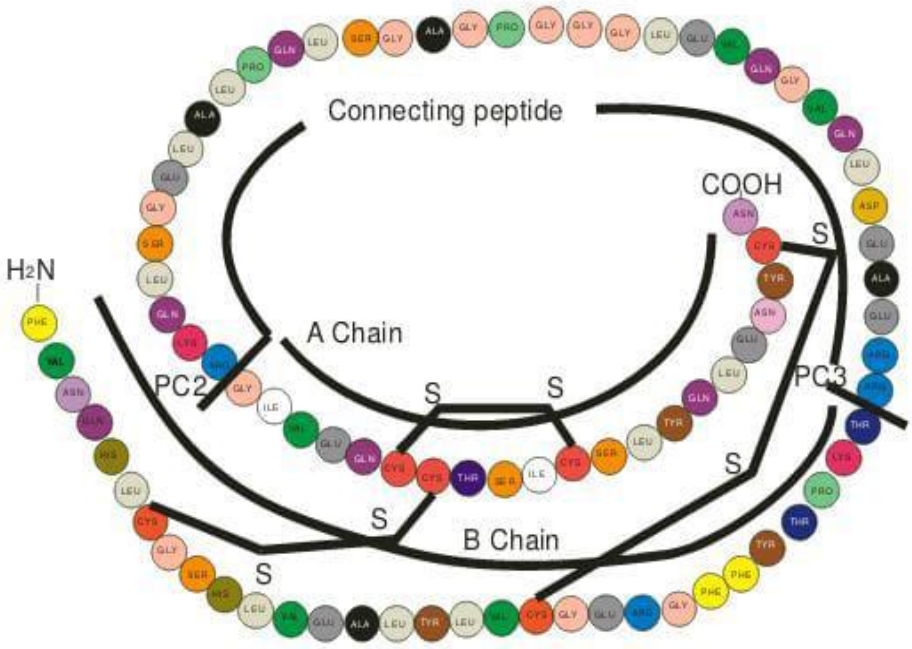


Figure 2.3. Structure of Human Proinsulin[16].

Insulin is shown as the shaded peptide chains, A and B.

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2.1.2.2 Type 2 Diabetes Mellitus

Discussions on the roles that insulin secretion abnormalities and deficiencies in peripheral hormone action play in the aetiology of diabetes mellitus have been and will continue to be relevant in the future. Patients with diabetes are identified as having type 2 diabetes mellitus (DM2) between 80 and 90 percent of the time[16]. The type of diabetes that is the most common is referred to as type II diabetes. Despite the fact that millions of people throughout the world have been diagnosed with type 2 diabetes, a significant proportion of people continue to go undetected. In the case that diabetes is not effectively detected or managed, individuals who have the condition are at a greater risk of experiencing cardiovascular events such as heart attacks and strokes.

Furthermore, patients have a higher risk of becoming blind, they need to have their feet and legs amputated because of damage to their nerves and blood vessels, and they develop renal failure that requires them to undergo dialysis or need a transplant[16]. The condition known as "prediabetes" is characterised by blood glucose levels that are higher than the normal range but do not meet the criteria for a diagnosis of diabetes. People who are at risk for developing type 2 diabetes are frequently found to have prediabetes, which is a precursor to the disease. According to the findings of a recent study, prediabetes may be causing long-term damage to the body, particularly to the cardiovascular system.

The presence of visceral obesity, which is often referred to as intra-abdominal obesity, is a common sign of type 2 diabetes. There is a strong correlation between insulin resistance and visceral adiposity. In addition, these individuals generally have hypertension and dyslipidaemia, which is marked by raised triglyceride levels and decreased HDL cholesterol levels. This condition is commonly referred to as postprandial hyperlipidaemia.

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There are a number of characteristics that are strongly connected with this prevalent form of diabetes mellitus, including advanced age, obesity, and insufficient level of physical exercise.[2] People of all ages, including children, are at risk of developing type 2 diabetes, which is also known as adult-onset diabetes. This condition can manifest itself in a variety of ways. In general, those who are middle-aged or older are the most common demographic of patients who have been diagnosed with type 2 diabetes. There is a correlation between those who are obese and lead a sedentary lifestyle and an increased likelihood of developing type 2 diabetes. The condition known as insulin resistance is characterised by the inability of adipose, muscular, and hepatic cells to utilise insulin for the purpose of transferring glucose into cells for the purpose of energy production.

This state is indicative of the initial stage of the disease. As a consequence of this, the body needs a greater supply of insulin in order to enable the uptake of glucose into the cells. In the beginning, the pancreas produced a greater supply of insulin in order to meet the growing demand. Following a meal, an increase in blood glucose levels causes the pancreas to gradually reduce the amount of insulin that it secretes. Insulin is produced in an insufficient amount by the pancreas, which ultimately results in the development of type 2 diabetes. People who have type II diabetes either have an insufficient amount of insulin produced by their bodies or the cells in their bodies neglect to produce insulin. If insulin is not present, the body will be unable to use glucose as a source of energy[17].

The body converts all of the sugars and carbs that are consumed during a meal into glucose, which serves as a source of energy throughout the body serves as the primary source of energy for the cells in the body. It is through the action of insulin that glucose can be transported from the bloodstream into the cells. When glucose levels in the bloodstream grow

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rather than being digested by cells, difficulties might emerge as a result of diabetes. The primary factor that contributes to the pathogenesis of type 2 diabetes mellitus in humans is an inadequate amount of insulin secretion. According to the data, the aberrations that the patients have are quantitative as well as qualitative, and they vary in terms of their severity. As the first sign of the secretory dysfunction that ultimately results in hyperglycemia, this is the initial signal. It has been demonstrated through research that persons who have substantial hyposecretion frequently exhibit elevated levels of glycemia. A graphical representation of the relationship between fasting and postprandial glycemia and insulinemia was used to construct the inverted U curve, often known as the Starling curve. When glycaemic levels are decreased, insulin levels are proportionately reduced as well. This is because the relationship between the two is inverse, which means that the relationship is inverse[17]. On the other hand, a decrease in insulinemia is shown to be connected with higher glycemia.

When it comes to patients who have type 2 diabetes, persistent hyperglycemia, also known as glucotoxicity, makes the progression of the illness worse by exacerbating the decline in insulin sensitivity against glucose. A damaging feedback loop is triggered when there is an increase in blood glucose levels. This is because glucose alone has the ability to change beta cells, which paradoxically results in a 17 percent reduction in insulin secretion. Individuals who have type 2 diabetes mellitus have anomalies in the biphasic insulin secretion curve after being stimulated with glucose through intravenous administration. An absence of the beginning segment of the curve, a decrease in height for the subsequent segment, and a duration that is greater than what is often observed in a conventional curve are all characteristics of this curve. As a consequence of these modifications, which have been

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present ever since the pre-diabetic stage, the efficiency of insulin is considerably reduced. In addition, studies have shown that individuals with type 2 diabetes have much lower insulin secretion pulses in response to glucose stimulation. As was said in reference, insulin, along with other hormones, performs its duties more efficiently when it is released in pulses rather than continually[17]. The presence of type 2 diabetes is associated with a wide variety of pathophysiological problems. Functional malfunction of β-cells and insulin resistance in the skeletal muscles and liver are the primary factors that contribute to this condition. When persons with type 2 diabetes experience the onset of glucose intolerance, it is linked to the interactions that occur between adipocytes, the gastrointestinal tract, α-cells, the kidneys, and the brain. Insulin resistance and insulin secretory dysfunction, which are both important indicators of type 2 diabetes, may also have a substantial role in predicting body mass index (BMI), fasting plasma glucose (FPG), and a history of diabetes in the family. When it comes to determining the severity of diabetes, these signs are absolutely necessary.

The presence of insulin resistance leads to an excessive amount of glucose being synthesised in the liver during the basal state. It is important to note that this holds true regardless of the presence of fasting hyperinsulinemia and the ineffectiveness of insulin on reducing hepatic glucose production [34]. The development of insulin resistance in skeletal muscle, which is the earliest detectable anomaly of type 2 diabetes, leads to postprandial hyperglycemia as a consequence of decreased glucose absorption following meals that are high in carbohydrates. Obesity and sedentary behaviour are two factors that significantly contribute to the prevalence of type 2 diabetes. Insulin resistance is made worse by obesity, which is connected with an increased risk of type 2 diabetes. Visceral fat buildup is particularly detrimental to the development of diabetes. In this particular circumstance, the pancreatic β-

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cells are subjected to a substantial amount of stress, which encourages them to produce and secrete increased quantities of insulin, as well as to generate additional β-cells from progenitor cells. All of this is done in order to restore the insulin function that has been damaged. Over time, the inability of β-cells to adapt for insulin resistance leads to an increase in blood glucose levels. This elevation reaches the thresholds of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), finally leading to the progression to the overt stage of type 2 diabetes. The annual probability of transitioning from type 1 diabetes to type 2 diabetes is estimated to fall somewhere in the region of five percent to ten percent. Patients who are severely obese and have high insulin resistance are approximately one-third more likely to acquire type 2 diabetes[17].

This is a statistics that is truly remarkable. There is a possibility that the diagnosis of type 2 diabetes could be delayed for years, despite the fact that the disease manifests itself gradually with few symptoms. Individuals experience a decrease in insulin secretion and sensitivity as they age, which results in an increase in the likelihood of acquiring type 2 diabetes. This also applies to people who are older, as their susceptibility to certain diseases is raised as they get older. As a result of the progressive nature of the disease and the decrease in insulin secretion, at least fifty percent of people who are diagnosed with type 2 diabetes acquire an absolute insulin deficit within ten years of receiving their diagnosis[15].

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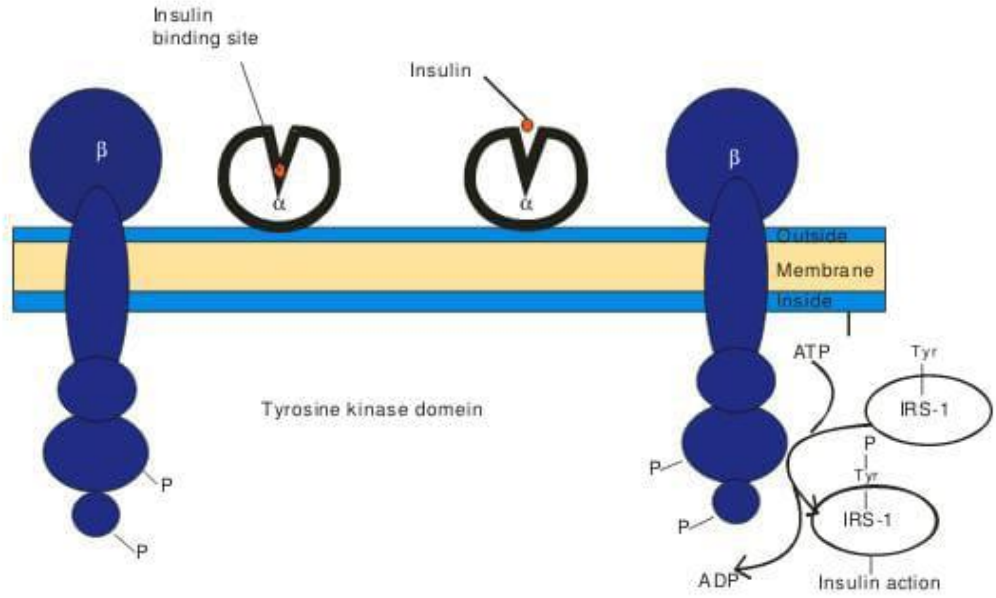


Figure 2.4 Probable Structure of the Insulin Receptor Tetramer in the Activated State[17]

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2.1.2.3 Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus is not a pathophysiological condition; rather, it is a categorisation system that determines how women who develop diabetes mellitus during pregnancy are classified functionally. A collective term known as gestational diabetes mellitus (GDM) is used to refer to both untreated, asymptomatic Type 2 diabetes mellitus identified during pregnancy as well as Type 1 diabetes mellitus that occurs in pregnant women. Both conditions are included in the category of gestational diabetes mellitus. In certain cases, women who go on to acquire gestational diabetes mellitus will exhibit symptoms of the condition during the third trimester of their pregnancies[17]. A total of around 415 million people around the world have been diagnosed with diabetes, with an estimated 318 million of those individuals exhibiting impaired glucose tolerance. The treatment of diabetes accounts for twelve percent of the total expenditures made in healthcare around the world, which has significant repercussions for the economy[18]. In order to accurately determine the true prevalence of gestational diabetes mellitus, there are a number of formidable challenges to overcome.

This is because the racial and ethnic composition of each group causes the incidence to vary from country to country and population to population. It has been found that women of Hispanic American, Native American, African, Pacific Islander, South Asian, and East Asian heritage are more likely to suffer from this illness than those of Caucasian descent[16]. According to estimates, the prevalence of gestational diabetes mellitus (GDM) in infants around the world is approximately 16.50%. Predictions indicate that this incidence is on the rise, which might be ascribed to the ongoing obesity pandemic[16]. The incidence of gestational diabetes mellitus (GDM) is influenced by a number of factors, including the diagnostic criteria, the screening procedures that are utilised (whether they are universal or selective),

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and the prevalence of type 2 diabetes in a particular nation. On the other hand, information from Western countries is consistently provided, whilst data from developed nations is difficult to obtain. The principal metabolic disorder that has an effect on glucose metabolism during lactation is referred to as gestational diabetes mellitus. The percentage of people who are affected by this disorder ranges anywhere from one to fourteen percent, depending on the demographic category that is involved. The identification of glucose intolerance that develops or is detected for the first time during pregnancy is essential to the process of diagnosing gestational diabetes mellitus (GDM) [19].

In 2013, sixteen percent of all live births around the world were affected by hyperglycemia during pregnancy, according to research that was published by the International Diabetes Federation[20]. According to the information presented in the article, it is projected that the prevalence of gestational diabetes mellitus (GDM) would increase in the future due to the increased prevalence of risk factors such as weight gain and physical inactivity for pregnant women. In the context of clinical research, the prevalence of gestational diabetes mellitus (GDM) in sub-Saharan Africa has not been adequately investigated. Over the course of the 1980s, the prevalence of the condition among pregnant patients in Nigeria increased from 0.3% to 15.30% in 2014[21]. The prevalence of gestational diabetes mellitus (GDM) in Nigeria is estimated to be around 500,000 women. Individuals who have been diagnosed with type 21 diabetes are at a greater risk of developing hypertensive diseases, such as preeclampsia, eclampsia, and pregnancy-induced hypertension. The findings of the study indicate that there is a positive link between the levels of glucose that were measured during the initial glucose tolerance test and the likelihood of developing preeclampsia. 5.90% of patients were found to have acquired gestational hypertension, although only 4.80% of patients were diagnosed with

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the illness, according to the findings of the study. During their pregnancies, 6.3% of women developed preeclampsia, and 5% of women experienced gestational hypertension, according to the findings of a different study that produced results that were equivalent to those above[23]. Diabetes mellitus occurring during pregnancy is linked to a wide range of problems, which can affect both the mother and the developing child. Both macrosomia and delivery issues, which are typically associated with foetal overgrowth, are included in this category. Such conditions as shoulder dystocia, Erb's palsy, and hypoxia, in addition to an increase in the number of caesarean sections that are performed, are mentioned here. In women who have macrosomia, shoulder dystocia is a delivery complication that happens more commonly than in other conditions. The delivery of babies who were born to mothers who were suffering from gestational diabetes mellitus (GDM) is usually accomplished through the use of caesarean sections[24].

Due to the fact that these newborns are dependent on maternal hyperglycemia, sometimes referred to as foetal hyperinsulinemia, they are at a greater risk of experiencing hypoglycemia after delivery. If this condition is not properly controlled, it can lead to brain damage. Additionally, there is evidence to show that gestational diabetes mellitus (GDM) is associated with an increased risk of stillbirth. Children who are born to mothers who were diagnosed with gestational diabetes mellitus have a higher likelihood of acquiring type 2 diabetes mellitus (T2DM), obesity, cardiovascular disease, and a number of metabolic problems. It is estimated that children who are born to mothers who have gestational diabetes mellitus have approximately twice the likelihood of becoming obese as children when compared to children who are born to moms who do not have diabetes. In addition, in children as young as five years old, poor glucose tolerance can be found. This is true even

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after taking into account the possibility of confounding variables, such as those associated with the body mass index of the mother. Hyperbilirubinemia, hypocalcaemia, respiratory distress syndrome, and polycythaemia are all illnesses that have the potential to manifest themselves; yet, the reported prevalence of these conditions varies from population to population. In recent ground-breaking research, it has been established that reducing maternal hyperglycemia can have an effect on certain adverse outcomes. The infant's birth weight reduced from 3.48 kilogrammes to 3.33 kilogrammes as a result of careful monitoring of blood glucose levels and management of gestational diabetes. Additionally, the prevalence of macrosomia, which is defined as birth weight that is greater than 4 kilogrammes, decreased from 21% to 10%.

A further point to consider is that those who have type 2 diabetes mellitus (T2DM) have a higher likelihood of experiencing thromboembolic events and coagulation abnormalities. When various signalling pathways and metabolic processes are disrupted, platelets exhibit an increased inclination to stick to one another, become activated, and aggregate. These abnormalities include insulin resistance, hyperglycemia, and dyslipidaemia, all of which have been described in patients who have gestational diabetes. The utilisation of platelets is typically employed for a particular and exclusive function. Inflammation throughout the body, oxidative stress, poor calcium metabolism, decreased nitric oxide bioavailability, and increased phosphorylation and glycosylation of cellular proteins all contribute to an increase in platelet activation and the production of chemicals that promote thrombosis and inflammation. Each of these characteristics is linked to the development of gestational diabetes. There is a considerable probability that gestational diabetes mellitus (GDM) can be caused by both low birth weights and high birth weights. The presence of maternal or

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placental insufficiency or undernutrition during pregnancy is a typical factor that contributes to the development of gestational diabetes mellitus. This can be attributed to the link that exists between insulin resistance and low birth weight in the development of this illness. Furthermore, it has been hypothesised that the foetus could ameliorate the symptoms of famine during pregnancy by epigenetically altering the expression of genes that are linked with the storage of fat, the utilisation of energy, and the regulation of appetite[25]. In the postpartum period, the incidence of gestational diabetes mellitus (GDM) is dramatically reduced in women who adhere to a nutritious diet and regular exercise while minimising high-risk variables before to becoming pregnant, according to research. A person must exercise extreme caution in order to prevent the development of gestational diabetes.

There is a possibility that the likelihood of being pregnant can be decreased by attaining quick weight loss and maintaining a healthy weight prior to becoming pregnant[25]. There has been an increase in the number of cases of diabetes mellitus during pregnancy. Before becoming pregnant, it has been demonstrated that consuming an excessive amount of red meat, animal fat, cholesterol, and blood products can increase the likelihood of developing gestational diabetes mellitus (GDM) [20]. There is a considerable relationship between obesity and overweight during pregnancy and the prevalence of gestational diabetes mellitus (GDM), which can either greatly increase or decrease the prevalence of the condition[25]. A reduction in the likelihood of acquiring type 2 diabetes can be achieved by the management of nutrition and the maintenance of a diet that is balanced. Reducing the amount of calories and fat that you consume from your meals will help reduce the weight gain that is associated with pregnancy. It has been established that physical activity is effective in

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regulating blood glucose levels and preventing, lowering, or delaying the need for insulin among those with diabetes. Exercising is beneficial for making adjustments to one's lifestyle after giving birth, to ensure proper nutrition for the infant, and to prevent the development of gestational diabetes in the future. There were six trials that included 322 women with type 2 diabetes and 20 participants. The results of these trials showed that dietary guidance and exercise led to considerable weight loss and decreased HbA1c levels among the participants[26]. According to the findings of study, a variety of exercise methods, ranging from low to high intensity, such as yoga, aerobics, and jogging, have been determined to be safe for both the mother and the unborn. By engaging large muscle groups through actions that are either continuous or intermittent, the most effective kind of aerobic exercise is called aerobic exercise. Its primary activities consist of activities such as climbing stairs, swimming, boating, jogging, walking, stationary cycling, skipping, and other exercises of a similar nature.

Out of these twenty different options for transportation, walking is the most common means of transportation[27]. An increased consumption of fat, cholesterol, and serum iron is linked to an increased risk of developing type 2 diabetes. This risk is connected with an additional risk factor. This indicates that nutrition is a crucial component in the course of treatment for the illness. It has been demonstrated that consuming foods that are high in fibre, such as cereals, legumes, nuts, and fish, can reduce the likelihood of acquiring type 2 diabetes. According to Neda Dolatkhah, a medical specialist, a proper eating regimen can assist pregnant women who have gestational diabetes mellitus in effectively regulating their blood glucose levels and promoting the growth of their foetuses. Furthermore, the utilisation of supplements in a prudent manner has the potential to enhance the metabolic processes of pregnant women who

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are diagnosed with gestational diabetes mellitus (GDM) and reduce the likelihood of foetal abnormalities. In the beginning, mothers who were diagnosed with gestational diabetes mellitus (GDM) would make use of dietary therapy in order to properly control their illness. In the event that nutritional therapy is shown to be ineffective, additional treatment management choices will be thoroughly examined. The patient's suffering will be exacerbated, and further medical costs will be incurred, to the extent that the pregnancy findings are negative[21].

The blood glucose levels of pregnant women normally fall during the duration of their pregnancy, with a level of 3.5 mmol/L being considered normal or asymptomatic. It is quite rare that women who are pregnant and have gestational diabetes and are not receiving pharmacological medication will experience hypoglycemia. The Clinical Guidelines for Queensland have been updated to their fifteenth version. Hypoglycemia is most commonly caused by medications that are used to treat diabetes. Insulin and sulfonylureas are two examples of medications that are commonly used to treat diabetes. Hypoglycemia is a condition that is more likely to occur in pregnant women who are undergoing insulin therapy for the treatment of gestational diabetes. Individuals who have diabetes and have a blood glucose level that is lower than 3.9 mmol/L are considered to be hypoglycemic by medical professionals[21].

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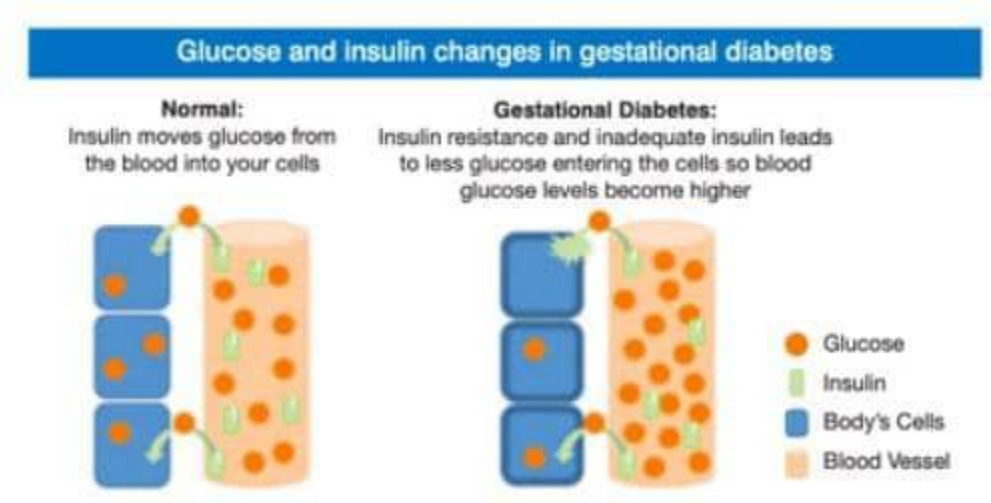


Figure 2.5 Gestational Diabetes Mellitus[1]

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2.1.2.4 Other Specific Type (Monogenic Diabetes)

The "Other Specific Forms" section contains further recognised etiological groupings of diabetes mellitus[27]. Fewer than 10% of the individuals in this group have pancreatic dysfunction that is caused by chemicals, medications, or infections. Exocrine pancreatic disorders account for ninety percent of the remaining occurrences. These include pancreatitis or cystic fibrosis, dysfunction associated with other endocrinopathies (such as acromegaly), or pancreatic impairment related to genetic problems in beta-cell function (previously known as MODY or maturity-onset diabetes of the young) [28].

2.1.3 Pathogenesis of Diabetes Mellitus

Between hyperglycemia and both physiological characteristics and behavioural responses, there is a direct association that occurs between the two. When hyperglycemia occurs, the brain is able to identify it and then sends a signal to the pancreas and other organs through the use of electrical impulses in order to alleviate the symptoms of the disease[29]. The risk factors for diabetes that can be managed include being overweight and leading a sedentary lifestyle. On the other hand, there are important risk variables that cannot be controlled, such as genetics and ethnicity, which have a big impact on the outcomes. Because type I diabetes is a chronic condition that lasts a lifetime, having a family history of the condition is the greatest risk factor for the illness. A history of diabetes in one's family is a substantial risk factor that should be taken into consideration to a significant degree. It is strongly recommended by the Standards of Medical Care in Diabetes published by the American Diabetes Association that those who have a parent or sibling who has been diagnosed with type I diabetes undergo screening for the condition. A straightforward blood test is all that is required to arrive at a diagnosis of type I diabetes. It is also possible for the

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pancreas to be damaged by diseases or injuries that hinder its ability to produce insulin, which could lead to the development of type I diabetes[29]. There are a number of extremely uncommon diseases and conditions that have the potential to cause damage to the pancreas, which could lead to the development of type I diabetes. The likelihood of getting type II diabetes can be increased on account of a number of different factors. Increasing age, insulin resistance, a history of diabetes in the family, genetic predispositions, obesity, dietary habits, physical inactivity, and racial or ethnic background are all factors that can contribute to the development of diabetes. Certain single nucleotide polymorphisms (SNPs) in gene 14 have been related to an increased risk of developing diabetes, according to analysis of genetic effects.

These genes have been associated with a number of health conditions, such as decreased insulin production, an increased risk of obesity, and reduced insulin sensitivity in the tissues of the body. It is believed that racial and ethnic traits are responsible for the increased prevalence of diabetes among a number of different ethnic groups, such as African Americans, Mexican Americans, American Indians, Native Hawaiians, and certain Asian Americans. Patients who fall into the aforementioned groups are more likely to suffer from cardiovascular disease and diabetes. Diabetes, obesity, and hypertension are all conditions that are more prevalent among these groups, which may be a significant contributor to the heightened incidence of these conditions. When compared to people of other ethnicities, the percentage of African Americans who have type 2 diabetes is significantly greater. Despite the fact that ethnicity and heredity are both substantial risk factors for diabetes, they are not the only factors that determine an individual's likelihood of getting the condition. Changes in eating habits and a decrease in physical activity, both of which have been brought about by

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urbanisation and rapid technological innovation, have been the primary contributors to the rise in the prevalence of diabetes. The presence of a history of substance abuse is a significant risk factor for the development of type II diabetes at an earlier age. There is a connection between it and the usage of illegal substances in the United States, according to research that has been accomplished. Despite the fact that cocaine and marijuana are the illegal narcotics that are used the most frequently, diabetic patients who are receiving primary care also frequently use heroin, amphetamines, hallucinogens, and inhalants that are not prescribed by their doctors[29].

2.1.3.1 Pathogenesis of Type 1 Diabetes Mellitus

The significant differences amongst pancreatic lesions make it difficult to understand the cause of selective cell death inside the islet in type 1 diabetes mellitus. In the beginning stages of overt hyperglycemia, pseudoatrophic islets are present. These consist of glycogen-producing alpha cells, somatostatin-secreting delta cells, and pancreatic polypeptide-producing PP cells, as well as normal islets and islets that contain both beta cells and infiltrating lymphocytes and monocytes. Lymphocytic infiltration is only seen in islets that have leftover lymphocytes[20]. This suggests that this sort of islet lesion may play a part in the long-term progression of type 1 diabetes mellitus. When identical twins donate their pancreas to their long-term diabetic twin siblings without the use of immunosuppressive drugs, the beta cells are destroyed quickly. This is different from the typical chronic course of the disease. Under these conditions, extensive insulitis progresses rapidly, and the presence of an anamnestic autoimmune response is indicated by T cell infiltration.[28] The autoimmune loss of pancreatic beta cells results in insufficient insulin production, which produces the metabolic abnormalities that are characteristic of T1DM. People with type 1 diabetes (T1DM) have

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pancreatic beta cells that do not work properly, produce more glucagon, and make less insulin. In healthy individuals, hyperglycemia decreases the amount of glucagon that is secreted. In people with Type 1 Diabetes Mellitus (T1DM), hyperglycemia does not have an impact on the secretion of glucagon. When glucagon levels are elevated, the metabolic issues that arise from low insulin levels become more severe[30]. The main problem with type 1 diabetes mellitus (T1DM) is that there is not enough insulin, and there are other challenges related to delivering it. When there is not enough insulin, lipolysis is unregulated, which leads to higher quantities of free fatty acids in the plasma. This hinders the metabolism of glucose in peripheral tissues, including skeletal muscle.

This decreases the amount of glucose that is absorbed, and the absence of insulin also results in a reduction in the expression of other genes, such as those that encode glucokinase in the liver and the GLUT-4 class of glucose transporters in adipose tissue. Both are essential for target tissues to respond to insulin properly[30]. In Type 1 Diabetes Mellitus (T1DM), the lack of insulin results in the body's inability to properly metabolise proteins, lipids, and glucose. These are the main metabolic problems that develop[20]. The significant differences amongst pancreatic lesions make it difficult to understand the cause of selective cell death inside the islet in type 1 diabetes mellitus. At the beginning of overt hyperglycemia, pseudoatrophic islets may be present. These consist of glycogen-producing alpha cells, somatostatin-secreting delta cells, and pancreatic polypeptide-producing PP cells, as well as normal islets and islets that contain both beta cells and infiltrating lymphocytes and monocytes[30]. Only islets with remaining lymphocytic cells exhibit lymphocytic infiltration, and it is likely that this variety of islet lesions contributes to the chronicity with which type 1 DM develops. When identical twin donors' pancreas is transplanted into their long-term diabetic twin mates

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without immunosuppression, -cells are quickly destroyed, in contrast to this chronicity in the disease's normal history. In these circumstances, an amnestic autoimmune reaction is indicated by the rapid development of a large insulitis with infiltrating T lymphocytes. Additionally, this discovery suggests that down-regulating events involved in the immunopathogenesis of the illness are the cause of the chronic time course in type 1 DM (but not in a transplanted pancreas) [31]. In all animal models of type 1 DM, activation of islet antigen-specific CD4+ T lymphocytes appears to be a necessary but not sufficient condition for the onset of diabetes. When injected into pre-diabetic or non-diabetic Fl mice, CD4+ islet-specific T-cell clones from diabetic NOD mice cause insulitis and diabetes. According to another paper, CD4+ T cells alone are enough to cause insulitis; however, CD8+ T cells help determine how severe the damage is [32]. These results show that CD4+ T cells may be the sole immunocompetent cells necessary for the disease process, along with evidence that insulitis in chronic graft versus host illness may develop in the absence of CD8+ T cells. However, it appears that the induction of illness is limited to one subset of CD4+ T cells[32].

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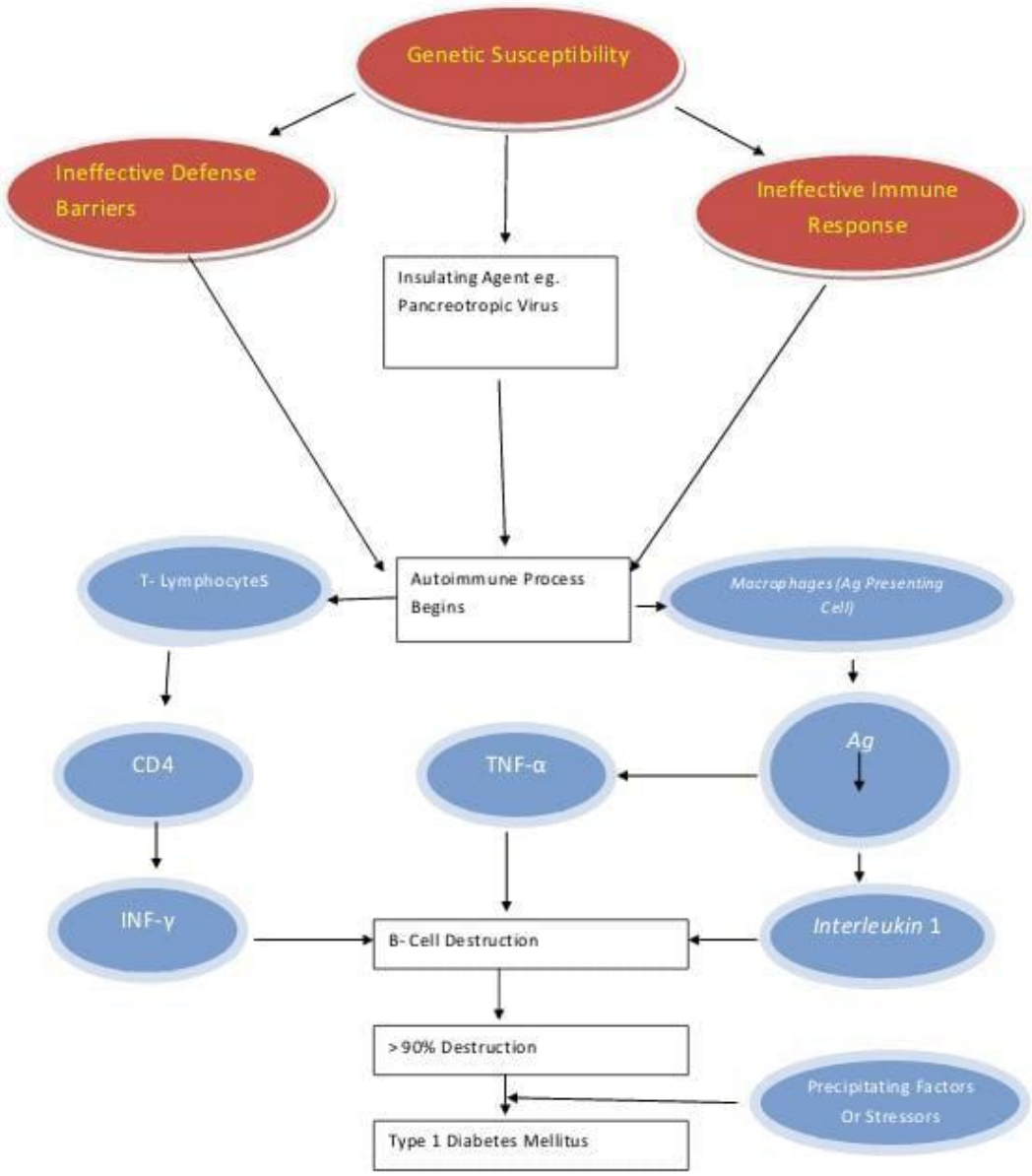


Figure 2.6 Pathogenesis of Type 1 Diabetes Mellitus[32]

2.1.3.2 Pathogenesis of Type 2 Diabetes

Through a tightly controlled and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and the secretion of insulin, blood glucose levels are kept within a narrow range under normal physiological conditions, despite significant fluctuations in supply and demand[20]. Due to the breakdown of these systems in type 2 diabetes, the two main pathophysiological abnormalities are reduced insulin production due to pancreatic beta-cell malfunction and impaired insulin action as a result of insulin resistance[33]. Compared to type 1 DM, type 2 diabetes mellitus has a stronger hereditary link, and its pathophysiology is marked by decreased insulin production and insulin resistance. It was reported that, the primary occurrences are thought to be an initial shortfall in the production of insulin and relative insulin insufficiency in many patients in conjunction with peripheral insulin resistance[34].

The effects of insulin resistance include insufficient regulation of hepatic glucose production, impaired triglyceride uptake by fat, and reduced insulin-mediated glucose uptake in the peripheral tissues (by muscle and fat). The amount of insulin released by islet cells will increase in order to combat insulin resistance. Patients with type 2 diabetes or impaired fasting glucose see an increase in endogenous glucose production. Hepatic insulin resistance is the primary source of the hyperglycemia associated with type 2 diabetes since this rise takes place in the context of hyperinsulinemia, at least in the early and intermediate disease phases[34]. Due to the breakdown of these systems in type 2 diabetes, the two primary pathophysiological abnormalities are reduced insulin production due to pancreatic beta-cell malfunction and impaired insulin action as a result of insulin resistance[20]. In the presence of insulin resistance, the quantity of insulin-secreting cells undergoes alterations that can

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augment insulin production to compensate for the heightened and irregular demand. Although the plasma insulin concentration (both fasting and meal-stimulated) is typically higher in absolute terms, it is typically insufficient to sustain healthy glucose homeostasis "related" to the severity of insulin resistance. Impaired glucose tolerance eventually results from insulin resistance and hyperinsulinemia[32]. The mode of inheritance for type 2 diabetes mellitus is unknown, with the exception of maturity-onset diabetes of the young (MODY). Mutations in the glucokinase gene on chromosome 7p may cause MODY, an inherited autosomal dominant condition.

When islet cell antibodies (ICA) are negative, MODY is defined as hyperglycemia diagnosed before the age of twenty-five and manageable for more than five years without insulin[33]. Type 2 diabetes is characterized by insulin insensitivity due to insulin resistance, diminished insulin production, and ultimately, the failure of pancreatic beta cells. Consequently, diminished glucose is conveyed to the liver, muscle, and adipose cells. Hyperglycemia leads to an elevation in lipolysis. It has recently been established that diminished alpha-cell function contributes to the etiology of type 2 diabetes. This dysfunction inhibits meals from reducing glucagon and hepatic blood glucose levels that increase during fasting[33]. Hyperglycemia arises from diminished insulin levels and increased insulin resistance. Incretins significantly mediate insulin release in the gut and, specifically for GLP-1, decrease glucagon. Although type 2 DM patients have reduced GIP activity, these patients nevertheless retain GLP-1's insulinotropic effects, making GLP-1 a potentially helpful therapeutic alternative. GLP-1 is quickly inactivated by DPP-IV in vivo, just like GIP is[34].

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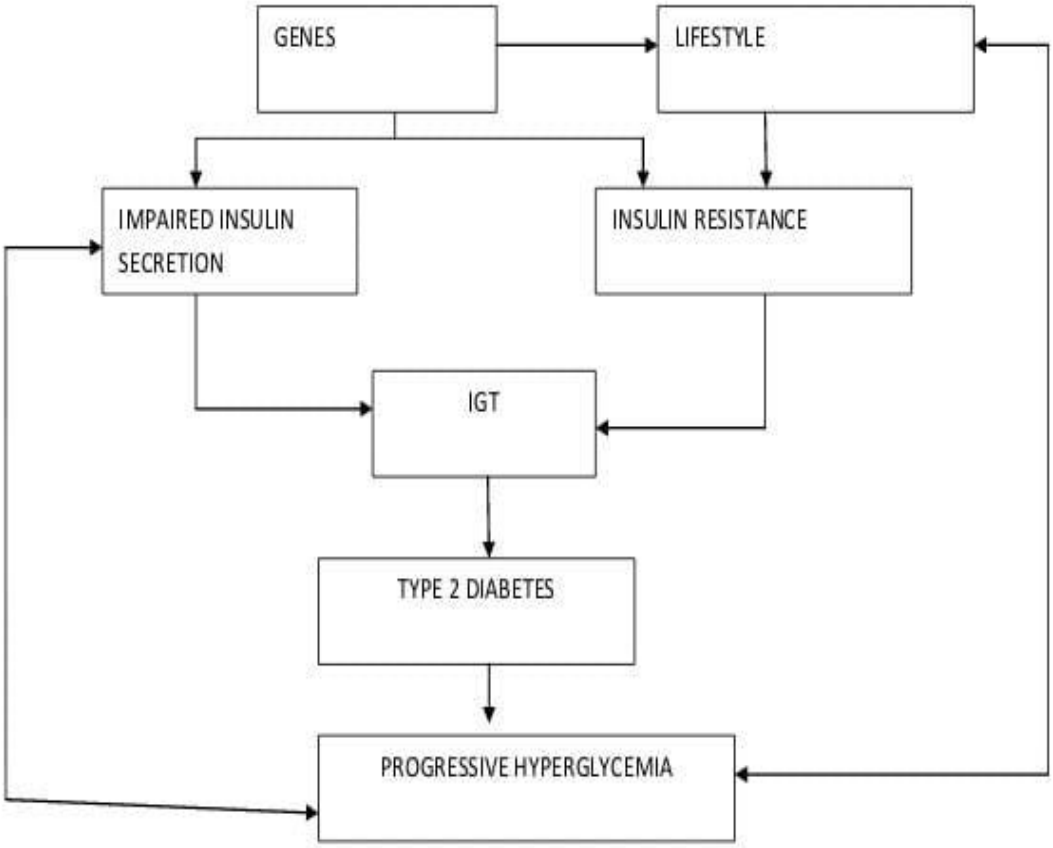


Figure 2.7 Pathogenesis of Type 2 Diabetes Characterized by Impaired Insulin Secretion and Insulin Resistance[33]

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2.1.3.3 Pathophysiology of Gestational Diabetes Mellitus

Over the course of a typical pregnancy, the maternal tissues gradually exhibit a decreased sensitivity to insulin concentrations. It is believed that hormones that are secreted by the placenta are the primary cause of this condition[34]. Additionally, there are other pathways that have not yet been discovered that are associated with pregnancy and obesity. It is possible for a pregnant woman to develop gestational diabetes mellitus (GDM) if she is unable to produce sufficient insulin to counteract the natural insulin resistance that she develops during her pregnancy[56]. There is no difference in the likelihood of developing gestational diabetes mellitus (GDM) between pregnant women who are underweight and those who are obese. As an additional point of interest, it has been asserted that the pathogenesis of the disease is believed to differ amongst different populations. Recordings have been made of this problem. Having a pre-existing increased level of insulin resistance that exacerbates the insulin resistance that is caused by pregnancy is the primary factor that determines the aetiology of pregnancy-induced insulin resistance in obese women.

One of the factors that is universally known to play a role in the development of metabolic syndrome is improved insulin resistance[35]. Despite the fact that a deficiency in the initial phase insulin response is more obvious, it seems that identical variables also influence women who are naturally thin. As a result of the cumulative effect of these impairments, insulin's capacity to regulate glucose levels is diminished, which finally results in hyperglycemia in moms who are 35 years old[18]. The placenta is responsible for the transmission of glucose, which is necessary for the growth of the foetus. Therefore, to offset the excess glucose transfer from the placenta, foetal hyperinsulinemia is triggered when the mother's blood sugar levels are elevated significantly. It is believed that the key cause

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contributing to foetal macrosomia, which is defined as a birth weight that is greater than 4000 grammes, is increased insulin levels throughout pregnancy[34]. Insulin levels that encourage growth are responsible for this condition. Additionally, the hormones that are secreted by the placenta during pregnancy have a variety of effects on the levels of glucose in the blood. In many cases, the lower blood glucose levels that are observed throughout the first half of the second trimester and the early phases of pregnancy can be attributed to the enhanced insulin sensitivity that is observed during these times[35]. Overt diabetes was diagnosed in women who were diagnosed with gestational diabetes mellitus and had blood glucose levels that were raised during the course of their pregnancy. During the later stages of pregnancy (after the second trimester), there is a correlation between rising blood sugar and free fatty acid concentrations and increased tissue resistance to insulin. Despite this, it is essential to maintain these elevated levels in order to fulfil the growing nutritional requirements of the foetus for its growth and development.

Post-receptor changes in the insulin signaling pathways are thought to be responsible for the pathophysiology of increasing insulin resistance, which has been discussed in the literature. It is possible for these abnormalities to become apparent at the beta subunit of the insulin receptor as well as at the level of insulin receptor substrate-1 level. For additional information, studies have shown that the cytoplasm of some cells has elevated quantities of the free p85 subunit of phosphatidylinositol 3 kinase. These characteristics are not exhibited by other cells. Inconsistencies in insulin signaling lead to a rise in insulin resistance, which in turn leads to a decrease in insulin-dependent glucose uptake in skeletal muscles, which is required for glucose utilisation. During the later trimesters of a typical pregnancy, it appears like the levels of glucose in the blood are increasing in this precise manner. Enhanced insulin

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production is activated by the mother's pancreatic beta cells through a physiological mechanism in order to maintain elevated blood glucose levels within the normal range. Insulin resistance can be eliminated due to the increasing insulin level, which helps in the process. Although there has been a rise, it is anticipated that the mother's glucose levels will remain within the normal range throughout the duration of the pregnancy[36]. There is a lot of complexity involved in the pathophysiology of gestational diabetes mellitus, which manifests earlier in pregnancy.

There is a possibility that this is connected to the inability of maternal pancreatic beta cells to produce a enough amount of insulin, which would help to reduce the level of insulin resistance in the tissues. There is a lack of complete comprehension on the pathways that lead to pancreatic dysfunction, which ultimately results in gestational diabetes mellitus. There are three different paths that have been identified as potential contributors to the birth of this illness. These pathways include genetic abnormalities that inhibit insulin secretion, autoimmune malfunction of beta cells, and beta cell damage that is connected to insulin resistance that has been present for a long period of time[36].

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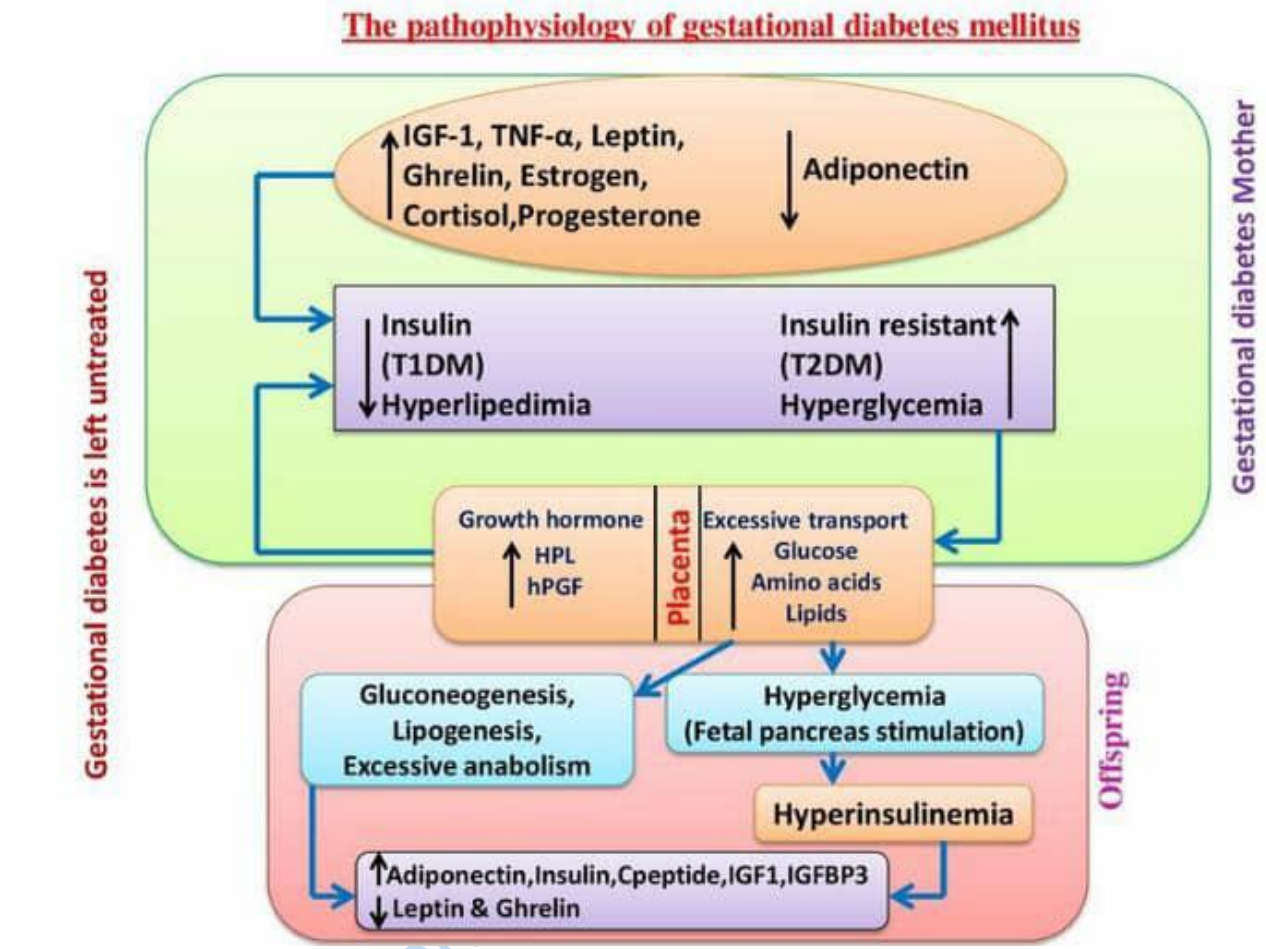


Figure 2.8 Pathophysiology of Gestational Diabetes Mellitus [36]

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2.1.4 Laboratory Diagnosis of Diabetes Mellitus

Diagnosis of diabetes and prediabetes, long-term glucose control, and detection and monitoring of dysfunctions and consequences are all goals of diabetes testing. It is a very efficient strategy to manage type-2 diabetes in overweight or obese individuals by decreasing a small amount of body weight. However, a proper diagnosis of diabetes and prediabetes necessitates the following tests: i) fasting plasma glucose (FPG; ideally done in the morning after a fast of more than 10 hours), ii) hemoglobin A1C or glycohemoglobin test, even though RPG (random plasma glucose; no overnight fasting) test[37]. Random plasma test is a straightforward test which does not call for fasting beforehand. If the blood glucose level is 200 mg/dl or more, diabetes is likely present but needs to be confirmed again[28]. Fasting plasma glucose test before taking the test, patients should have fasted for eight hours. Diabetes is diagnosed when the blood glucose level is greater than 126 mg/dl on two or more occasions and on distinct days[39].

Oral glucose tolerance test is performed when the fasting plasma test results are 110–125 mg/dl and the random plasma glucose test results are 160–200 mg/dl. This blood test measures how the body reacts to glucose. For this test, you must fast for at least eight hours but no longer than sixteen. After determining the fasting blood sugar level, 75 grams of glucose are given and 100 grams for pregnant women. Over the course of two or three hours, the blood is examined every 30 to 60 minutes. If the glucose level is less than 140 mg/dl after two hours, the test is considered normal. The diagnosis of diabetes is confirmed by a fasting glucose level of 126 mg/dl or higher and a two-hour glucose level of 200 mg/dl or higher[40]. Glycated proteins the blood, proteins and glucose react naturally to generate glycated derivatives. The amount of reactive amino groups present in the protein that are available to

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glucose for reaction and the blood glucose concentration both affect how much glycation occurs. All proteins containing reactive sites have the potential to be glycated, and the quantity of glycated proteins that can be detected in blood serves as a proxy for changes in blood glucose levels over time. Glycated proteins having a longer life span in blood are of relevance from a clinical diagnostic perspective, as they represent the exposure of these proteins to glucose for extended periods of time[39, 28]. Glycated Hemoglobin has a 90–120 day in vivo life span. Glycated hemoglobin A, a ketoamine molecule created by the interaction of hemoglobin A with glucose, develops during this period.

It has been possible to separate a number of glycated hemoglobin subfractions. Glycated hemoglobin A fraction HbA1c, which serves as a retroactive measure of the average glucose Concentration, is the one of these that is of most interest. It is advised that HbA1c be used as a crucial indicator for tracking blood glucose control. Diabetes is regarded as having a blood HbA1c above 6.5%[40]. Fructosamine test the plasma proteins primarily consist of albumin. Plasma glucose and albumin interact non-enzymatically because albumin also includes free amino groups. As a result, glycated albumin can also be used as a blood glucose measure. In order to assess the average blood glucose concentration over a period of 1 to 3 weeks retrospectively, glycated albumin is typically obtained. 205–285 micromol/L is the reference range[28].

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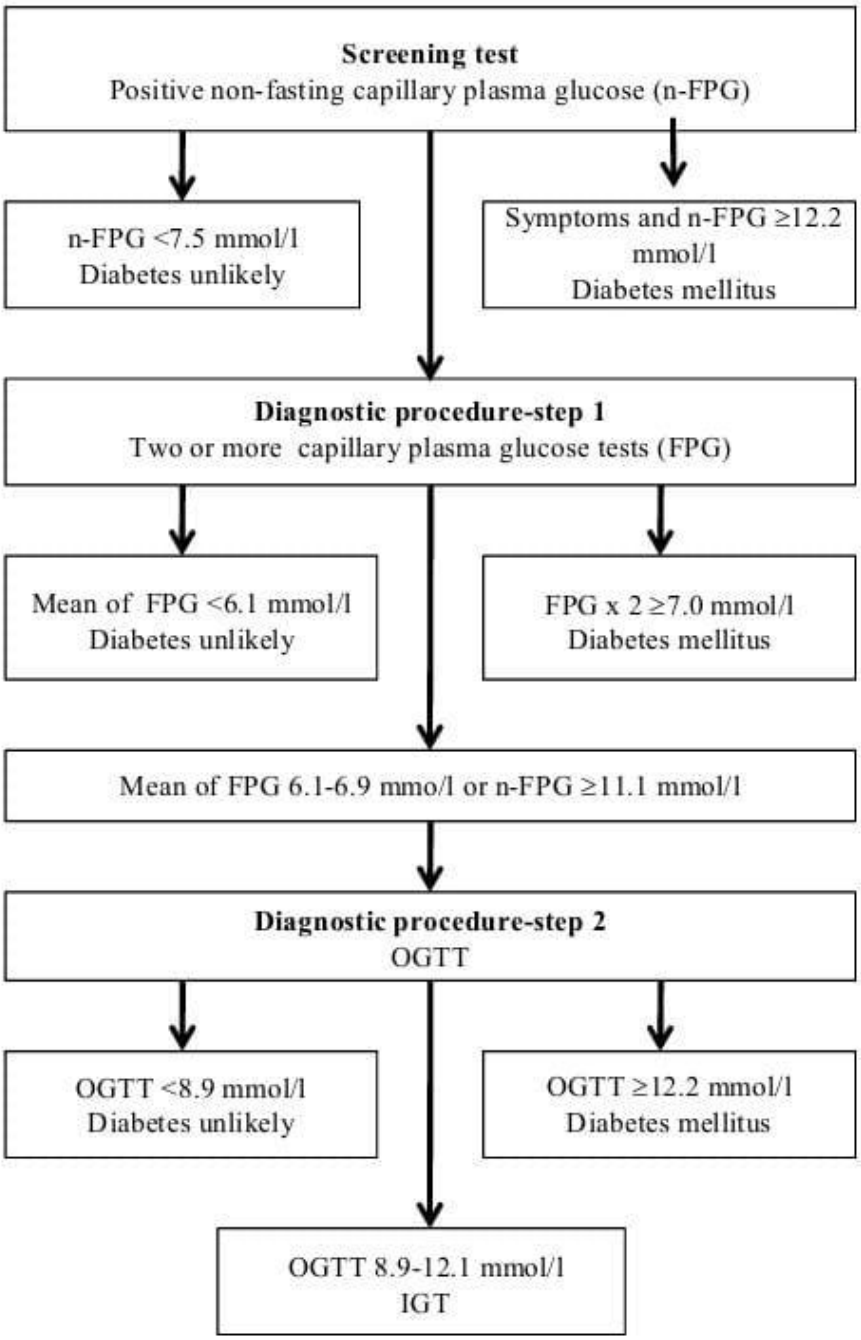


Figure 2.9 Work-up Schedules after Positive Screening Test[40].

2.2 Overview of *HNF1A* Gene

The gene known as hepatocyte nuclear factor 1A (HNF1A) is responsible for encoding an essential component of an auto-regulatory transcriptional circuit that is present in both mature and developing pancreatic tissue. It is the HNF1AMODY subtype that is responsible for the major variant of MODY, which is caused by heterozygous mutations in the *HNF1A* gene. This classification of monogenic diabetes is distinguished by the presence of an autosomal dominant inheritance pattern, an early onset, and a gradual deterioration of beta cells that ultimately results in severe hyperglycemia. This particular subtype of MODY has the highest prevalence and is observed more frequently than other variants, particularly in the regions of Europe, North America, and Asia[42]. It has a connection to the highest amount of prevalence. People who have HNF1A MODY are more likely to experience extrapancreatic symptoms, such as glycosuria, since their renal glucose threshold is lower than their normal threshold. It's possible that these symptoms will appear for the first time before diabetes actually starts.

In the majority of cases, this phenomenon can be attributed to the expression of *HNF1A* in beta-cells as well as organs such as the kidney, liver, and small intestine. In light of the fact that the risk of micro- and macro-vascular consequences in HNF1A-MODY is comparable to that of Type 1 and Type 2 Diabetes Mellitus, it is absolutely necessary for these patients to maintain rigorous control over their glucose levels. When patients with pathogenic mutations in the *HNF1A* gene are exposed to sulfonylureas, even at low doses, they demonstrate sensitivity to the medication[42]. One of the genes that encodes the protein known as hepatocyte nuclear factor-1 alpha (HNF-1α) is the *HNF1A* gene. The HNF-1α protein performs its job as a transcription factor by binding to certain areas of DNA in order to

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regulate the activity of specific genes. It has been found that this protein is present in a number of organs and tissues; however, it appears to be of special significance in the liver and the pancreas. Throughout the process of growth and development of pancreatic beta cells, the HNF-1α protein plays a crucial role in regulating the activity of genes. Beta cells are responsible for the production of the hormone insulin as well as its release into the bloodstream. The transport of glucose from the bloodstream into cells for the purpose of energy utilisation is managed by insulin, which is responsible for regulating blood glucose levels. Protein HNF-1α is responsible for regulating genes that are related with the development of the liver[42]. It is hypothesised that the HNF-1α protein has a role in the suppression of tumours by regulating genes that influence the development and survival of cells, hence avoiding excessive or uncontrolled cell proliferation. Within the structure of HNF-1α, there are various crucial regions that play a crucial role in facilitating the execution of its tasks. When it comes to protein interactions, the dimerisation domain is absolutely necessary.

In order to facilitate the formation of a dimer that functions as a transcription factor, this particular region plays a crucial role in the process of HNF-1α proteins forming proteins that are structurally similar to one another or to other proteins[10]. It is necessary for the DNA binding domain to engage with particular areas of individual DNA molecules in order for the dimer to be able to regulate gene activity. The *HNF1A* gene, which is found on chromosome 12, is responsible for encoding a transcription factor that is composed of 631 amino acids. An N-terminal dimerisation domain, a DNA-binding domain, and a C-terminal transactivation domain are the three distinct domains that are contained inside this transcription factor. Through its interaction with hepatocyte nuclear factor-1-beta, *HNF1A* is capable of

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producing either a homodimer or a heterodimer, depending on the circumstances. There are three different isoforms of *HNF1A*, and each of these isoforms exhibits tissue-specific expression as well as distinctive transcriptional properties by themselves. *HNF1A* is mostly expressed in the pancreatic islet beta-cells which are the principal location of expression. Within these cells, it exerts its influence on the regulation of other transcription factors that are particular to the islets. Both the kidney and the gut, as well as the liver, have been found to express *HNF1A*. It has been determined that the *HNF1A* gene has around 400 different gene variants that are responsible for the development of MODY. These mutations have a variety of effects on the properties of proteins and the behaviours of cells throughout the body[9].

The most common forms of mutations are missense mutations, which are primarily found in the dimerisation or DNA-binding domains, and truncating variations, which are primarily found in the transactivation domain[43]. In order to produce the protein known as hepatocyte nuclear factor-1 alpha (HNF-1α), the *HNF1A* gene is responsible for encoding the transcriptional instructions. The protein known as HNF-1α functions as a transcription factor, interacting with particular areas of DNA in order to regulate the expression of genes. The liver and the pancreas are particularly important organs for the function of this protein, despite the fact that it is present in a number of organs and tissues. To ensure the growth and development of pancreatic beta cells, it is necessary for the HNF-1α protein to exercise control over the activity of genes. Beta cells are responsible for the production of the hormone insulin as well as its release into the bloodstream. By limiting the amount of glucose that enters cells from the bloodstream for the purpose of energy utilisation, insulin is able to control the levels of glucose in the blood[41].

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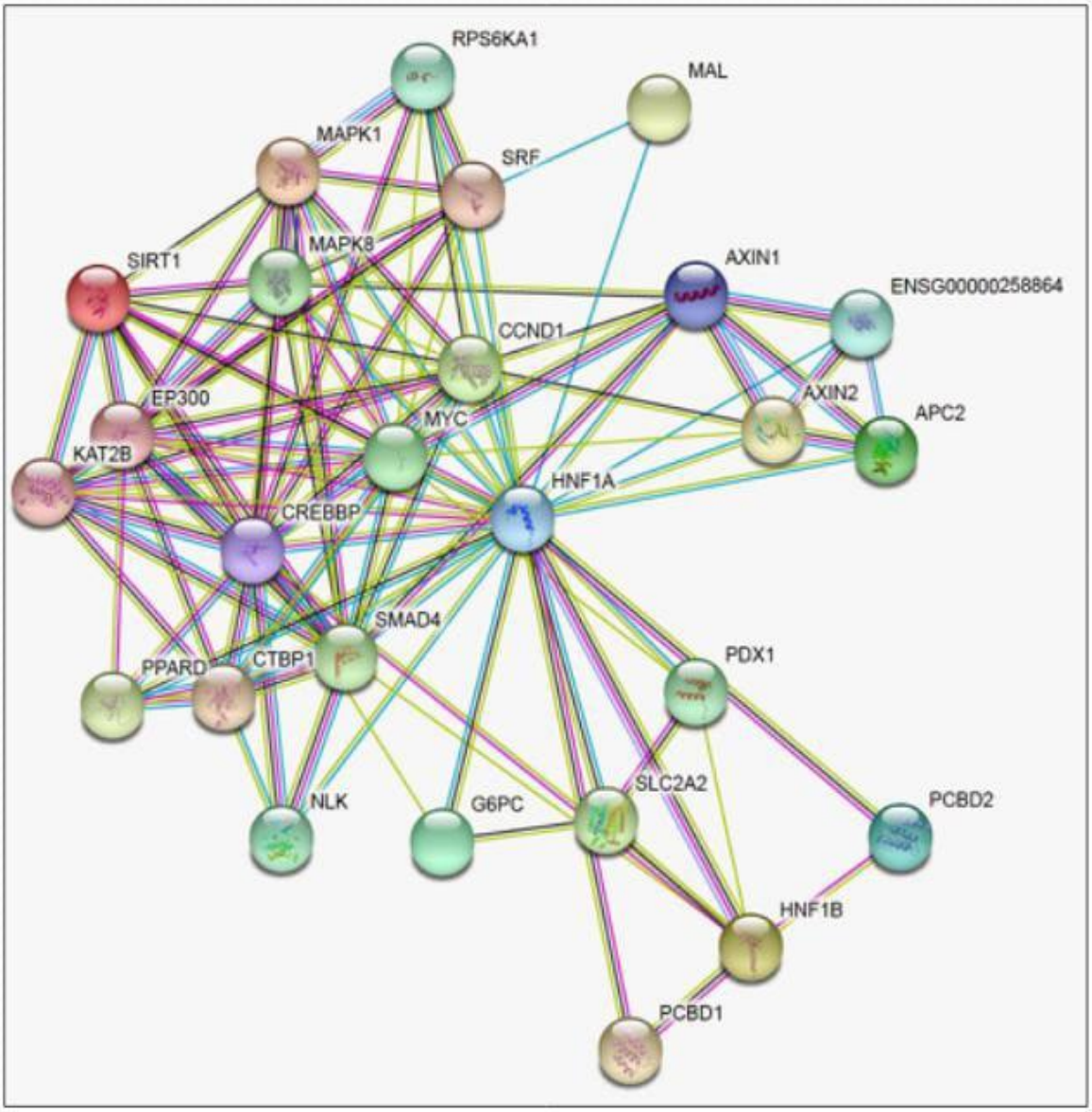


Figure 2.10 HNF1α Protein Interaction Partners[41]

Furthermore, the HNF-1α protein modulates genes associated with hepatic development. The HNF-1α protein, identified as a tumour suppressor, is believed to contribute to the inhibition of excessive or uncontrolled cellular proliferation and division. This is accomplished regulating genes that control cell development and viability. Multiple essential regions of the HNF-1α structure are critical to its functionality. The dimerisation domain is crucial for protein interactions. Consequently, HNF-1α proteins in this particular region can interact with one other or with other structurally analogous proteins. This results in the formation of a two-protein complex known as a dimer, which functions as a transcription factor. The dimer can modulate gene expression by attaching to certain DNA areas through a specialised region termed the DNA binding domain.[43] A group of illnesses is referred to as maturity-onset diabetes of the young (MODY).

These disorders are characterised by significantly increased glucose levels in the bloodstream and are caused by mutations in the *HNF1A* gene. The symptoms of this particular kind of diabetes usually appear before the age of thirty. A mutated form of the *HNF1A* gene responsible for the primary type of MODY, known as HNF1A-MODY (or MODY3). [41] The earliest symptoms, including recurrent skin infections, fatigue, blurred vision, weight loss, excessive thirst (known as polydipsia), and frequent urination (known as polyuria), are caused by increased blood glucose levels. Unregulated hyperglycemia may result in the onset of renal and ocular problems over time. HNF1A-MODY arises from two mutations in the *HNF1A* gene, with each mutation affecting one of the two gene copies in each cell. These mutations result in the production of a mutated and dysfunctional HNF-1α protein. Specific changes hinder the HNF-1α protein's capacity to dimerise, translocate to the nucleus for DNA contact, and limit transcription factor binding to DNA for gene control. These

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alterations hinder transcription, resulting in variations in gene expression inside cells. This obstructs the development and functionality of beta cells. This renders the regulation of blood glucose levels impractical, as cells demonstrate a reduced ability to synthesise insulin in response to blood glucose. The manifestations of MODY result from increased blood glucose concentrations. In exceedingly rare cases, individuals with HNF1A-MODY may develop one or more hepatocellular adenomas, which are benign liver tumours that do not result in cancer. A mutation exists in the second allele of the *HNF1A* gene observed in the hepatic cells of these individuals. The second mutation, commonly referred to as a somatic mutation, is non-heritable. The processes by which mutations provoke unregulated development and cancer in liver cells are not fully understood; nonetheless, it is confirmed that this phenomena occurs independently[41].

HNF1α is crucial in embryonic development for its ability to regulate the proliferation of intestinal epithelial cells and the differentiation of particular cell lineages. HNF1α expression was seen in the kidneys, liver, stomach, and pancreas of adult humans. Through genome-wide integration of transcriptomics and antibody-based proteomics, an analysis of the tissue-specific expression of the protein *HNF1A* revealed that various human tissues exhibited differing levels of *HNF1A* expression[40]. HNF1α associates with no fewer than 106 target genes in human pancreatic islets and at least 222 target genes in the human liver. Furthermore, it can participate in mitochondrial metabolism due to its role as the transcriptional regulator of angiotensin-converting enzyme 2. Furthermore, HDL metabolism is dependent on HNF1α, the transcriptional regulator of bile acid transporters in the kidneys and intestines[41].

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The expression of organic cation transporter 1 (OCT1), governed by HNF1α, enables the hepatic absorption of small, hydrophilic, positively charged organic molecules. OCT1 substrates, including metformin, tropisetron, ondansetron, tramadol, and morphine, may undergo modifications in hepatic absorption and drug metabolism due to genetic variations in HNF1α[41]. HNF1α directly modulates the low affinity/high capacity glucose cotransporter (SGLT2), which promotes glucose reabsorption in the kidneys. Moreover, HNF1α governs the transcription of acute phase inflammatory proteins, including fibrinogen, C-reactive protein (CRP), and interleukin 1 receptor. Both pancreatic tumours and hepatocellular adenomas exhibited significantly decreased amounts of HNF1α protein compared to the neighbouring normal tissues. This information indicates that HNF-1α may act as a protein that suppresses cancer progression.

The following table presents a comprehensive analysis of the function of HNF1α in different tissues. The Gene Ontology (GO) Consortium offers web access and analytical tools that leverage the GO knowledgebase, in addition to experimental support for GO annotations. The GO database presents 29 unique ontologies that define the biological processes related to the HNF1α protein[43]. Human HNF1a is a transcription factor unique to certain tissues, demonstrating extensive expression. It is located on chromosome 12 at q24.31[41]. HNF1a modulates several liver-specific genes in its function related to the metabolism of lipids, glucose, and other compounds. HNF1a regulates many pancreas-specific genes that are crucial for the maturation, development, and production of insulin by beta cells. The Exome Aggregation Consortium (ExAC) database has recorded 894 unique variants of the HNF1a gene. The gene is characterised by many polymorphisms; nevertheless, there are no specific mutation hotspots. The Genome Aggregation Database (GnomAD) allows users to query

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1,231 unique variants[39]. Diverse changes may occur, ranging from the HNF1a promoter to the 3' untranslated region (UTR). These variants include missense, translocation, nonsense, splice mutations, in-frame amino acid deletions, insertions, duplications, and both partial and complete gene deletions. Mutations have been detected in all exons; however, exons 2 and 4 demonstrate the highest frequency of alterations. The mutation in exon 4 (p291fsinsc) is the most common among them[43]. A study analysing 414 unique variants across 1,247 families with the HNF1a gene revealed that deletions (1.2%), splice site alterations (9%), frameshift mutations (22%), missense mutations (55%), and promoter area modifications (2%) were the most prevalent mutation types.

Single nucleotide polymorphisms (SNPs) are responsible for the mutations listed below: A missense mutation occurs due to a mutation in the exon area of HNF1a, leading to the replacement of a single amino acid. The mutation occurs in the exon region, leading to abnormal splicing of the HNF1a transcript. This characteristic is ascribed to the mutation. A synonymous mutation impacting the terminal nucleotide of exon 8 in HNF1a (c.1623 G > A, p.Gln541Gln) was expected to disrupt RNA splicing. However, there is no evidence to support this notion, as no RNA has been collected from patients. Mutations in intronic regions can lead to the formation of pseudoexons, hence creating new splice sites. A mutation in the promoter region 43 may result in diminished gene expression[43].

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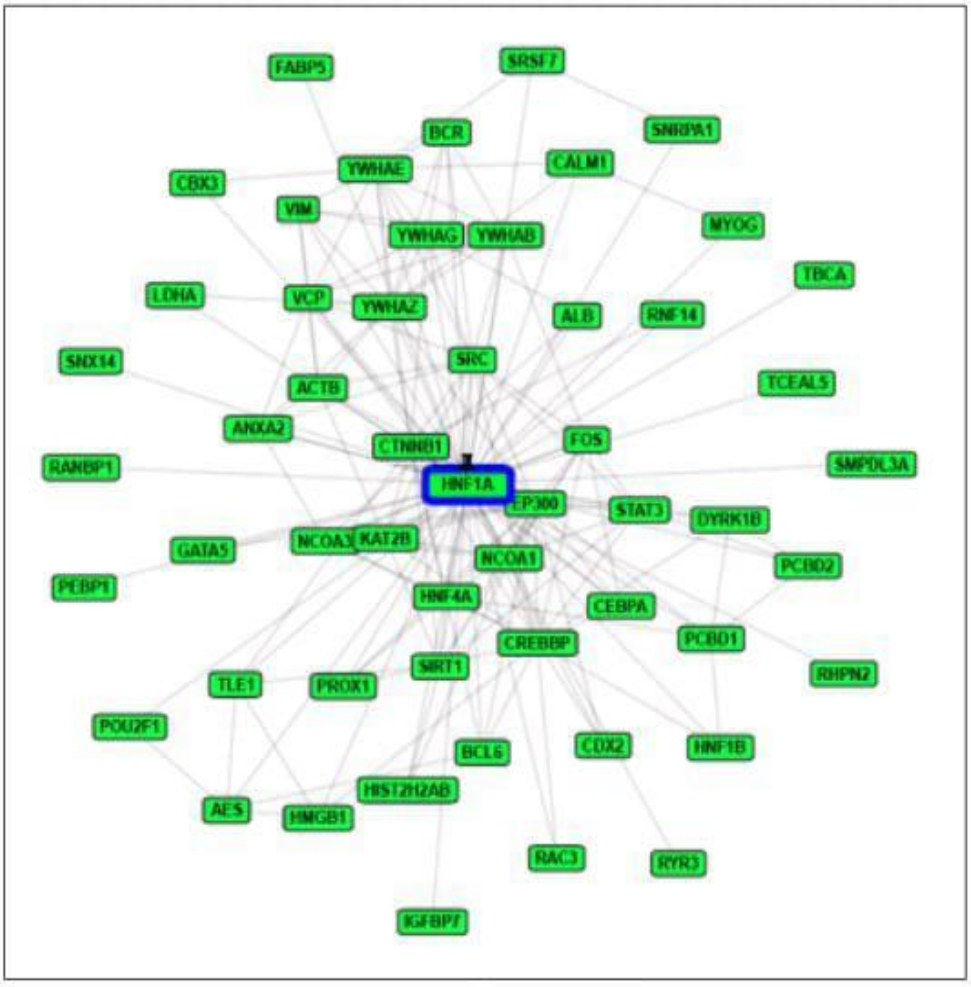


Figure 2.11 HNF1α Protein Interaction Partners[43]

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2.2.1 Extra Pancreatic Organs and *HNF1A*

There is a transcription factor known as *HNF1A* that belongs to the family of homeoboxes known as HNF1. The endogenous ligands of *HNF1A* have not yet been discovered; nevertheless, aspirin and resveratrol have been recognized as possible exogenous ligands through the use of in silico studies. However, despite the fact that aspirin and resveratrol are not specific ligands for *HNF1A*, they are able to exert an influence on a number of molecules, including cyclooxygenases and sirtuins, respectively. *HNF1A* activity may be influenced by these medications[44]. Furthermore, it has been demonstrated that *HNF1A* and sirtuins44 are engaged in the processes that involve protein interaction. Sirtuin 1 in the liver engages in a physical interaction with *HNF1A*, which ultimately leads to the control of genes that are targets of *HNF1A*. *HNF1A* is mostly expressed in the liver, intestines, pancreas, and kidneys, which are the key organs that are involved in this process[45].

The physiological functions of *HNF1A* in a number of organs are summarized in Figure 2.11, which may be found there. In the course of mouse embryo development, HNF1A mRNA was found in the yolk sac at embryonic day (E) 8.5, as well as in the liver, stomach, and kidneys at E14.5. The *HNF1A* gene contains three different isoforms: A, which is encoded by exons 1–10, B, which is encoded by exons 1–7, and C, which is encoded by exons 1–6. Isoform A is more commonly found in the liver, kidneys, and fetal pancreas, whereas isoforms B and C are more commonly found in the pancreatic and islets of the adult pancreas[44].

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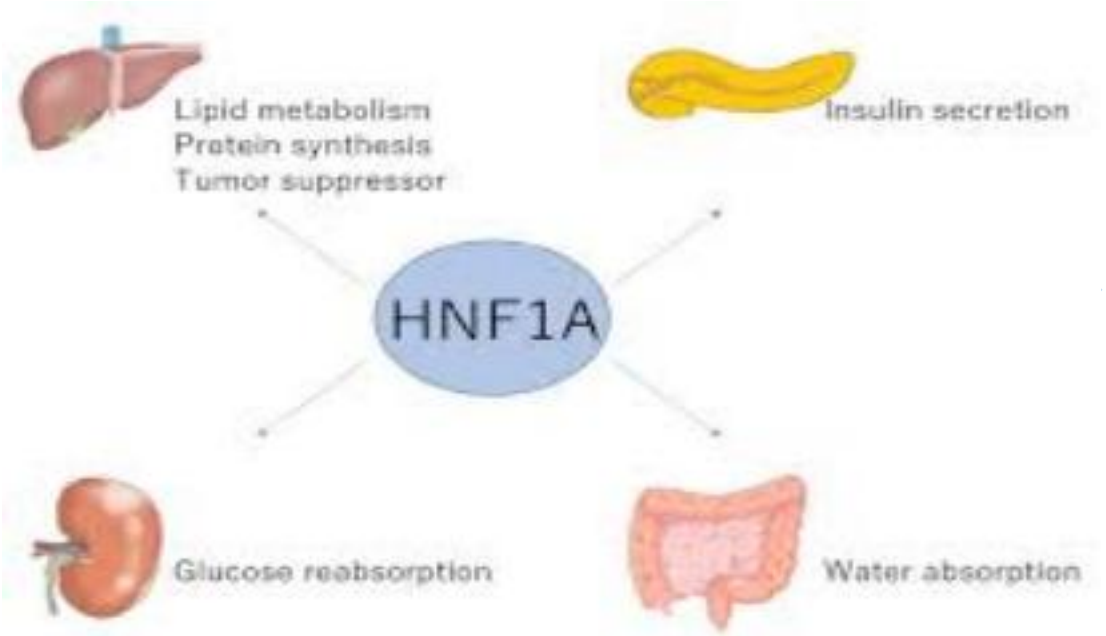


Figure 2.12. Physiological Role of *HNF1A* in the Liver, Pancreas, Kidneys, and Intestine[44].

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2.2.1.1 *HNF1A* and Liver

There is a connection between HNF families, particularly *HNF1A*, and the development, function, and malignancy of the liver. There are eight distinct types of hepatocellular adenomas (HCAs), which are classified according to the genotype of the individual. Inactivating *HNF1A* mutations and demonstrating histologically steatosis of the tumour are two characteristics that distinguish one classification from another within the category[47]. Through their expression, the vast majority of HCAs that fall within this category demonstrate biallelic somatic inactivation of *HNF1A*. There are specific HCAs that are identified in MODY3 families that have a somatic mutation in one allele within the tumour and a heterozygous germline mutation in the other allele.

This shows that *HNF1A* in the liver has the ability to inhibit the proliferation of cancer cells. To achieve optimal liver function, lipid metabolism is absolutely necessary[44]. Histopathological hepatomegaly and stunted growth were observed in mice that were homozygous for the Hnf1a gene defect. In addition to exhibiting hypercholesterolaemia and hyperbileacidemia, mice lacking Hnf1a exhibited aberrant gene expression in relation to the formation and absorption of bile acids, as well as de novo cholesterol biosynthesis [44]. In a particular study, it was found that mice who lacked the Hnf1a gene had higher levels of triglycerides in their blood, a fatty liver, and increased synthesis of fatty acids. The process of lipid accumulation in these neoplasms was clarified through the use of a lipidomic study of HCAs that was caused by the inactivation of *HNF1A*. This investigation demonstrated an increase in de novo lipogenesis in cells that were tumours. It was shown that the lipid profiles of those with MODY3 and those who did not have diabetes were comparable. The individuals who were diagnosed with MODY3 had higher levels of high-density lipoprotein

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cholesterol and lower levels of triglycerides when compared to those who were diagnosed with type 2 diabetes. Therefore, it is highly improbable that heterozygous *HNF1A* mutations are sufficient to cause aberrant lipid metabolism. In the process of hepatic activity, protein synthesis plays an essential role. The liver is responsible for the production of a number of essential proteins, including albumin and C-reactive protein (CRP). Elevated CRP levels have been linked to a wide range of diseases, including but not limited to cardiovascular disease, type 2 diabetes mellitus, obesity, psychological discomfort, depression, and cancer.

The SNPs that are found in the *HNF1A* gene are correlated with the levels of plasma CRP. Furthermore, comparisons between individuals who do not have diabetes and various diabetic populations, such as type 1, type 2, MODY1, MODY2, and MODY548 patients, show that high-sensitivity CRP levels are lower in MODY3 cohorts. Two *HNF1A* binding sites are present in the human CRP promoter, and these sites have the ability to activate CRP transcription. When people with MODY3 symptoms are present in their livers, there is a possibility that the transcriptional activity of *HNF1A* is diminished[48]. Through the modulation of lipid and glucose metabolism, HNF1a has the potential to adversely impair the liver's ability to maintain blood glucose homeostasis. It does this by affixing itself directly to the promoter region of the glucose 6-phosphate transporter (G6PT) gene, which is the major enzyme of the glucose-6-phosphatase (G6Pase) system. The following genes are more likely to be transcribed when this promoter region is present[45]. By contrasting the HNF1a (-/-) mice with their HNF1a (+/+) and HNF1a (+/-) littermates, it is clear that the HNF1a (-/-) mice have significantly lower levels of hepatic G6PT mRNA and a lower level of microsomal G6P transport activity [45]. For the purpose of maintaining glucose homeostasis within the body, the G6Pase system is absolutely necessary.

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It is possible that the alteration in HNF1a, which took place with the assistance of the G6Pase system, is the primary cause of the disruption in glucose homeostasis. In the liver, after the deletion of HNF1a, there was an increase in the expression of genes that code for enzymes that are involved in the process of fatty acid production. These enzymes include fatty acid synthase and acyl-CoA carboxylase, as well as peroxisomal β-oxidation enzymes including CYP4A3, bifunctional enzyme, and thiolase. On the other hand, there was a notable reduction in the expression of the gene that is responsible for the production of liver fatty acid binding protein, which is identified as L-FABP. Upon conducting an analysis of the 5' promoter region of L-FABP, it was discovered that there are two HNF1a binding sites present. Cellular investigations, which verified this requirement, indicated that HNF1a is essential for the transactivation of L-FABP[47]. This requirement was determined by the studies. Our hypothesis was that a mutation in HNF1a might potentially alter the homeostasis of blood glucose by changing the metabolism of glucose and the process of adipogenesis.

Additionally, HNF1a is responsible for facilitating the transcription of the apolipoprotein gene [48]. In Oji Cree individuals who were diagnosed with type 2 diabetes, a strong association was seen between the HNF1a G319S genotype and the amounts of apolipoprotein B, low-density lipoprotein cholesterol, and total plasma cholesterol. Oji Cree individuals who were investigated and did not have type 2 diabetes showed a significant link between the HNF1a G319S genotype and the plasma levels of HDL-C and apolipoprotein AI. This correlation was found to be significant. Due to the fact that there was no connection between the two variables, the phenotype did not exhibit any connection with either lipoprotein or plasma triglycerides (a). Based on the findings obtained from these individual samples, it was determined that I27L exhibited a preventive effect against hypertriglyceridemia 48. HNF1a

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has been shown to have a significant impact on the lipid profile of diabetic patients, according to researchers who conducted the study. According to the findings of these studies, a more in-depth comprehension of the intricate relationships that exist between genes, hyperglycemia, and cardiovascular risk factors in type 2 diabetes has been achieved. According to the findings of a number of research, a decrease in the expression of HNF1a is linked to an increased risk of developing fatty liver, which is intimately connected to insulin resistance[48]. In conclusion, the HNF1a SNPs that are linked to abnormal blood glucose levels may be the result of changes in the maturation, activation, and death of beta cells. These changes may also be the result of the maintenance of mature pancreatic function and the regulation of glucose and lipid metabolism in the liver. It is possible that these alterations are responsible for aberrant amounts of glucose in the blood.

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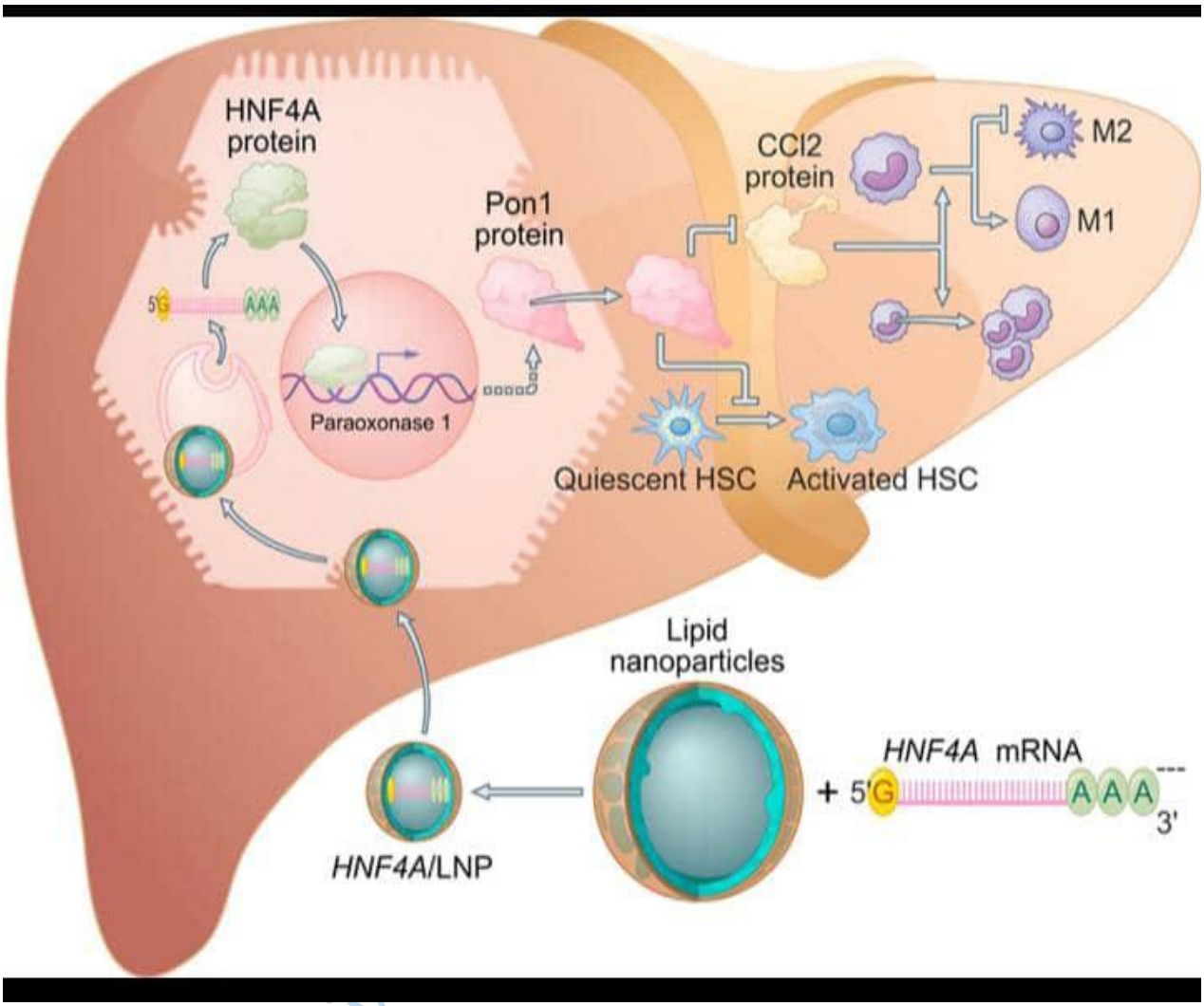


Figure 2.13 Physiological Role of *HNF1A* in the Liver[48].

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2.2.1.2 Kidney and *HNF1A*

In the early stages of renal development, it was possible to see the expression of both *HNF1A* and HNF1B levels[61]. Embryonic kidney disorders, including renal cysts, solitary kidneys, renal hypoplasia, and electrolyte abnormalities, have been linked to HNF1B mutations due to their association with these conditions. On the other hand, the vast majority of *HNF1A* mutations do not have any association with aberrant renal morphology [50]. In comparison to people who exhibit symptoms of type 1 and type 2 diabetes, patients who have MODY3 syndrome have been shown to have a lower rate of glucose reabsorption in their urine, according to studies. In mice that lacked the Hnf1a gene, the binding of phlorizin, which is a competitive inhibitor of sodium–glucose cotransporter (SGLT) 1 and 2, to the surface of the proximal tubule cell was reduced. *HNF1A* was shown to directly promote the transcription of SGLT2 in both rodents and humans, and the expression of SGLT2 was found to be decreased in the kidneys of mice that lacked the Hnf1a gene[44].

When it comes to the treatment of type 2 diabetic mellitus (T2DM), SGLT2 inhibitors are the most commonly used medications. An SGLT2 inhibitor called dapagliflozin was given to patients who had been diagnosed with MODY3 during a clinical development trial[52]. The patients received a single dose of the drug. The surprising discovery that changes in urine glucose excretion after pharmacological therapy were more evident in MODY3 patients than in those with type 2 diabetes disproved the theory that the drug's efficacy would decrease in MODY3 patients due to reduced SGLT2 expression. This finding was made possible by the fact that the medicine was shown to be more effective in MODY3 patients. It is possible that those who have type 2 diabetes will have greater glucose reabsorption in urine through SGLT1 rather than SGLT2, which is still a possibility[44]. There is still a lack of clarity

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regarding the origin of this outcome. On top of that, phenylketonuria and Fanconi syndrome were discovered in mice that were missing the Hnf1a gene. On the other hand, these characteristics were not found in any of the other investigations. Additionally, there are just a few cases of renal failure in people who have MODY3, whereas Fanconi syndrome can be caused by HNF4A mutations (MODY1)[44].

2.2.1.3 Gut and *HNF1A*

There is a substantial amount of expression of *HNF1A* and HNF1B in the crypts of the small intestine, and these two proteins work together to control the proliferation of intestinal cells. There were no significant problems observed in mice that had deficits in either Hnf1a or Hnf1b across the gastrointestinal system. Dehydration occurred in mice that had concurrent defects in both HNF1A and HNF1B immediately after birth. This was due to the mice's inability to absorb water in the colon. LPH, which stands for lactase-phlorizin hydrolase, is an enzyme that is produced in the small intestine and is necessary for the digestion of lactose. The DNA-binding domain of *HNF1A* is able to physically connect with the C-terminal zinc finger of GATA5, which results in a considerable increase in the activation of the human LPH promoter[44].

The function of *HNF1A* in the gastrointestinal tract in relation to diabetes has not been properly examined; however, ghrelin, which is a hormone that stimulates appetite and is mostly produced in the stomach and intestines, may be a possible target of *HNF1A*. A large rise in ghrelin-positive cells was observed in the colons of Hnf1a-null specimens. This was accompanied by an increase in the expression of the ghrelin gene as well as an increase in the levels of both total and active ghrelin in the bloodstream. Patients diagnosed with MODY3 had considerably greater quantities of total ghrelin in their blood while they were fasting, in

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comparison to persons who were diagnosed with type 1 or type 2 diabetes. On the other hand, it was shown that the levels of acylated ghrelin, also known as active ghrelin, were comparable in patients with MODY3 to those reported in healthy individuals and individuals with type 2 diabetes[57].

2.2.2 Pancreas and *HNF1A*

2.2.2.1 Physiological and Pathophysiological Roles of *HNF1A* in Endocrine and Exocrine Cells of the Pancreas

HNF1A is expressed in endocrine cells, such as alpha, beta, delta, and pancreatic polypeptide (PP) cells, as well as exocrine cells, such as acinar and duct cells, inside mouse pancreatic tissue. These cells are responsible for the production of pancreatic oil. Pancreatic cells are produced by these cells, which are responsible for their creation. In the majority of pancreatic epithelial cells in the E10.5 stage, *HNF1A* was found, and at the E15.5 stage, it was found in hormone-positive and amylase-positive cells. On the other hand, studies have shown that its expression in duct cells is practically nonexistent. All during the process of hepatic development, HNF4A acts as a positive regulator of *HNF1A* expression. On the other hand, *HNF1A* has the ability to directly connect with the P2 promoter of HNF4A, which allows it to moderate the expression of HNF4A in differentiated pancreatic cells. This hierarchical structure of transcriptional processes is present during the development of the liver. Furthermore, *HNF1A* is responsible for the regulation of the production of PDX1, which is an essential component for the growth of the pancreas as well as the maintenance of beta cell activity[44]. Within the beta cells of rodents, this interaction takes place concurrently with PDX1. When it comes to the transformation of human induced pluripotent stem (iPS) cells into beta cells, the relationship between *HNF1A* and HNF4A is absolutely necessary.

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Consequently, the significance of the HNF1A–HNF4A axis in the development of the pancreas is highlighted by this. Many other types of pancreatic endocrine cells are present, such as alpha, beta, delta, and PP cells, in addition to cells that are responsible for the secretion of ghrelin[58]. The expression of SGLT1 in murine alpha cells was directly regulated by *HNF1A*, which made it possible for it to participate in the production of glucagon during circumstances of hypoglycemia[59]. The fact that Hnf1a-null animals demonstrated reduced insulin synthesis, which resulted in higher blood glucose levels, provided evidence that *HNF1A* plays a role in the process of insulin secretion within mouse beta cells. On the other hand, animals with heterozygous Hnf1a deficiency showed normal levels of glucose in their blood glucose[44]. Furthermore, Hnf1a-null mice that possessed a C3H or CBA genetic background exhibited non-diabetic traits.

This indicates that there are phenotypic differences between subjects with MODY3 and Hnf1a-null mice[60]. In addition, these mice exhibited characteristics that were not associated with diabetes. A considerable proliferation rate of these cells was seen in Hnf1a-null animals, despite the fact that their acinar cells contained both endocrine and exocrine granules. It has been determined through this discovery that *HNF1A* is necessary for the formation of exocrine cells in the correct manner. Despite the fact that genome-wide association studies (GWAS) have shown a connection between *HNF1A* single nucleotide polymorphisms and pancreatic cancer, it is still unknown whether or not *HNF1A* mutations are responsible for malfunction in human exocrine cells[61]. The exocrine gland is frequently linked to the development of pancreatic cancer, with pancreatic ductal adenocarcinoma (PDAC) being the most common form of the disease. According to the findings of a recent study, *HNF1A* is significantly expressed in pancreatic cancer stem cells (PCSCs), which suggests that it may

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play a role in regulating the functional capabilities of PCSCs. Within a number of PDAC cell lines, the inhibition of *HNF1A* resulted in the cessation of tumour growth and even the death of the tumour, in addition to a reduction in the markers that are associated with stem cells. Because of the overexpression of *HNF1A* in PDAC cell lines; there was an increase in the number of stem cell markers, which in turn stimulated the growth of tumours. With regard to pancreatic cancer, the observations indicate that activation of *HNF1A* leads to an increase in the proliferation of tumours, despite the fact that *HNF1A* has also been shown to have a role in preventing the growth of tumours in this context. Through the formation of a complex with lysine-specific demethylase 6A, which is expressed by the KDM6A gene, *HNF1A* is able to exercise its efficacy in suppressing tumour growth. A mouse acinar cell line is used for this purpose, and this complex is responsible for blocking the carcinogenic route.

The fundamental cause of hyperglycemia that is brought on by HNF1a is the impairment of mature B lymphocytes. During resting states, mature B cells are responsible for the release of insulin, and when glucose levels are increased, they are responsible for the manufacture of insulin. These are the two primary functions of mature B cells. Individuals who have MODY3 have issues with their ability to secrete insulin, and as the condition worsens, the insulin-secreting islets experience a gradual loss in their effectiveness[59]. Forty HNF1a mutant carriers from the United Kingdom and France demonstrated abnormal levels of insulin while fasting as well as abnormal amounts of insulin produced in response to glucose stimulation[62]. These patients had higher insulin sensitivity, as well as elevated proinsulin to insulin ratios. Additionally, their insulin sensitivity was elevated. Furthermore, the findings suggested that an HNF1a mutation modifies basal insulin secretion directly, as opposed to the insulin secretion that is connected with glucose sensing. A recent islet study conducted on a patient

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with MODY3 who was 33 years old and had been misdiagnosed as having type 1 diabetes for 17 years demonstrated that HNF1a influences the generation of glucose-stimulated insulin, which ultimately results in insulin-deficient diabetes[62]. One possible explanation for the disparity between the two outcomes is that different forms of HNF1a were present in the experiment. Following further investigation, it was discovered that individuals who possessed the G319S mutation demonstrated a reduction in insulin production prior to the initial manifestation of diabetes. Additionally, individuals who have HNF1A-T2D present with this hypothesis malfunction of the islets. Therefore, we hypothesis that HNF1a has two distinct and significant functions in the regulation of insulin secretion at regular intervals and in insulin synthesis in response to increased glucose levels. Both of these activities are important in regulation of insulin secretion.

For the purpose of determining which domain of HNF1a is accountable for baseline insulin secretion and which domain is responsible for mediating insulin secretion in response to elevated glucose levels, it is vital to evaluate the islet function of HNF1a mutants with varying degrees of clinical severity. Given this information, a better understanding of the role that HNF1a plays in the dysfunction of beta cells will be made possible[63]. The hormone HNF1a 60 has the ability to exert control over a number of genes that are involved in the mechanism of insulin secretion. Additionally, it has the ability to directly bind itself to the promoter region of the insulin gene, which may ultimately result in an increase in the gene's transactivation. It has been demonstrated that HNF1a exerts a direct influence on the transcription of insulin, as well as the GLUT2 and HNF4 pyruvate kinase genes, which are both necessary for the production of insulin within cells respectively. Furthermore, it has been revealed that heterozygous HNF1a variations can change the expression of critical

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enzymes that are involved in the glucose metabolism of mitochondria[63]. Despite the fact that HNF1a is an essential transcription factor that makes a major contribution to the maintenance of B cell activity, additional research is necessary in order to have a better understanding of the specific mechanisms that are involved. Nkx6 has been shown to have the ability to directly boost the expression of HNF1a, according to research findings. Insulin-positive cells that have undergone differentiation are the only cells that express the transcription factor MafA. MafA is immediately activated by HNF1a and encodes transcription factors that are produced later in the pancreatic developmental pathway[62].

According to the findings of several studies, HNF1a may have an effect on the development of beta cells as well as the growth of cells. Six weeks after birth, HNF1a whole-body knockout mice, which were distinguished by the presence of minuscule islets and a high alpha/beta cell ratio, passed away unexpectedly. These mice had a ratio of a to b that was in the negative. This phenomenon is consistent with the findings of the islet study, which focused on diabetes patients who have a mutation in the HNF1a 60 gene. Attributing the enhanced a/b cell ratio to the presence of a pancreas in these patients that has a marginally superior a cell population is one possible explanation for the observed improvement. Individuals who are diagnosed with MODY3 display a variety of symptoms, including decreased negative feedback to glucose, higher glucagon secretion, diminished intestinal glucagon impact, and hyperactivity of cells[63]. According to the findings of a study that was carried out in the year 2020 by Kazuya Yamagata60, HNF1a has the ability to reduce glucagon synthesis via controlling the expression of SGLT1 in cells. It was discovered that HNF1a inhibits a cellular characteristic that is generated by mutations in HNF1a. This conclusion was reached by the simulation of monogenic diabetes using human embryonic

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stem cells[63]. Human intervention was necessary in order to discover this. Despite the fact that further research is required, it is clear that HNF1a has an effect on islet-specific transcription factors. These transcription factors, in turn, play a part in the development of pancreatic organs, the differentiation of endocrine and exocrine cells, and growth processes [63].

2.2.2.2 Roles of *HNF1A* in Beta Cells

It was discovered through the use of microarrays to examine the pancreatic islets of mice lacking HNF1a that HNF1A is responsible for regulating genes that are linked with the metabolism of glucose and amino acids. Glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation are all processes that are regulated by these genes. In contrast to type 1 and type 2 diabetes, MODY3 is defined by a distinct phenotype that is characterised by decreased insulin production prior to the onset of diabetes[44]. It was observed that the insulin production response of ex vivo islets from mice that were defective in the Hnf1a gene was reduced in response to increased doses of glucose or arginine. Researchers made the discovery that MIN6 cells, which are a murine beta cell line, had a decreased production of insulin in response to arginine. These cells expressed a dominant negative mutation of *HNF1A*, which was denoted by the symbol P291fsinsC. After a maturation period of six months following implantation into mice, human iPS cell-derived beta cells with a MODY3 mutation (HNF1A+/H126D) demonstrated decreased glucose-stimulated insulin secretion (GSIS) [64]. This was observed after the cells had been implanted into animals. A diabetic individual with HNF1A+/T260M was found to have an impaired GSIS, which was verified to be the result of a deficiency in *HNF1A* from the islets of the individual[65]. The impact that type 2 diabetes mellitus has on insulin secretion can be attributed to the interaction between genetic variables, which include the accumulation of disease-associated common variants,

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and environmental impacts, which include metabolic stress and the effects of ageing. Modifications to the glucose metabolism caused by *HNF1A* mutations (MODY3) reduce the amount of insulin that is produced by beta cells. In addition to a reduction in glucose absorption, there is a connection between *HNF1A* mutations and decreased levels of glucose transporter 2 (GLUT2) in the body. In mouse beta cells, *HNF1A* has the ability to modulate both GLUT2 and liver-type pyruvate kinase, which is an enzyme that is responsible for providing a rate-limiting enzyme in glycolysis[64]. The widespread *HNF1A* mutation that is related with MODY3 (P291fsinsC) is also responsible for a reduction in the amount of ATP that is produced by mitochondria in murine beta cells.

As a consequence of this, it is highly probable that *HNF1A* has a role in the production of ATP, mostly inside the mitochondria, and the subsequent secretion of insulin through the regulation of glucose flux (Figure 2.12). The direct role that *HNF1A* plays in the regulation of genes that are related with mitochondrial function is a topic that is consistently the subject of debate. This set of genes might be controlled by a network of transcription factors, which might include PDX1 and HNF4A, among other possible candidates. There is a disruption in mitochondrial respiration when there is no *HNF1A* present in beta cells that have been produced from human embryonic stem cells. There is a correlation between this disruption and a decrease in LINKA, which is a human-specific long non-coding RNA[66]. This suggests that *HNF1A* serves a specialised role in the function of mitochondria. Furthermore, *HNF1A* is known to directly control TMEM27 and hepatocyte growth factor activator, both of which are known to cause an increase in the proliferation of murine beta cells. While this is happening, the primary metabolic route is also taking place.

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Through its involvement in the formation of the SNARE complex, collectrin (TMEM27) is responsible for regulating the process of insulin exocytosis. Additionally, *HNF1A* is responsible for the regulation of genes that are connected with roles in beta cells that are not well understood, some of which are AKR1C19[61].

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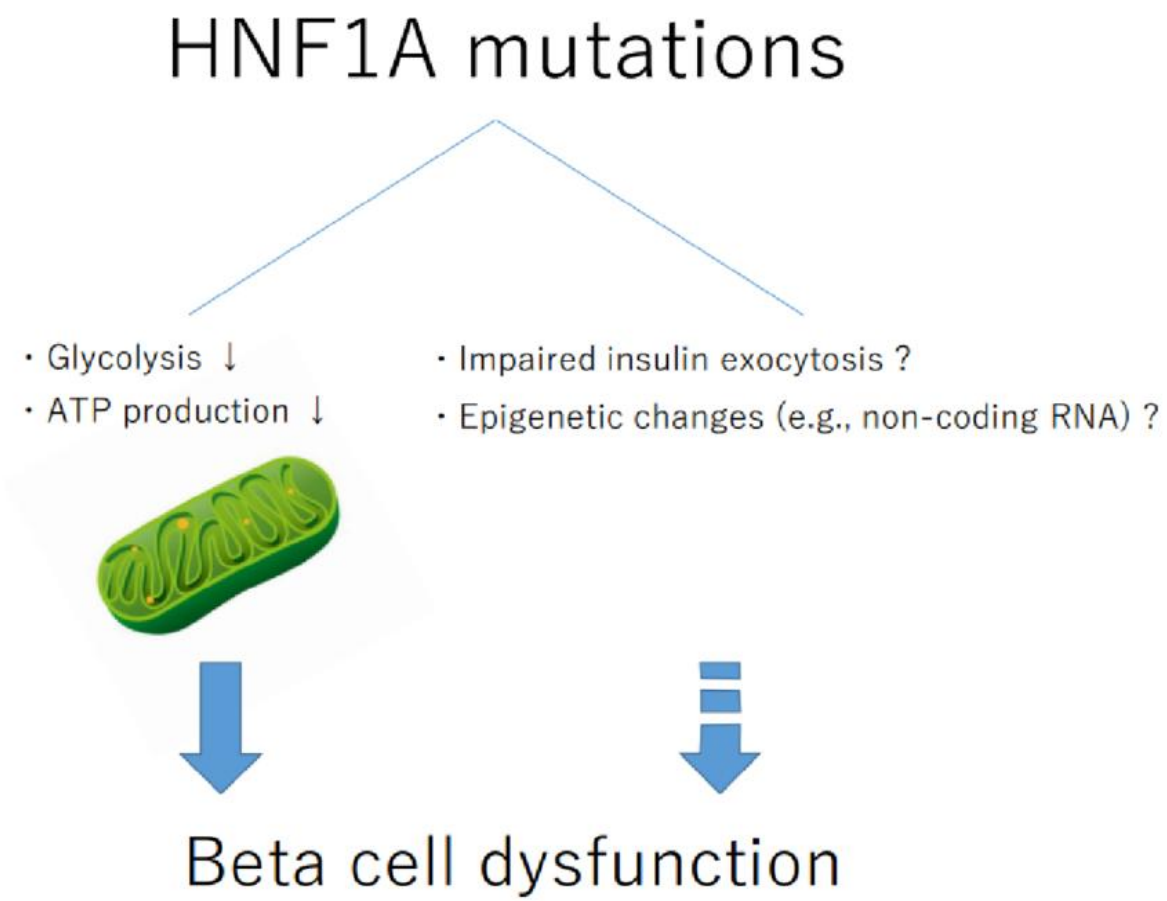


Figure 2.14 Schematic Representation of Beta Cell Dysfunction Caused by *HNF1A* Mutations[61].

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2.2.2.3 Epigenetics and *HNF1A* in Beta Cells

The genome-wide association studies (GWAS) that have been conducted over the past 15 years have helped us gain a better knowledge of the genetics that are related with type 2 diabetes mellitus (T2DM). With that being said, there is still a hurdle to be faced in terms of enhancing the accuracy of individualised risk assessment for type 2 diabetes[63]. There is a complicated interaction between inherited and environmental factors, including personal lifestyle choices, which is likely the reason why it is difficult to precisely anticipate the start of type 2 diabetes[67]. Additional genetic components also require identification and analysis in order to be well understood. Without regard to the DNA sequence, epigenetic modifications, which include histone modification, DNA methylation, and non-coding RNA, have the potential to influence transcription factor binding to the genome as well as gene expression. Despite the fact that there is growing evidence that links epigenetic modifications to the development of type 2 diabetes, the biological processes that are at the root of the disease are not yet properly understood[68].

When it comes to the proliferation and activity of beta cells, histone modification is absolutely necessary. As a result of its ability to relax the structure of chromatin, histone acetylation is regarded as an active epigenetic marker. This is because it makes it possible for transcription factors to bind to DNA. The two principal histone acetyltransferases are known as CREB-binding protein (CBP) and P300. They are all engaged in the acetylation of H3K2761 and have structures that are very similar to one another[61]. One of the most important functions of *HNF1A* is to ensure that histone acetylation is preserved in the promoter regions of its target genes, such as GLUT2, within the mouse islets. This describes a fundamental biological process. Previous research has demonstrated that CBP is connected

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with the N-terminal domain of *HNF1A*, whereas the P300/CBP associated factor is found to interact with the C-terminal region of *HNF1A*. Also noteworthy is the fact that *HNF1A* is responsible for recruiting P300 to the promoter of human GLUT2, which is another important element. The genes that were lowered in islets that lacked Hnf1a were identical to those that were diminished in islets that had faulty CBP or P300. It is possible that *HNF1A* and CBP/P300 work together to increase gene expression. This could be accomplished by the modification of histones, namely the acetylation of H3K27. There is a possibility that this potential exists, according to our findings collectively[61].

2.3 Diabetes and the *HNF1A* Gene

When it comes to genetic changes and clinical manifestations, HNF1A-MODY3 is characterized by considerable variety. It is possible to regard the two human variants of HNF1a, P408H and P409H, to be distinct variants [70]. One of the hypotheses that may be considered is that these two adjacent sites are responsible for regulating the promoters of two different genes that are influenced by HNFPA. This occurrence is anticipated, and it can be accounted for by the interactions that take place between HNF1a and a number of other co-activators[8]. These interactions are absolutely necessary for the full activation of a number of HNF1a target promoters that are located farther downstream. In the past, observations have been made that demonstrate the various functions that HNF1a plays, as well as its substantial involvement in the careful regulation of a number of genes that are associated with metabolic management. The presence of many HNF1a SNPs is associated with an increased risk of developing type 2 diabetes; however, these SNPs do not directly produce MODY3. These incidents are of a very interesting kind. We will be able to enhance our care of diabetes on an individual basis if we are able to determine the underlying cause of these episodes. First, let's

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take a look at the distinctions that exist between type 1 diabetes, type 2 diabetes, and those that are associated with pregnancy. One of the most important differences is the age at which the competition began. The condition known as diabetes can be broken down into three distinct categories: type 2 diabetes (T2D), maturity-onset diabetes of the young (MODY), and gestational diabetes mellitus (GDM) [61]. Diabetes mellitus during pregnancy, also known as gestational diabetes mellitus (GDM), manifests itself throughout the second to third trimester of pregnancy. When diseases manifest themselves at an earlier stage, the influence of hereditary variables on the process is significantly stronger.

The influence of environmental and other physical elements is expected to be more evident when the beginning occurs later. This is because the beginning happens later. While a woman is pregnant, her body is subjected to a substantial amount of stress. The second significant difference pertains to the nature of the family history that appears to have originated. In circumstances in which the history of the family is more prominent, the influence of hereditary elements becomes more significant. As a consequence of heterozygous mutations in HNF1a, MODY3 is produced. In patients with MODY, the presence of a normal HNF1a allele is indicative of the fact that HNF1a expression is thought to be significant in this population. The optimal functioning of B cells, in particular, is impossible in the absence of MODY. Inadequate protein levels cause a significant drop in the activity of mutant alleles[59], which in turn leads to the formation of inactive heterodimers as a consequence of contacts between mutant products and wild-type forms that are dominated by negative interactions [61]. The HNF1A-MODY mutations function by one of the two mechanisms described above: a dominant-negative mechanism or a direct loss of function. Both of these pathways are described in the previous sentence.

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Both type 2 diabetes and gestational diabetes mellitus are conditions that can be caused by a combination of different circumstances. We propose that the HNF1a SNPs that are connected to type 2 diabetes or type 2 diabetes mellitus partially degrade the functionality of the HNF1a protein. This is because the HNF1a SNP has an effect on the abnormal control of blood glucose, which is an essential component of both type 2 diabetes and type 2 diabetic mellitus. The HNF1a protein is made up of three different functional domains: a DNA binding region that contains a nuclear localization signal, a C-terminal transactivation domain, and an N-terminal dimerization domain. In addition, a nuclear localization signal is contained inside the DNA binding domain. Residues 1–32, which are part of the N-terminal dimeric domain, contain two four-helix bundles that are responsible for promoting dimerization. Located at circle [61], these bundles serve to split the a-helix.

Additionally, when HNF1a links with the structurally similar transcription factor HNF1b, it often results in the formation of a heterodimer. According to reference 66, the formation of a homodimer occurs when two HNF1a molecules interact with one another. There is a connection between the reverse palindrome 5'-gttaatnataac-3' and the DNA binding domain (DBD) of HNF1a (131), which is an essential component in the formation of a structure that makes use of helix-to-helix communication. Both the POU-specific domain (POUs, amino acids 91–181) and the POU-homologous domain (POUH, amino acids 203–279) are subdomains of the DBD. The POU-specific domain is the more prominent of the two. The DBD incorporates both of the subdomains within its structure. The locations of the amino acids that are found in DNA-binding domains (DBDs) can vary somewhat from one another because of these differences. It is the responsibility of POUs, which are necessary for HNF1a, to maintain the stability of proteins. The key single nucleotide polymorphisms (SNPs) of

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HNF1a that are associated with MODY3 are found in exons 1, 2, and 4, which are responsible for encoding the DNA-binding region of the C and transactivation domain of HNF1a. Contrast this with the SNPs that are responsible with the development of type 2 diabetes. In exons 8 and 9, the HNF1a SNP that has been associated to type 2 diabetes is frequently found. Within the transactivation domain, the HNF1a SNP sites that have been associated to the beginning of type 2 diabetes are situated along the margin of the proline-rich activation domain II. Through the following mechanisms, SNP mutations have the potential to influence the functionality of the HNF1a protein: 1) the binding affinity of DNA to the transcriptional regulatory region of the target gene; 2) the transcriptional efficacy of HNF1a; 3) the capability of HNF1a to translocate into the nucleus; 4) the stability of the HNF1a protein; and 5) the expression levels of the HNF1a protein, particularly SNPs located in the promoter region 61 where they are located.

Research that was carried out in 2017 by Najmi and colleagues (134) reveals that the HNF1A-MODY variant and the wild-type protein exhibit transcriptional activity and DNA binding proficiency that are equivalent to one another[61]. According to the aforementioned research, HNF1a appears to have a role in a number of different signaling pathways that ultimately result in elevated levels of glucose in the blood. Furthermore, the HNF1a variants that are observed in patients who have type 2 diabetes may not have sufficient penetrance to directly cause the condition; nonetheless, they do raise the likelihood that the condition will develop. In conclusion, the HNF1a gene exhibits a high amount of variety, and the clinical manifestations that result from the presence of several SNPs might greatly differ from one another[61]. For this reason, genetic identification is absolutely necessary in order to acquire comprehensive information regarding HNF1a. It is essential that this be done in order to

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effectively treat aberrant blood glucose levels that are brought on by mutations in the HNF1a gene. *HNF1A* is made up of 631 different amino acids. The amino acids are made up of three distinct domains: the DNA-binding domain (residues 203–276), the transactivation domains (residues 281–631), and the amino-terminal dimerization domain (residues 1–32). P291fsinsC (formerly known as p.G292fs) is the *HNF1A* mutation that is most prevalent in MODY3[61]. This mutation has a predominantly negative effect on wild-type *HNF1A*. It has been determined that the enzyme is lacking in the part of the transactivation domain that is most prominent. There is a high prevalence of missense mutations in the DNA-binding domain and the amino-terminal dimerization region, whereas deletion mutations are frequently found in the transactivation domain. There is a possibility that this is the reason for the clinical variability observed in persons with MODY3[61].

Single nucleotide polymorphisms, sometimes known as SNPs, are the form of variation in DNA sequences that occurs most frequently within the genome. It is anticipated that there will be ten million SNPs in the human genome. Through the statistical analysis of the link between diseases and SNPs, it is possible to facilitate the evaluation of disease risk, the elucidation of the molecular causes of the disease, and the development of pharmacological targets for patients who have certain SNPs. Through the use of statistical analysis, each of these objectives can be accomplished. More than one hundred single nucleotide polymorphisms (SNPs) that are frequently associated with type 2 diabetes mellitus (T2DM) in individuals have been discovered using genome-wide association studies (GWAS), with a minor allele frequency (MAF) that is greater than one percent. With the help of simulated models of type 2 diabetes, it has been suggested that these widespread variations could account for around 75% of the heritability of the illness. A statistically insignificant

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correlation exists between individual common variants and type 2 diabetes, as indicated by an odds ratio that is less than 1.3[70]. It is anticipated that individuals who possess a greater number of disease-associated variations will have a higher probability of getting the ailment. When it comes to the heritability of type 2 diabetes, infrequent genetic mutations that occur at a frequency that is lower than 0.5% have a pretty insignificant impact. In light of the fact that occasional variants have a small impact, it would suggest that common mutations have a significant influence on the inheritance of type 2 diabetes type [71]. A finding that is supported across a number of different ethnic groupings is that there is an association between HNF1A SNPs and the chance of people developing type 2 diabetes[72]. Common coding variations of *HNF1A* include I27L, A98V, and S487N, all of which have the potential to influence the risk of developing type 2 diabetes. 17 individuals who had unique *HNF1A* coding variants (minor allele frequency < 1%) were subjected to an oral glucose tolerance test (OGTT) consisting of 75 grams of glucose.

Only one of these individuals had an abnormally high glucose level after two hours, which is defined as a difference that is greater than ninety milligrams per deciliter between the glucose readings taken after a meal and the readings taken while the individual was fasting. However, it is feasible that the loss in insulin production was partially compensated by an enhancement in insulin sensitivity and a reduced threshold for urine glucose excretion, as shown in patients with MODY3[61]. Insulin levels were not evaluated in these uncommon variation carriers; however, it is possible that this enhancement occurred. Based on the findings, it is clear that the functional validation of *HNF1A* variants is of extremely high importance.

Furthermore, the findings indicate that predicting type 2 diabetes in the general population by using rare variations that were discovered in genome-wide association studies (GWAS) may

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result in an exaggerated impact of these alterations, which ultimately leads to an increased prevalence of false positives. Particularly for rare *HNF1A* mutations linked to MODY3, ascertainment biases may develop in Mendelian genetic studies[73]. This is because the selection of patients with manifest diabetes or a familial history of MODY may lead to the selection of patients with these characteristics. Typically, it is assumed that variations of uncommon diseases have a significant influence on the phenotype of the disease themselves. In Mendelian genetic investigations, however, there is a possibility of ascertainment biases occurring[61]. Approximately thirty to seventy percent of all cases of MODY are characterized by the presence of maturity-onset diabetes of the young type three (MODY3).

Despite the fact that it is classified as belonging to the same subtype, MODY3 has been linked to almost 200 distinct heterozygous mutations in the hepatocyte nuclear factor 1a (HNF1a) gene[60]. Multiple factors, such as the kind and position of HNF1a mutations, the patient's genetic background (Lango Allen et al., 2010), and environmental impacts that occur both prenatally and postnatally, all have a role in determining the many clinical symptoms that are associated with MODY3 individuals. There is a wide range of treatment regimens that are necessary for individuals to follow in order to effectively control their diabetes[61]. In the beginning, younger individuals are treated with dietary modifications and sulfonylureas; but, as they get older, they require more medication, which may include insulin. There is currently a lack of clarity on the precise biochemical mechanism that links specific HNF1a mutations to the increase of B cells. It is possible that modifications in the dimerization domain will have a significant impact on the functionality of HNF1a. Because of these alterations, HNF1a is unable to interact with co-factors and DNA targets as effectively as it should. Mutations in the DNA-binding homeodomain have been linked to a

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number of other diseases in addition to MODY3, which is one of them. Due to the absence of a definitive connection between decreased transactivation activity and decreased DNA binding, the ramifications of these alterations are not entirely obvious[61]. It is quite likely that the altered transcriptional activation that occurs as a consequence of mutations in the HNF1a promoter is the cause of the extremely unusual occurrence of these mutations. This is because these mutations have the potential to have enormous consequences, which is why they are so uncommon. Additional mutations have been found in the transactivation domain, which has resulted in changes to the cellular localization of HNF1a as well as its interactions with DNA and other factors 59, 60. Both of these mutations have been found in that location. Therefore, the site of the HNF1a-specific mutation is crucial for both the method and the consequences that are produced as a consequence of it.

The HNF1ap291fsinsC mutation is one of the alterations that can be discovered in the domain that facilitates transactivation[61]. This mutation is found in twenty to fifty percent of cases among the majority of patients who have MODY3 syndrome. The creation of an early stop codon and a frameshift mutation61 is the result of inserting a cytosine (C) into the polycytidine sequence of HNF1a's codon 291, which is denoted by the notation p291fsinsC[59]. When compared to individuals who have other HNF1a mutations, those who have this mutation exhibit clinical symptoms that are more severe. Frequently, they display symptoms that are comparable to those of type 1 diabetes, and in certain instances, they may develop hepatocellular adenoma[59]. Unlike the other alterations that can be identified in the transactivation domain that is connected to MODY3, this mutation does not duplicate those modifications. Among the negative consequences that are connected with HNF1ap291fsinsC phenotypes in MODY3, the most significant one is the synthesis of a shorter HNF1a protein

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or haploinsufficiency.[61] This could be the result of nonsense-mediated decay. Additionally, it is of the utmost importance to underline that HNF1a+/ mice do not exhibit diabetes, but HNF1a/ animals exhibit growth retardation, decreased insulin production, and altered aerobic glucose metabolism. As a result of the pleiotropic consequences that are caused by the total deletion of HNF1a61, persons who have MODY3 report experiencing a number of renal issues. This demonstrates quite clearly that mice do not fully replicate the etiology of MODY3, which is caused by mutations that are either missense or nonsense.

As a result of being subjected to genetic alteration and being lacking in either one or both HNF1a alleles, human embryonic stem cells (hESCs) exhibit decreased mitochondrial function, insulin synthesis, glycolytic activity, and a tendency to differentiate. Despite the fact that human induced pluripotent stem cell (hiPSC) lines have been produced from patients who have HNF1a mutations, there is a lack of understanding regarding the capacity of these cells to grow into hematopoietic cells. More severe symptoms are displayed by mice who overexpress truncated forms of HNF1a. These symptoms include decreased insulin synthesis and poor beta cell proliferation[61]. Consequently, this suggests that the shorter versions of HNF1a that are the result of the p291fsinsC mutation may display additional functions that are independent of HNF1a. This may be the case because of the interactions that these variants have with other members of the HNF family. Despite the fact that HNF1b, which is the homologous protein to HNF1a, is expressed in a greater variety of organs, the ventral endoderm is the primary organ that is affected by the homozygous deficiency observed in this protein[59]. There is a significant role that the expression of HNF1b plays in the identification of multipotent progenitor cells. This expression can be observed as early as the primitive gut tube stage of differentiation. The process of pancreatic agenesis takes place

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in mice when there is no expression of HNF1b at all. Heterozygous mice, on the other hand, exhibit symptoms that are consistent with renal dysfunction and pancreatic hypoplasia. In a similar vein, mutations in human HNF1b primarily appear in phenotypes that are related with renal diseases, diabetes mellitus, and MODY[61]. A mutation known as HNF1ap291fsinsC is responsible for the development of MODY3 diabetes, which is characterized by varied penetrance and is typically detected before the age of 25. To this day, the utilization of human pluripotent stem cells (hiPSCs) for the purpose of elucidating the mechanisms that underlie the diseases associated with MODY3 has produced limited results.

The deletion of HNF1a in genetically modified embryonic stem cells (ESCs) has a number of significant effects, including a reduction in insulin secretion, a decrease in glycolysis, a compromise in mitochondrial function, and a developmental tendency toward a particular differentiated cell type[55]. Putting an emphasis on the fact that these consequences are present is absolutely necessary. It is not yet known if the whole or partial deletion of HNF1a in embryonic stem cells (ESCs) faithfully imitates the mutation that is most commonly found in MODY3 (HNF1ap291fsinsC)[61]. Through the utilization of CRISPR/Cas9-engineered lines, healthy donor hiPSCs, and MODY3 patients, we were able to successfully identify defects in the development of progenitor cells and B cells that were attributed to the HNF1ap291fsinsC mutation. These findings were made possible as a result of the adoption of these three strategies. As human embryonic stem cells (ESCs) that are devoid of both HNF1a alleles mature into β cells, there is a minor decrease in the quantity of β cells, which is accompanied by a significant decrease in the expression of PDX1.

In contrast, our data reveal that the target genes of both HNF1a and HNF1b are affected, and that the additional dominant negative function of HNF1ap291fsinsC over HNF1b results in a

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more severe phenotype that cannot be compared to the absence of HNF1a. The HNF1a/HNF1b target genes PKHD1 and KIF12, whose disruption correlates with developmental abnormalities, as well as HNF1b-associated components of the Wnt and Notch pathways, which are critical for early pancreatic development, were consistently downregulated in HNF1ap291fsinsC lines. This was the case in all of the lines. In this way, the circumstance was established. For additional information, our findings suggest that HNF1ap291fsinsC forms a dimer with HNF1b, which leads to a decrease in the amount of PP differentiation and transcription that is dependent on HNF1b[60].

Patients who had truncating mutations had an early onset of the disease, which was accompanied by a gradual decline in the function of their B cells during the course of disease progression. The severity of the phenotype that was observed in the HNF1aMODY3 lines from the very beginning is confirmed by these results[54]. The manifestation of more severe symptoms is a consequence of a variety of factors, including differing genetic origins and other obstacles. Despite the fact that the severity of the problem varied from patient to patient, the degree of the PP differentiation defect was consistently seen across all five patient lines. According to the findings that we obtained, this variation can be attributable to the fact that HNF1ap291fsinsC has an effect that is complementary to that of HNF1b function. Following the partial restoration of B cells with HNF1b overexpression in HNF1ap291fsinsC organisms, it is possible that the amounts of HNF1b in each patient line could shed light on the various MODY3 phenotypes and the heterogeneity that has been seen.

It has not been possible to mimic diabetes using human induced pluripotent stem cells (hiPSCs). On the other hand, organoid models for progenitor growth have been constructed utilizing human embryonic stem cells (hESCs) as well as both murine and human adult

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tissues. We have devised a method that is capable of producing progenitors from human embryonic stem cells (hiPSCs) in an effective manner and then developing these progenitors into cells that are similar to b. Using healthy, MODY3, and CRISPR/Cas9 hiPSC-derived pancreatic organoids, we were able to show that HNF1ap291fsinsC influences the development of beta-like cells in a relevant human model through a mechanism that has not yet been described. Similar traits were observed in mice and pigs that were developing MODY3 symptoms. People who have a mutation in the HNF1ap291fsinsC gene are more likely to experience pancreatic problems.

The abnormalities include a decrease in the dimensions of the pancreas and the volume of beta cells. These findings are in agreement with our research, which shown that a reduced progenitor pool derived from mutant induced pluripotent stem cells significantly restricts the growth of the pancreas as well as the number of beta cells. Our research has uncovered a unique method by which the shorter HNF1ap291fsinsC protein, which is produced by the common MODY3 p291fsinsC mutation, inhibits the function of HNF1b while simultaneously inhibiting the proliferation of pancreatic polypeptide and beta cells. In order to investigate the pancreatic developmental defects that are linked with monogenic diabetes, the PP organoid system that we developed offers a suitable theoretical foundation[61].

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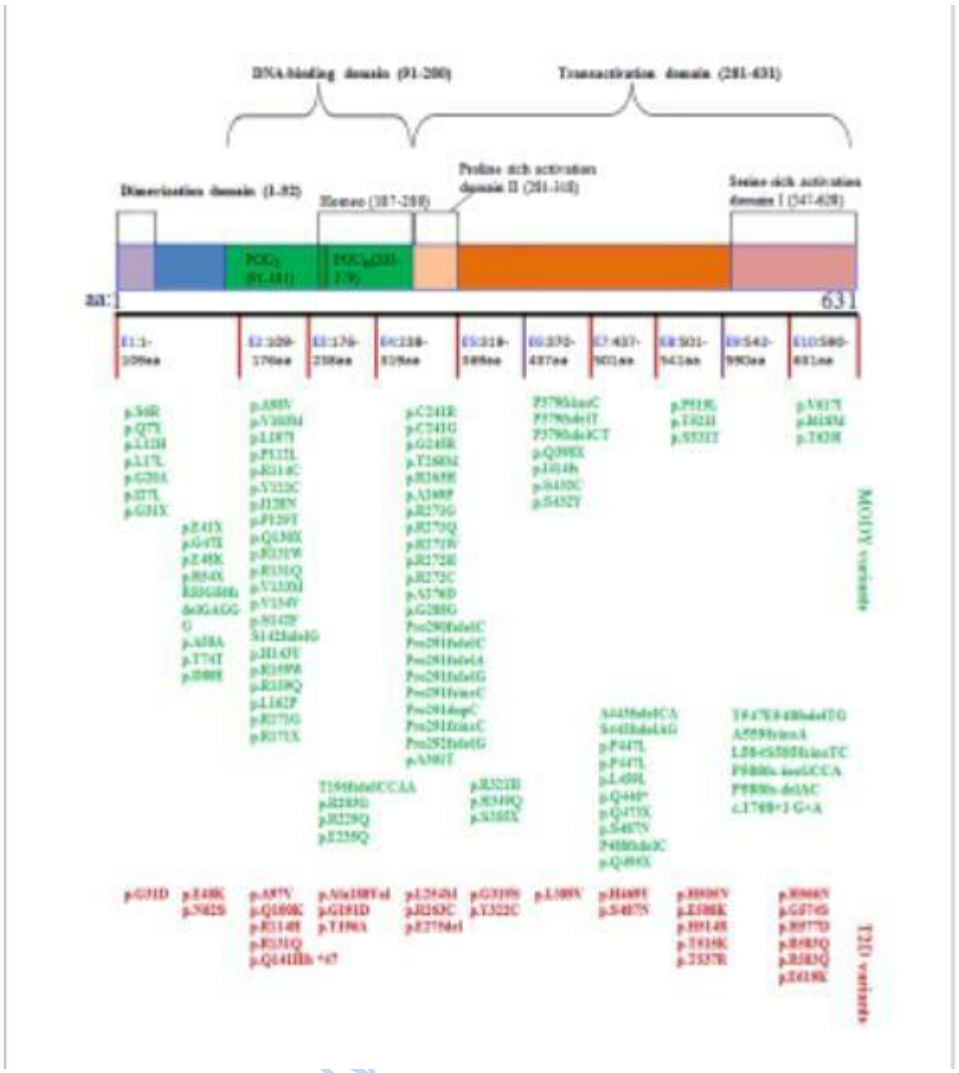


Figure 2.15. The Structure of HNF1a Protein and the SNPs Associated with MODY3 or T2D. The SNPs in Blue Often Cause MODY3. The SNPs in Red Often Cause T2D[61]

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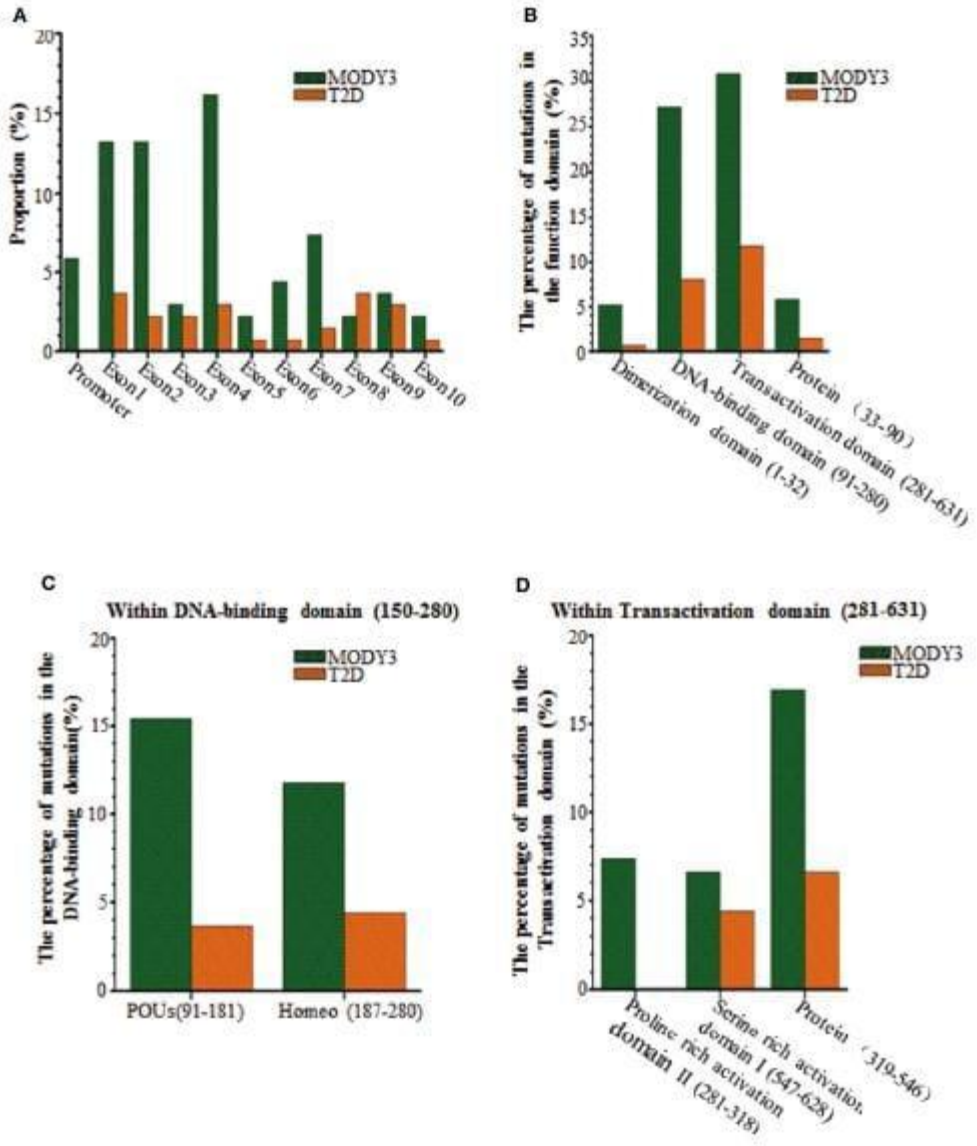


Figure 2.16. Comparative analysis of HNF1a SNPs associated with MODY3 and those linked to T2D. (A) Position within the promoter and ten exons, (B) Position within the functional domains of the HNF1a protein, (C) Position within the DNA-binding domain of the HNF1a protein, (D) Position within the transactivation domain of the HNF1a protein[61]

2.3.1 Pharmacologic Treatment for Diabetic Patients with *HNF1A* Mutations

Metformin's ability to lower glucose levels was found to be equivalent in people with MODY3 and those with type 2 diabetes. On the other hand, the sensitivity of individuals with MODY3 to sulfonylureas (SU) was found to be higher than that of adults with type 2 diabetes. People who have MODY3 have a higher sensitivity to sulfonylureas (SU), which may be due to the fact that they possess a higher insulin sensitivity[61]. When they are given SU, they are more likely to have hypoglycemia as a consequence of this[74]. In patients diagnosed with MODY, monotherapy with glucagon-like peptide-1 receptor agonists (GLP-1 RAs) or dipeptidyl peptidase-4 (DPP-4) inhibitors has the potential to prevent hypoglycemia and provide effective glycemic control. In patients diagnosed with MODY3, the use of a combination of sulfonylureas and GLP-1 receptor agonists has the potential to increase insulin secretion[61].

Additionally, the combination of DPP-4 inhibitors with sulfonylureas has the potential to improve glucose regulation in individuals diagnosed with MODY3 while simultaneously lowering the risk of hypoglycemia[74]. In patients with MODY3, the responsiveness to sulfonylurea medication implies that *HNF1A* mutations may not significantly affect the insulin secretion pathway. This is because membrane depolarization occurs in beta cells. The manifestation of a favorable reaction lends credence to this assertion. Insulin exocytosis is regulated by *HNF1A*, and mutations in *HNF1A* have the potential to change the production of mature insulin secretory granules through their effects. In order to have a better understanding of the effects that *HNF1A* mutations have on other endocrine cells, such as alpha cells, it is essential to do additional research using human participants[61].

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Among the many issues that might arise as a consequence of HNF1A-MODY3, severe diabetic retinopathy, diabetic nephropathy, and diabetic peripheral neuropathy are potentially among them. The prompt application of identification and administration of when it comes to improving one's quality of life, delaying the start and progression of disorders, and managing blood glucose levels, medicine is absolutely necessary. The hyperglycemia that is accompanied by extra-pancreatic symptoms, rising hyperglycemia, and stable mild hyperglycemia are the characteristics that define the HNF1A-MODY phenotype characteristics[74]. Adjustments to one's diet are a realistic and successful therapy option for those who have mild hyperglycemia; nevertheless, pharmacological therapies are indicated for individuals whose hyperglycemia is getting worse. The therapeutic approach that is taken for persons who have HNF1A-MODY is contingent upon their age as well as their HbA1c levels[64]. Patients who have mutations in the HNF1a gene are at a significant risk when they are given sulfonylurea, which is a medicine that is used to treat hypoglycemia as an oral preparation.

Those individuals who have mutations in the HNF1a gene are expected to have decreased hepatic clearance of certain sulfonylurea derivatives, which is considered to be the source of higher serum levels. It is possible that the high amounts of these drugs that are currently in circulation are the reason for their increased effectiveness. When compared to the response rate seen with regular metformin, the response rate shown with sulfonylureas was five times higher in patients who were diagnosed with HNF1AMODY. On the other hand, evidence suggests that the two medications are practically equivalent in terms of their effectiveness in treating type 2 diabetes. It is typically observed that individuals who have HNF1A-MODY exhibit a more favorable response to sulfonylureas as opposed to insulin therapy when it

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comes to the regulation of blood glucose levels. As an additional point of interest, sulfonylureas have been shown to have a beneficial hypoglycemic effect when they are provided while fasting. HNF1A-MODY patients exhibit a high level of sensitivity to medications that are taken orally that contain sulfonylureas. The occurrence of sulfonylurea medication that is ineffective is extremely uncommon, and it only happens to a very small percentage of people who have the c.618G>A mutation. Seventy-four are. Low-dose sulfonylureas, such as gliclazide, which should be administered at a daily dosage of 20–40 mg, are recommended for long-term maintenance and should be considered the first-line treatment for HNF1A-MODY [74]. A large number of studies have shown that individuals who have specific variations, on the other hand, do not react to the medications that were mentioned earlier. P.His126Asp individuals exhibit a lack of sensitivity to glucagon-like peptide-1 receptor agonists (GLP-1Raa), which are more commonly referred to as incretin analogues within the medical community[72].

They exhibit a lack of reactivity to sulfonylureas and dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors), regardless of the dosage that is administered to them. On the other hand, despite the fact that low-dose sulfonylurea therapy is the primary treatment for MODY3, it does not exhibit any particular vulnerability to type 2 diabetes and is not related with HNF1a. Those Mexicans who have the HNF1a p.E508K mutation do not exhibit an increased susceptibility to sulfonylureas, according to the scientific literature[72]. HNF1A-MODY genes are characterized by a wide variety of clinical and genetic variants, as the conclusion of this discussion suggests. With that being said, MODY3 continues to display a considerable amount of character. Reduced levels of high-sensitivity C-reactive protein, often known as hsCRP, are typically observed in individuals who have been diagnosed with HNF1A-MODY

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conditions[74]. To further complicate matters, even though MODY3 is a form of monogenic diabetes, the severity of the ailment varies depending on the individual's genetic predisposition as well as the environmental variables that they are exposed to. In the past, people who were diagnosed with MODY3 were frequently fat or overweight, and they did not have any additional risk factors for type 2 diabetes, such as dyslipidemia or hypertension. In addition, they lacked the necessary qualities for this condition. In light of the fact that the prevalence of overweight and obesity is on the rise, it is not out of the question that individuals who have MODY3 who are also classed as overweight or obese. The distinction between type 2 diabetes and HNF1A-MODY3 is more difficult to make due to the fact that statistics show that more than thirty percent of people diagnosed with HNF1A-MODY in the United States are either overweight or obese. As a consequence of this, despite the fact that there are clinical standards that have been established for the identification of MODY3, patients who have HNF1A MODY3 may on occasion fail to meet all diagnostic criteria or may suffer from an inaccurate diagnosis of type 1 or type 2 diabetes[78].

2.4 Review of Empirical Studies

In the *HNF1A* gene, a total of nine different variants were discovered. These variants included seven mutations, including one novel mutation identified as 538G≥C in the promoter region, as well as six novel mutations in the coding area. Additionally, there were two polymorphisms. Based on the findings of the functional test, it was shown that the *HNF1A* promoter displayed a decreased transcriptional activity for two different promoter variants specifically. The mutation in the Arg263His coding region was shown to be associated with diabetes in eight individuals belonging to a single MODY family, however it was not found in any members of the family who did not have diabetes[76]. Our findings in

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Paper II show that none of the 27 HNF1A variants that were considered to be harmful were associated with an increased risk of developing type 2 diabetes in the population cohorts (odds ratio = 2.02; 95% confidence interval = 0.73-5.60; p-value = 0.18). The utilization of bioinformatics prediction tools allowed for the evaluation of the anticipated adverse consequences, which were used to establish this[77]. The functional repercussions of these changes were investigated further by analyzing the influence that these variations had on the transcriptional activation of HNF-1A, the binding of DNA, and the subcellular (cytoplasmic/nuclear) localization of the protein. A further investigation was conducted to look at the connection between type 2 diabetes and various other functional test models.

A transcriptional activity that is lower than sixty percent, in comparison to the activity of wild-type HNF-1A, was found to be the most significant predictive factor that correlates type 2 diabetes with the phenotype of a carrier for type 2 diabetes (odds ratio = 5.04; 95% confidence interval = 0.0007). The statistics show that around forty-four percent of the population possesses *HNF1A* mutations, which considerably raises the chance of disease onset. This is a huge rise[77]. The presence of protein-coding variants that have a mean absolute frequency (MAF) that is less than 0.01% is indicative of an increased propensity for pathogenicity. These changes are also linked to uncommon disorders like MODY. In addition, the missense variants that were found to be the most abundant were investigated in population frequency databases, such as gnomAD, as well as clinical variant databases in Cape Town, particularly ClinVar[77]. Two possible variants in *HNF1A* were discovered, each of which had a minor allele frequency that was lower than 0.1%. In this case, the variations are c.1124delG; p.(Gly375AlafaTer9) and c.1243G>A; p.(Gly415Arg). It was discovered that the p. (Gly375AlafaTer9) mutation was present in three people: two people who had

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poor glucose tolerance and one person who did not have any symptoms of diabetes. Furthermore, the presence of p. (Gly415Arg) was identified in a single person who had a decreased glucose tolerance. On the basis of this and other clinical criteria, it would appear that these polymorphisms do not cause MODY; nonetheless, it is possible that they are a risk factor for type 2 diabetes (p.(Gly415Arg))[77]. Out of the fourteen modifications that were found in Indians, four of them were classified as pathogenic, six of them were classified as potentially pathogenic, three of them were classified as variants of unknown consequence, and one of them was classified as benign. The formation of heterodimers of HNF1B was a possibility for patients who possessed mutations that were either pathogenic or potentially disruptive. Sanger sequencing and next-generation sequencing were performed on four hundred fifty Hungarian index patients who were suspected of having MODY disease[42]. The results of these tests indicated that approximately thirty percent of the patients presented with a positive result. It was discovered that the GCK gene included more than seventy percent of the pathogenic mutations, the *HNF1A* gene contained more than twenty percent, and other genes that are connected to MODY contained fewer than ten percent of the defects. An evaluation was performed on a total of 48 patients and their family members, and the results revealed that the *HNF1A* gene had 8 pathogenic mutations and 9 potentially pathogenic mutations[78].

2.5 Theoretical Framework

In an attempt to explain molecular characterization of *HNF1A* gene among diabetic patients in Lagos, the health belief model serves as a useful framework in identifying the outcome on the level of understanding and perception on the molecular characterization of *HNF1A* gene among diabetic patients.

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2.5.1 Health Belief Model

The health belief model is a psychological framework that was developed with the purpose of informing and predicting actions that are related to health. The initiative, which was initiated in the 1950s by a group of social psychologists working for the United States Public Health Service, aimed to obtain a better understanding of the variables that were contributing to the low level of participation in the computerized program that was developed for the detection and prevention of disease[76]. According to this theory, two elements that impact an individual's behavior about health-seeking are the individual's assessment of the threat that is posed by a health condition and the value that is attributed to techniques that can minimize that threat. It examines the relationship between a person's beliefs and the behavior that they exhibit in their daily life[76].

2.5.2 Major Concepts of the Model

The term "perceived susceptibility" refers to the degree to which an individual is aware of their sensitivity to a certain health problem[76]. According to this point of view, a health worry may be personally significant, or it may imply that a medical diagnosis is accurate[76]. Individuals will refrain from taking action until they can determine that the severity of the situation is severe enough to result in significant effects for their bodies or for their social lives. Despite the fact that they admit their special sensitivity to the circumstance, this can still be considered valid. It is referred to as the patient's perceived advantages when they are convinced that a particular treatment would either cure their illness or reduce the likelihood of them becoming sick in the future[76]. In the context of the therapy or operation, the phrase "perceived cost" relates to the difficulty, time, and accessibility involved with the treatment

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or procedure[96]. In the context of the concept of motivation, the propensity to comply with a therapy and the conviction that persons should engage in actions that would enhance their health state are both included. There are a number of elements that are subject to change, including personality characteristics, the degree of pleasure that patients experience, and socio-demographic factors[76]. The health belief model is comprised of three fundamental components, which are described in the following manner[76]:

1. Individual Perception: Individual perception or view of susceptibility to disease and the seriousness of the disease combine to form his perceived threat of illness. For example, a patient needs to understand the familial link for mental illnesses particularly when other people have died from mental illness, the patient may perceive the personal risk of mental disorder[76].

2. Modifying Factors: Age, gender, race, and ethnicity are some of the demographic factors that are taken into consideration in this research. Furthermore, personality, social status, and the influence of a reference group are addressed, in addition to demonstrations of approaches that can be put into practice. Requests for guidance from friends, family members, professionals in the field of mental health, and the media[76].

3. Variables Affecting the Likelihood of Initiating Actions: The third component is the variable affecting the likelihood that the individual will take any action, the extent to which action is believed to be beneficial is viewed in conjunction with existing barriers to that action[76].

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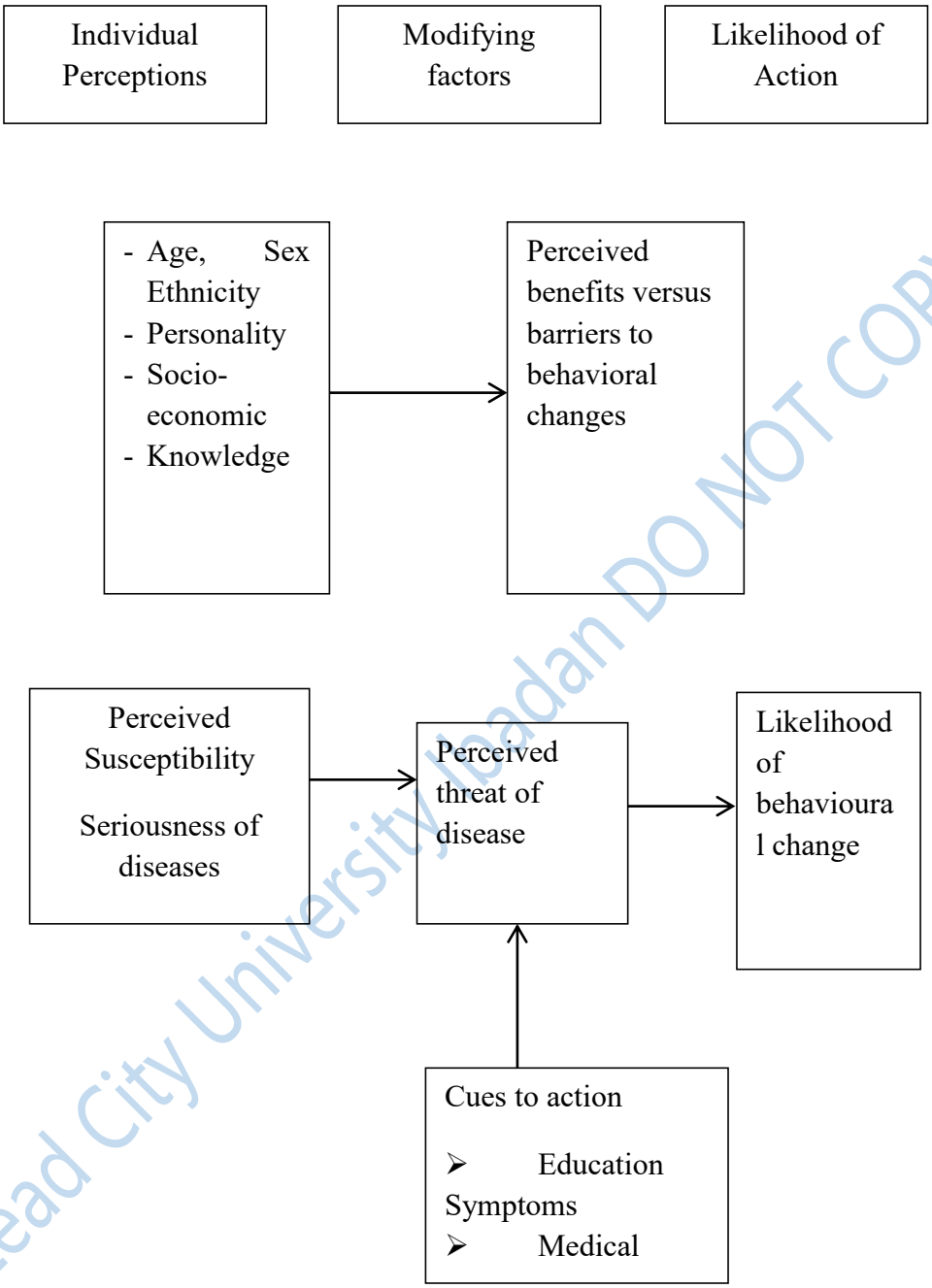


Figure 2.17: Schematic Representation of Health Belief Model[76].

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2.5.3 Adaptation of Health Belief Model to Study

1. Perceived Severity

On the other hand, it could be the result of an individual's misunderstandings regarding the repercussions of diabetes or the difficulties that it would provide in their life. The anticipation of a potential risk is an essential step that must be taken before putting into action a preventative approach. The people who live in Lagos State ought to participate in preventative actions in order to solve the problems that are caused by diabetes.

2. Perceived Susceptibility

The greater a person's perceived susceptibility is, the greater the likelihood that they will engage in practices to reduce the risk. Lagos state resident are more likely to partake in good practice if he/she perceives they are at risk of developing health complications and irreversible health condition associated with diabetes. An increase in perceived susceptibility has been shown to enhance engagement in good practices.

3. Perceived Benefits

It is the conviction that an individual has regarding the manner in which altering their behavior would enhance their life in comparison to the activities that they have been engaging in in the past, or the manner in which the modification will be useful in reducing the likelihood of obtaining an illness. Those who live in Lagos State and have a comprehensive awareness of the elements that contribute to diabetes are more likely to steer clear of those causes.

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4. Perceived Barriers

In the context of the acquisition of a new habit, the term "individual assessment" refers to the appraisal that an individual makes of the factors or challenges that create barriers to the process. It is the purpose of this article to investigate the negative perceptions that are connected to preventative actions. Perceived obstacles are the construct that outperforms all other dimensions in terms of their ability to accurately predict changes in behavior. The difficulties may include, among other things, a lack of information, cultural viewpoints and customs, socioeconomic conditions, and other factors.

5. Self-efficacy

Under most circumstances, people will refrain from seeking out new career chances unless they are certain that they will be successful in the position. In the event that an individual considers a novel technique to be advantageous but is uncertain about her capacity to implement it, it is quite probable that she will refrain from attempting to put it into practice. When taking into consideration the level of pressure that is exerted by society, this is extremely relevant.

2.6 Summary of Gaps in Literature Reviewed

There have been numerous regions and ethnic groups all over the world that have been investigated for the presence of gene mutations in nuclear and mitochondrial DNA, as well as single nucleotide polymorphisms (SNPs) that are associated with diabetes. A number of studies have been carried out in order to investigate the genetic factors that influence diabetes incidence. There are between one and two and a half percent of all instances of diabetes that are caused by the MODY type of monogenic diabetes[79].

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This is the most common form of the disease. MODY is distinguished by the presence of an autosomal dominant inheritance pattern, the onset of diabetes in individuals under the age of 25, and a reduction in insulin production as a consequence of mutations in transcription factors that are responsible for the formation and function of beta cells. There are currently fourteen distinct subtypes of MODY that have been discovered[79]. MODY1 (HNF4A), MODY2 (GK), MODY3 (HNF1A), MODY4 (PDX1), MODY5 (HNF1B), and MODY6 (NeuroD1) are the subtypes that are included in this description. When it comes to these three categories of abnormalities, the most common cause of MODY is a mutation in the *HNF1A* gene. At the same time, single nucleotide polymorphisms (SNPs) that are located within and close to *HNF1A* have been associated with an increased likelihood of developing type 2 diabetes.

Although it has been established that there is a correlation between *HNF1A* SNPs and type 2 diabetes, the nature of the causal interaction between the two is still still unknown. These findings are consistent with the correlation that has been shown between MODY3 and *HNF1A* mutations. Multiple studies have made use of mice lacking *HNF1A* in order to gain a better understanding of the pathophysiological role that *HNF1A* plays in diabetes[79]. Nonetheless, the molecular foundation remains insufficiently explored owing to the phenotypic variability revealed in Hnf1a-null creatures and individuals with diabetes. In order to better understand the *HNF1A* gene in diabetes patients in Lagos, it is necessary to do molecular characterization of the gene.

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Endnotes

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Chapter Three

Methodology

3.1 Research Design

The present study employed a quantitative (analytical), cross sectional research design

Study population: All patients attending and confirmed to have elevated blood sugar and HbA1C in Federal Medical Centre, Ebute-Metta, Lagos.

Study location: According to estimates, the population of Lagos was approximately 21 million in the year 2015, making it the most populated metropolitan area in Nigeria. After the Nigerian government made the decision in December 1991 to move the capital to Abuja, which is located in the middle of the country, Lagos continued to serve as the nation's capital until that time. Awori, a subset of the Yoruba people, founded Lagos as their place of residence[1]. They were originally from the areas that are now known as the Local Government Areas (LGAs) of Lagos Island, Eti-Osa, Amuwo-Odofin, and Apapa. These individuals are referred to as the Awori subgroup. As a result of the rapid progression, the city expanded westward beyond the lagoon, encompassing regions that are now known as Lagos Mainland, Ajeromi-Ifelodun, and Surulere. The two primary regions that are recognized as Lagos are the Island, which is where the city of Lagos was initially established, and the Mainland, which is where it has also evolved since then. In Lagos, there are sixteen different local government areas, and Ikeja is one of them. Ikeja is the capital of Lagos State.

Study Sites: The Federal Medical Centre Ebute-Metta, Lagos was used in this study. Federal Medical Centre Ebute-Metta, Lagos, was chosen due to various factors such as its facilities, patient population and its track record in management of diabetes.

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3.2 Sample Size

The sample size (N) was determined using sample size determination formula for health studies. The overall pooled prevalence of DM in Nigeria is 5.77%[2].

$$N = \frac{Z^2P\ (1 - P)}{D^2}$$

N = sample size required

Z = confidence level at 95% (standard value of 1.96)

P = estimated prevalence of DM in Nigeria is 5.77% (0.0577)

D = margin of error at 5% (standard value = 0.05)

$$N = \frac{1.96^2 \text{ X } 0.0577\ (1\text{-}0.0577)}{0.05^2}$$

$$\frac{3.8416 \text{ X } 0.0577\ (0.94)}{0.0025}$$

N =83 minimum sample sizes

Therefore, a total of 100 patients were used in this study.

The study population for the present study consist of 48 patients diagnosed with diabetes mellitus aged 20years and above with no pre-existing chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, smokers, alcoholics and having acute infectious diseases who are residents of Lagos state. 52 apparently healthy individuals were employed as controls.

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3.3 Sampling Techniques and Sample Collection

The sampling technique that was adopted for this research study was the simple random sampling technique. This method was ensured that all diabetic patients who are residents of Lagos State, have equal chance of being selected. There was no bias in the participant's selection. Ten milliliters (10ml) of venous blood were obtained from the anterior cubital vein of qualified patients using sterile veni-puncture procedure using 10ml disposable, sterile syringe. The blood was collected in ethylene diamine tetra acetic acid anticoagulated sample bottle and fluoride oxalate sample bottle for genomic and biochemical analysis and was stored at 5^0C until required for analyses. The fluoride oxalate anticoagulated blood sample was centrifuged at 3000RPM for 5minutes and the plasma was obtained into a sterile bottle for biochemical analysis within 4hours.

3.4 Description of Research Instruments

3.4.1 Estimation of Fasting Blood Glucose

3.4.1.1 Principle

The enzyme glucose oxidase (GOD) catalyzes the conversion of glucose into gluconic acid upon exposure to atmospheric oxygen. This procedure yields hydrogen peroxide (H_2O_2). The enzyme peroxidase (POD) facilitates the liberation of nascent oxygen (O_2) from hydrogen peroxide. This oxygen subsequently reacts with 4-amino antipyrine and phenol to yield a crimson molecule known as quinoneimine dye. A direct association exists between the concentration of glucose in plasma and the intensity of the color[3].

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3.4.1.2 Reagents

1. Glucose colour reagent; it contains:

Glucose oxidase (GOD),

Peroxidase (POD),

4-amino antipyrine,

Phenol, and

Phosphate buffer (pH 7.5)

2. Glucose standard solution, Concentration = 100 mg/dl. 100 mg of anhydrous glucose was dissolved in 100 ml of distilled water

3.4.1.3 Procedure

1. The test tubes were arranged as blank, standard and test (Sample).

2. 20microlitre of the sample, standard and distilled water were added into their respective labelled test-tube

3. 2000microlitre of glucose reagent were added into each test-tube.

4. The solution were mixed and incubated at 37^0C for 15minutes

5. The absorbance were read at 520nm.

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3.4.1.4 Calculation

Results were calculated as follows:

$$\text{Result} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{standard concentration}$$

3.4.1.5 Reference range

70-110mg/dl

3.4.2 Genomic analyses

Primer Design: The primer design is as shown below:

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PCR primer sequences for the *HNF1A* gene

Site	Forward	Reverse
Exon 1	TGCAAGGAGTTTGGTTTGTG	GAAGGTCATGGGGACTCAAC
Exon 2	GCCATGGCAATGAGAAAGAA	GGCAACTGGACAGCCTTTTA
Exon 3	GGCAGAGCTCAHCTTCTCAG	AAGGAGTGGCATGAATGGAA
Exon 4	CTCTGGGAAGGAGAGGTGGT	GTCCCAGAGACACATGCAGA
Exon 5	TGAGTCCCCTAGGGACAGG	CCTGCCTTCCCTGTTAGCTT

Source[4]

3.4.2.1: Primer synthesis: The primers were synthesized using Oligosthesizer machine at the department of Nigerian Institute of Medical Research (NIMR). Include primer sequence data which were given me.

3.4.2.2 DNA extraction: Genomic DNA were isolated from EDTA anticoagulated whole blood using the standard protocol (QIAamp Blood Mini kit) [4].

3.4.3 PCR QIAGEN Procedure

1. Pipet 200microlitre of whole blood, 20microlitre of QIAGEN protease and 200microlitre of buffer AL into a 1.5ml low binding micro-centrifuge tube and mix by vortexing.

2. Incubate at 56^0C for 10minutes.

3. Spin down briefly to remove drops from the inside of the tubes.

4. Add 200microlitre of 96-100% ethanol and mix by vortexing.

5. The mixture were carefully applied to a QIAamp spin column.

6. Centrifuge for 1minute at full speed (15,000 xg).

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7. Discard the tube containing the filtrate. The DNA was bound to the filter in the spin columns.

8. Place column in a 2ml collection tube.

9. Open the QIAamp spin column and add 500microlitre of buffer AW1 without hitting the rim. Close the cap and centrifuge for 1minute at full speed, the place the spin column in a clean 2ml collection tube.

10. The QIAamp spin column were carefully opened and 500microlitre of buffer AW2 were added. The cap was closed and the QIAamp columns were centrifuged for 3minutes at maximum speed.

11. Discard the 2ml collection tube containing the filtrate, place the QIAamp spin column in a new collection tube and spin for 1minute to remove residual buffer AW2.

12. Place the spin column in a clean 1.5ml low binding micro-centrifuge tube and add 200microlitre of buffer AE or distilled water to the spin column.

13. Incubate at room temperature for 5minutes to elute the DNA and centrifuge for 1minute at full speed.

3.4.4 ACMG classification

All HNF1A variations will be assessed using the ACMG guidelines, which classify variants as pathogenic (class 5), likely pathogenic (class 4), uncertain significance (class 3), likely benign (class 2), or benign (class 1).

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3.5 Data Management

3.5.1 Data Collection

Biodata and social demographic data was collected using a well-structured self-administrated pretested questionnaire which was specifically designed to obtain information that helped in either including or excluding certain individuals from this study respectively.

3.5.2: Data entry: Data was entered into excel spread sheet

3.5.3 Data analysis

The data were analyzed utilizing Statistical Package for the Social Sciences software (SPSS) version 26.0. Results will be presented as percentages, proportions, and mean ± standard deviation, and will be illustrated in suitable tables and figures. To assess associations and comparisons between groups, One-way ANOVA and Student's t-test will be employed as appropriate, with $p<0.05$ deemed statistically significant.

3.6 Ethical Approval

A letter of approval to collect data and samples from the participants was obtained from institutions used as study sites. Approval from Research Ethics Committee of the Nigerian Institute of Medical research will be obtained for the study. The researcher was obtained informed consent from the participants. Informants/ Participants have the right to remain anonymous. Principles of confidentiality and anonymity were maintained throughout the study. No personal details will appear on the instrument of data collection (questionnaire). Only the researcher, supervisor and statistician have access to the information and data that was obtained for the purpose of this study only. Participation was entirely voluntary and participants were informed that they are free to decline to participate any time without

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suffering any negative consequences. Participants were given adequate information about the study and no part of the research process is expected to pose a source of physical or emotional harm to the participants.

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Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

Among the 100 study participants, 47 of them (47%) were males while 53% were females. Mean age of the participants was 46.45 years while 40% of the participants were aged 51-70 years (Table 1). Also, 63% of the study participants had normal body mass index (BMI) of 24.3 while 37% were overweight or obese. The mean values showed significant difference for both age and sex with $p < 0.000$ *and* 0.003 respectively (Table 2). The mean values showed significant difference for age but no significant difference was observed within sex and BMI at 95% confidence level ($p < 0.05$) (Table 3). The parameters tested for showed no significant difference for age, sex and BMI with respect to the genetic variants at 95% confidence level ($p < 0.05$) (Table 4).

Also, the study showed that there is polymorphism of the HNFIA gene in the participants. Different band sizes were detected in the different exons used in the study while some participants did not have the specific exon. All the participants had different types, band sizes and numbers. Exon 1 had three (3) bands while exons 2-5 had only one band but different band weights showing variations in the exons and by extention in the HNAFIA gene (Tables 5-10 and plates 1-5).

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Table 4.1: Frequency Distribution and Percentage of Non Genetic Risk Factors for the Non Diabetic and Diabetic Mellitus Participants with their Respective Mean ± SD

Parameters		Frequency (N = 100)	Percentage (%)	Mean ± SD
Sex	Male	47	47	
	Female	53	53	
Age (years)	20 – 30	16	16	46.45 ± 12.14
	31 – 40	13	13	
	41 – 50	31	31	
	51 – 70	40	40	
BMI (Kg/m^2)	Normal	63	63	24.30 ± 3.88
	Overweight	27	27	
	Obese	10	10	
Fasting Blood Sugar (mmol/L)	Non Diabetes	43	43	7.36 ± 3.93
	Diabetes	57	57	
2HrPP (mmol/L)	Non Diabetes	21	21	14.25 ± 4.03
	Diabetes	79	79	
Hb1Ac (%)	Non Diabetes	52	52	6.73 ± 3.94
	Diabetes	48	48	
Height (m^2)		100	100	1.74 ± 0.06

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Table 4.2: Distribution of BMI (kg/m^2) across the Age Categories and Sex of Respondents with their Respective Mean ± SD

Parameters		**BMI (kg/m^2)**			**Mean**	***p-value***
		Normal	**Overweight**	**Obese**		
Age	20 – 30	14 22.2%	2 7.4%	0 0.0%	20.01 ± 2.90	*0.000*
	31 – 40	12 19.0%	0 0.0%	1 10.0%	22.78 ± 2.53	
	41 – 50	21 33.3%	8 29.6%	2 20.0%	24.87 ± 2.67	
	51 – 70	16 25.4%	17 63.0%	7 70.0%	26.07 ± 3.97	
Sex	Female	29 46.0%	19 70.4%	9 90.0%	25.30 ± 4.15	*0.003*
	Male	34 54.0%	8 29.6%	1 10.0%	22.98 ± 3.98	
	Mean ± SD ***p-value***	24.30 ± 3.88 *0.003*				

The Mean Values Showed Significant Difference or Both Age and Sex at 95% Confidence Level ($p < 0.05$)

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Table 4.3: Influence of Age, Gender and Body Mass Index in Assessing the Non Diabetic and Diabetic Mellitus of the Participants

Parameters		**Fasting blood sugar (mmol/L)**		**2HrPP (mmol/L)**		**HbA1c (%)**	
		Non Diabetes	Diabetes	Non Diabetes	Diabetes	Non Diabetes	Diabetes
Age	20 – 30	14	2	4	12	15	1
		32.6%	3.5%	19.0%	15.2%	28.8%	2.1%
	31 – 40	9	4	5	8	10	3
		20.9%	7.0%	23.8%	10.1%	19.2%	6.2%
	41 – 50	12	19	4	27	17	14
		27.9%	33.3%	19.0%	34.2%	32.7%	29.2%
	51 – 70	8	32	8	32	10	30
		18.6%	56.1%	38.1%	40.5%	19.2%	62.5%
	Mean ± SD	7.36 ± 3.62		14.25 ± 4.03		6.73 ± 3.94	
	p-value	*0.001*		*0.009*		*0.001*	
Sex	**Female**	20	37	8	49	33	24
		46.5%	64.9%	38.1%	62.0%	63.5%	50.0%
	Male	23	20	13	30	19	24
		53.5%	35.1%	61.9%	38.0%	36.5%	50.0%
	Mean	7.36 ± 3.62		14.25 ± 4.03		6.73 ± 3.94	
	p-value	*0.546*		*0.688*		*0.228*	
BMI (kg/m²)	**Normal**	37	26	17	46	42	21
		86.0%	45.6%	81.0%	58.2%	80.8%	43.8%
	Overweight	2	25	2	25	8	19
		4.7%	43.9%	9.5%	31.6%	15.4%	39.6%
	Obese	4	6	2	8	2	8
		9.3%	10.5%	9.5%	10.1%	3.8%	16.7%
	Mean ± SD	7.36 ± 3.62		14.25 ± 4.03		6.73 ± 3.94	
	p-value	*0.232*		*0.082*		*0.208*	

The Mean Values Showed Significant Difference For Age But No Significant Difference Was Observed Within Sex and BMI at 95% Confidence Level ($p < 0.05$)

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Table 4.4: Influence of Age, Gender and Body Mass Index (BMI) on the *HNF1A* Genetic Distribution within the Study Participants before Digestion

Parameters		Before digestion													
		Exon 1		300bp		118bp		Exon 2 (418bp)		Exon 3 (368bp)		Exon 4 (469bp)		Exon 5 (919bp)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Sex	**Female**	3	54	32	25	52	5	6	51	10	47	1	56	11	46
		60.0%	56.8%	59.3%	54.3%	57.8%	50.0%	60.0%	56.7%	66.7%	55.3%	50.0%	57.1%	61.1%	56.1%
	Male	2	41	22	21	38	5	4	39	5	38	1	42	7	36
		40.0%	43.2%	40.7%	45.7%	42.2%	50.0%	40.0%	43.3%	33.3%	44.7%	50.0%	42.9%	38.9%	43.9%
Age	**20 – 30**	0	16	10	6	16	0	4	12	1	15	0	16	4	12
		0.0%	16.8%	18.5%	13.0%	17.8%	0.0%	40.0%	13.3%	6.7%	17.6%	0.0%	16.3%	22.2%	14.6%
	31 – 40	2	11	8	5	13	0	2	11	3	10	0	13	2	11
		40.0%	11.6%	14.8%	10.9%	14.4%	0.0%	20.0%	12.2%	20.0%	11.1%	0.0%	13.3%	11.1%	13.4%
	41 – 50	0	31	15	16	29	8	2	11	3	28	1	30	6	25
		0.0%	32.6%	27.8%	34.8%	32.2%	20.0%	20.0%	12.2%	20.0%	32.9%	50.0%	30.6%	33.3%	30.5%
	51 – 70	3	37	21	19	32	8	2	38	8	32	1	39	6	34
		60.0%	38.9%	38.9%	41.3%	35.6%	80.0%	20.0%	42.2%	53.3%	37.6%	50.0%	39.8%	33.3%	41.5%
BMI (kg/m^2)	**Normal**	4	59	32	31	59	4	8	55	6	57	2	61	12	51
		80.0%	62.1%	59.3%	67.4%	65.6%	40.0%	80.0%	61.1%	40.0%	67.1%	100.0%	62.2%	66.7%	62.2%
	Overweight	0	27	15	12	22	5	1	26	6	21	0	27	5	22
		0.0%	28.4%	27.8%	26.1%	24.4%	50.0%	10.0%	28.9%	40.0%	24.7%	0.0%	27.6%	27.8%	26.8%
	Obese	1	9	7	3	9	1	1	9	3	7	0	10	1	9
		20.0%	9.5%	13.0%	6.5%	10.0%	10.0%	10.0%	10.0%	20.0%	8.2%	0.0%	10.2%	5.6%	11.0%

The Parameters Tested For Showed no Significant Difference For Age, Sex and BMI With Respect to the Genetic Variants at 95% Confidence Level ($p < 0.05$)

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Table 4.5: Influence of Age, Gender and Body Mass Index (BMI) on the *HNF1A* Genetic Distribution within the Study Participants after Digestion

Parameters		**After digestion**							
		Exon 1 digest (248bp)		**Exon 1 digest (145bp)**		**Exon 5 digest (725bp)**		**Exon 5 digest (165bp)**	
		Neg	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**
Sex	**Female**	1 25.5%	56 58.3%	11 44.0%	46 61.3%	21 55.3%	35 58.1 %	57 57.6%	0 0.0%
	Male	3 75.5%	40 41.7%	14 56.0%	29 38.7%	17 44.7%	26 41.9 %	42 42.4%	1 100.0 %
Age	**20 – 30**	1 25.0%	15 15.6%	5 20.0%	11 14.7%	5 13.2%	11 17.7 %	16 16.2%	0 0.0%
	31 – 40	0 0.0%	13 13.5%	2 8.0%	11 14.7%	5 13.2%	8 12.9 %	13 13.1%	0 0.0%
	41 – 50	2 50.0%	29 30.2%	7 28.0%	24 32.0%	10 26.3%	21 33.9 %	31 31.3%	0 0.0%
	51 – 70	1 25.0%	39 40.6%	11 44.0%	29 38.7%	18 47.4%	22 35.5 %	39 39.4%	1 100%
BMI (kg/m^2)	**Normal**	4 100.0%	59 61.5%	19 76.0%	44 58.7%	21 55.3%	42 67.75 %	63 63.6%	0 0.0%
	Overweight	0 0.0%	27 28.1%	4 16.0%	23 30.7%	11 28.9%	16 25.8 %	26 26.3%	1 100.0 %
	Obese	0 0.0%	10 10.4%	2 8.0%	8 10.7%	6 15.8%	4 6.5%	10 10.1%	0 0.0%

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Table 4.6: Frequency and Percentage Prevalence of *HNF1A* Gene Variants Amongst the Study Populations Before Digestion and After Digestion

Parameters		**Frequency**		**Percentage (%)**	
		Neg	Pos	Neg	Pos
Before Digestion	Exon 1 (505bp)	5	95	5	95
	300bp	54	46	54	46
	118bp	90	10	90	10
	Exon 2 (418bp)	10	90	10	90
	Exon 3 (368bp)	15	85	15	85
	Exon 4 (469bp)	2	98	2	98
	Exon 5 (919bp)	18	82	18	82
After Digestion	Exon 1 digest (284bp)	4	96	4	96
	Exon 1 digest (145bp)	25	75	25	75
	Exon 5 digest (725bp)	38	62	38	62
	Exon 5 digest (165bp)	99	1	99	1

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Table 4.7: Influence of *HNF1A* Gene Variants on the Parameters Assayed for Non-Diabetic and Diabetic Mellitus Patients Before Digestion

Parameters		Before Digestion													
		Exon 1		300bp		118bp		Exon 2 (418bp)		Exon 3 (368bp)		Exon 4 (469bp)		Exon 5 (919bp)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Fasting blood sugar (mmol/L)	**Non diabetes**	2 40.0 %	41 43.2 %	20 37.0 %	23 50.0 %	40 44.4 %	3 30.0 %	7 70.0 %	36 40.0 %	6 40.0 %	37 43.5 %	1 50.0 %	42 42.9 %	7 38.9 %	36 43.9 %
	Diabetes	3 60.0 %	54 56.8 %	34 63.0 %	23 50.0 %	50 55.6 %	7 70.0 %	3 30.0 %	54 60.0 %	9 60.0 %	48 56.5 %	1 50.0 %	56 57.1 %	11 61.1 %	46 56.1 %
2HrPP (mmol/L)	**Non diabetes**	3 60.0 %	18 18.9 %	8 14.8 %	13 28.3 %	18 20.0 %	3 30.0 %	0 0.0 %	21 23.3 %	2 13.3 %	19 22.4 %	0 0.0 %	21 21.4 %	0 0.0 %	21 100.0%
	Diabetes	2 40.0 %	77 81.1 %	46 85.2 %	33 71.7 %	72 80.0 %	7 70.0 %	10 100.0%	69 76.7 %	13 86.7 %	66 77.6 %	2 100.0%	77 78.6 %	18 100.0%	61 74.4 %
HbA1c (%)	**Non diabetes**	4 80.0 %	48 50.5 %	25 46.3 %	27 58.7 %	49 54.4 %	3 30.0 %	9 90.0 %	43 47.8 %	6 40.0 %	46 54.1 %	2 100.0%	50 51.0 %	11 61.1 %	41 50.0 %
	Diabetes	1 20.0 %	47 49.5 %	29 53.7 %	19 41.3 %	41 45.6 %	7 70.0 %	1 10.0 %	47 52.2 %	9 60.0 %	39 45.9 %	0 0.0 %	48 49.0 %	7 38.9 %	41 50.0 %

The Parameters Tested For Showed No Significant Difference Between the Non-Diabetic and Diabetic Mellitus With Respect to the Genetic Variants at 95% Confidence Level ($p < 0.05$) Before Digestion

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Table 4.8: Influence of *HNF1A* Gene Variants on the Parameters Assayed for Non-Diabetic and Diabetic Mellitus Patients After Digestion

Parameters		After Digestion							
		Exon 1 digest (248bp)		Exon 1 digest (145bp)		Exon 5 digest (725bp)		Exon 5 digest (165bp)	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Fasting blood sugar (mmol/L)	Non diabetes	2	41	12	31	13	30	43	0
		50.0%	42.7%	48.0%	41.3%	34.2%	48.4%	43.4%	0.0%
	Diabetes	2	55	13	44	25	32	56	1
		50.0%	57.3%	52.0%	58.7%	65.8%	51.6%	56.6%	100.0 %
2HrPP (mmol/L)	Non diabetes	0	21	3	18	4	17	20	1
		0.0%	21.9%	12.0%	24.0%	10.5%	27.4%	20.2%	100.0 %
	Diabetes	4	75	22	57	34	45	79	0
		100.0 %	78.1%	88.0	76.0%	89.5%	72.6%	79.8%	0.0%
HbA1c (%)	Non diabetes	3	49	12	40	16	36	52	0
		75.0%	51.0%	48.0%	53.3%	42.1%	58.1%	52.2%	0.0%
	Diabetes	1	47	13	35	22	26	47	1
		25.0%	49.0 %	52.0%	46.7%	57.9%	49.1%	47.5%	100.0 %

** The Parameters Tested For Showed No Significant Difference Between the Non Diabetic and Diabetic Mellitus with Respect to the Genetic Variants at 95% Confidence Level ($p < 0.05$) After Digestion

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Table 4.9: Number(s) of Bands and Molecular Size of *HNF1A* Gene Variants Before Digestion Amongst the Participants

Parameters	No. of bands	Molecular size (bp)	No of participants	Percentage (%)
Exon 1	3	505bp, 300bp and 118bp	100	100
Exon 2	1	418bp	100	100
Exon 3	1	386bp	100	100
Exon 4	1	469bp	100	100
Exon 5	1	919bp	100	100

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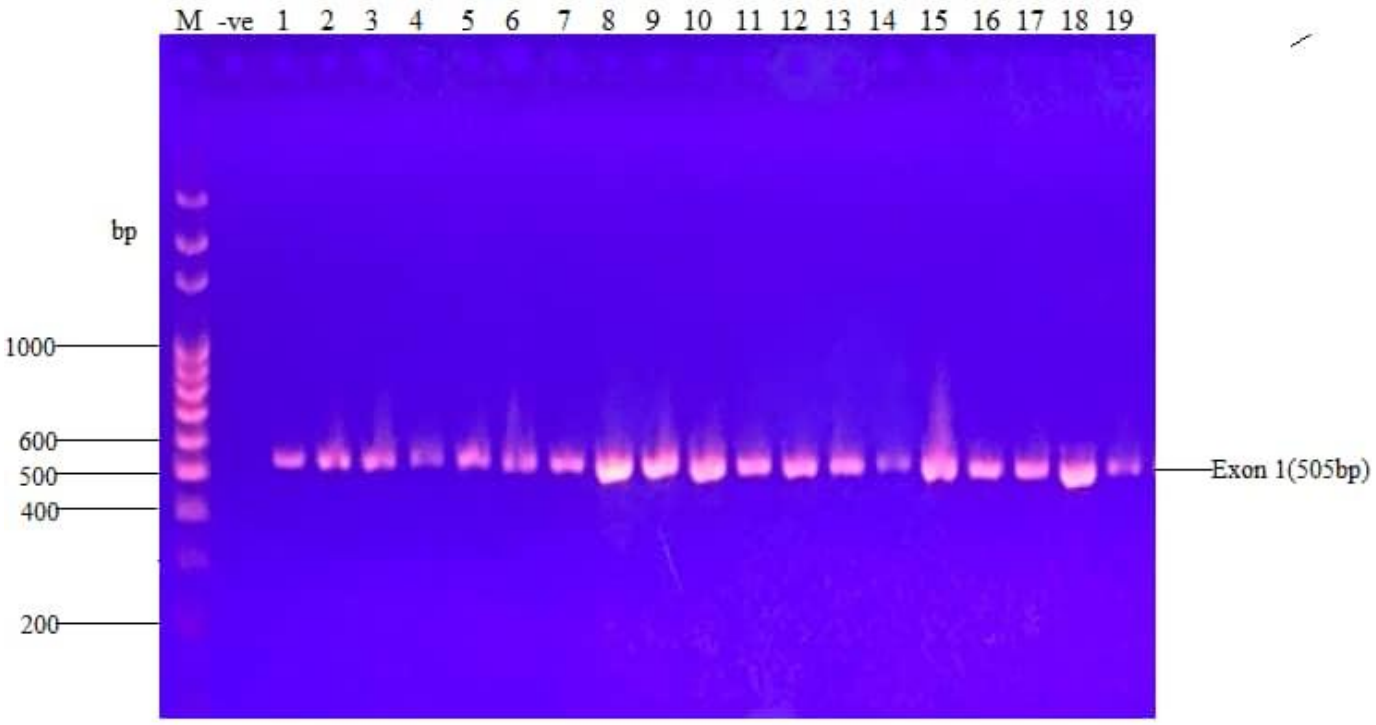


Plate 4.1 Exon 1 Before Digestion

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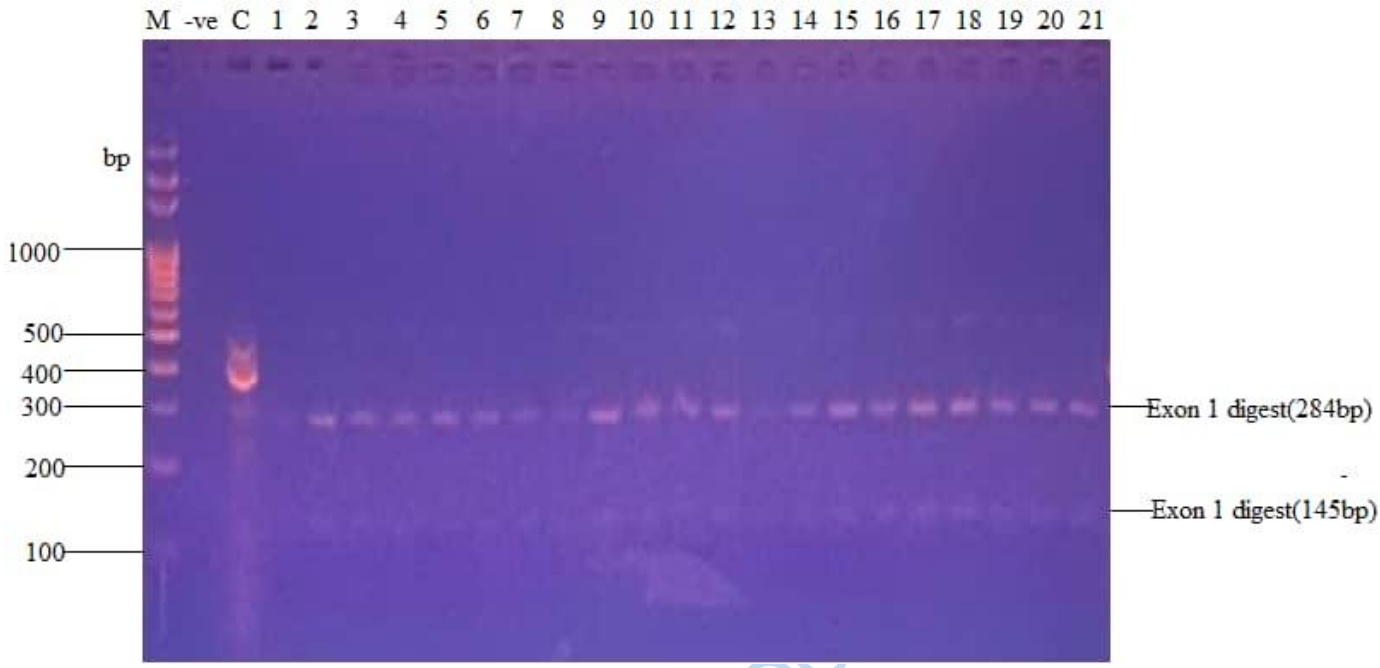


Plate 4.2 Exon 1 After Digestion

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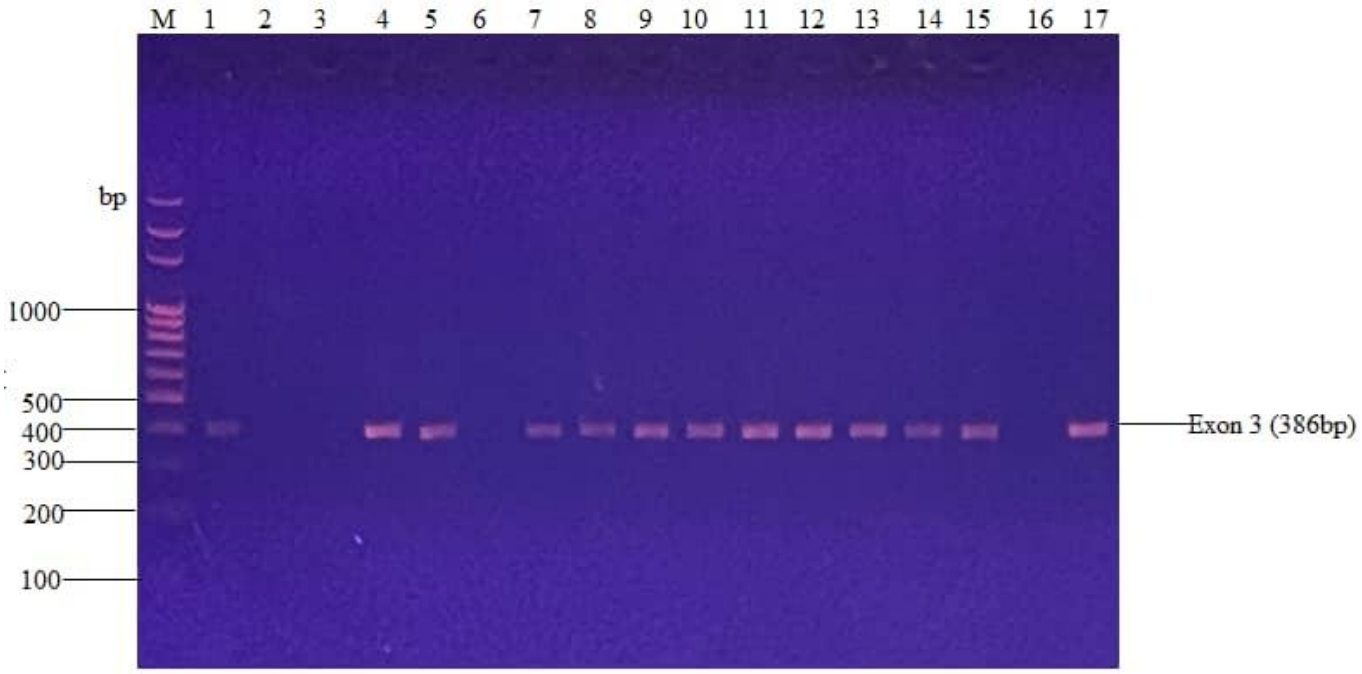


Plate 4.3 Exon 3 Digestion

Laboratory Analysis, 2024

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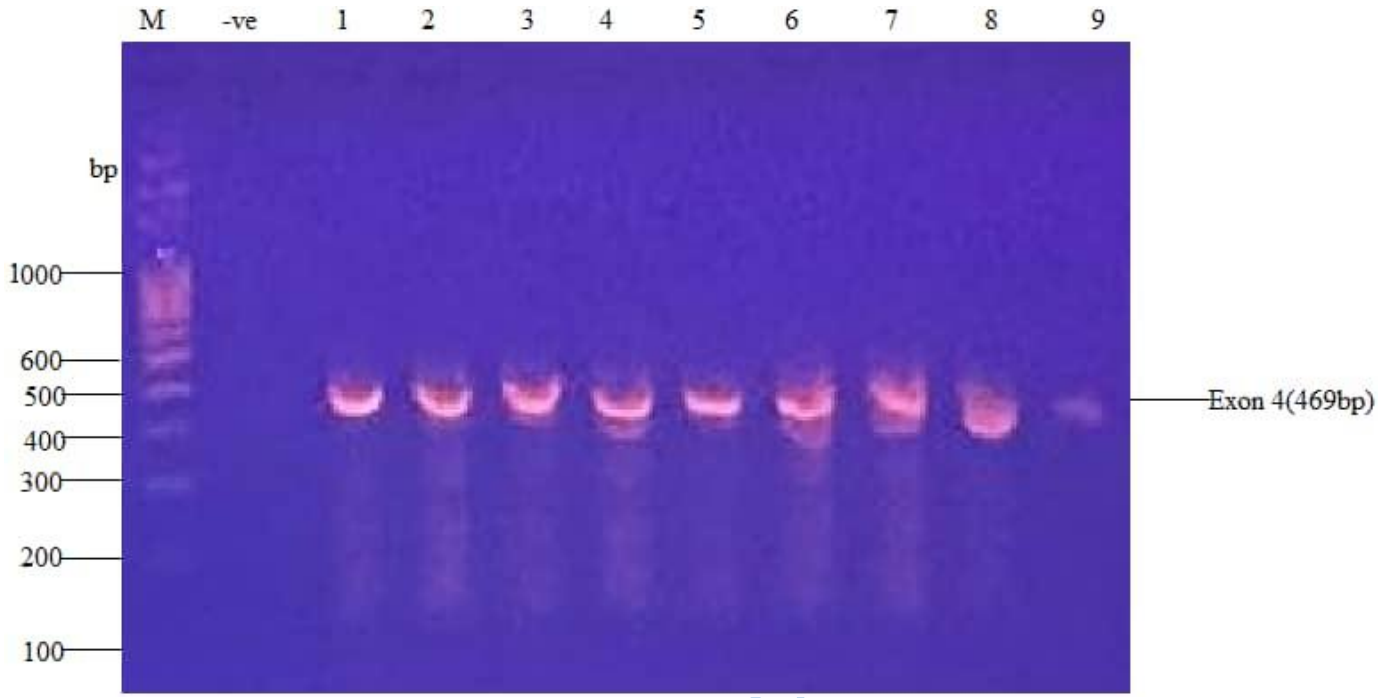


Plate 4.4 Exon 4 Digestion

Laboratory Analysis, 2024

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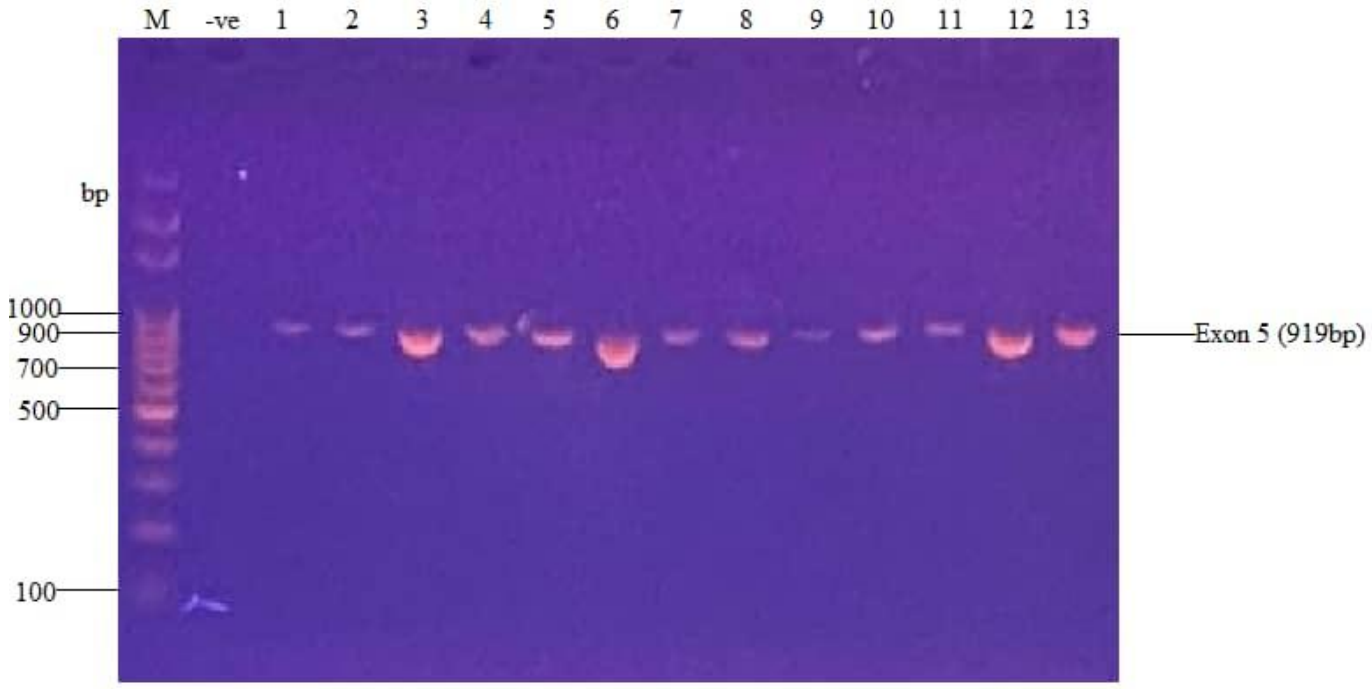


Plate 4.5 Exon 5 Before Digestion

Laboratory Analysis, 2024

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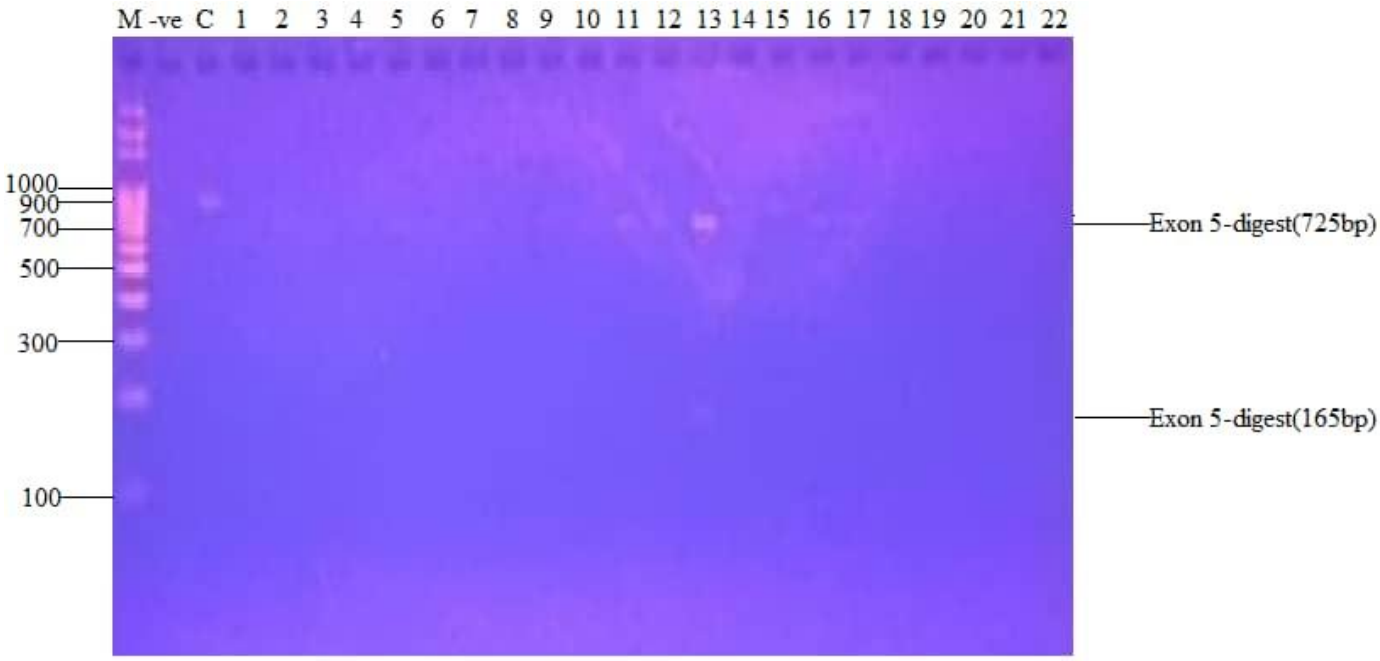


Plate 4.6 Exon 5 After Digestion

Laboratory Analysis, 2024

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Table 4.10: Number(s) of Bands and Molecular size of *HNF1A* Gene Variants after Digestion amongst the Participants

Parameters	No. of bands	Molecular size (bp)	No. of participant	Percentage (%)
Exon 1 digest	2	4284bp and 145bp	100	100
Exon 2	1	418bp	100	100
Exon 3	1	386bp	100	100
Exon 4	1	469bp	100	100
Exon 5 digest	2	725bp and 165bp	100	100

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Table 4.11: Mean ± SD of the Non Genetic Values of Patients with Non Diabetic and Diabetic Mellitus

Parameters	**Fasting blood sugar (mmol/L)**		**2HrPP (mmol/L)**		**HbA1c (%)**	
	Non diabetes	Diabetes	Non diabetes	Diabetes	Non diabetes	Diabetes
Age	38.58 ±	52.39 ±	43.76 ±	47.16 ±	40.81 ±	52.56 ±
p- value	10.94	9.34	12.68	11.97	11.85	9.20
	0.000	*0.000*	*0.255*	*0.277*	*0.000*	*0.000*
Height	1.75 ±	1.74 ±	1.75 ±	1.74 ±	1.74 ±	1.74 ±
p-value	0.06	0.06	0.06	0.06	0.06	0.06
	0.445	*0.445*	*0.598*	*0.591*	*0.660*	*0.661*
BMI	22.40 ±	25.73 ±	22.98 ±	24.65 ±	22.63 ±	26.11 ±
p-value	3.88	3.24	3.94	3.82	3.53	3.43
	0.000	*0.000*	*0.080*	*0.093*	*0.000*	*0.000*

The Parameters Tested For Showed Both Significant and No Significant Differences Between the Non Diabetic and Diabetic Mellitus with Respect to the Non Genetic Factors at 95% Confidence Level ($p < 0.05$) After Digestion For Both Age and BMI But not for Height

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4.2 Discussion of Findings

This chapter presents a detailed discussion of the results obtained from the investigation of non-genetic risk factors and the molecular characterization of *HNF1A* gene variants in diabetic and non-diabetic participants. *HNF1A* gene regulates liver-specific genes, and genes that have a role in glucose metabolism, transport, and secretion of insulin. *HNF1A* gene mutations are frequently associated with type 2 diabetes [1]. The analysis showed a statistically significant difference between the ages of diabetic and non-diabetic participants ($p = 0.000$), which confirms that older age is associated with a higher risk of developing diabetes. This finding aligns with the body of research that highlights how aging contributes to the progressive loss of beta-cell function and increasing insulin resistance, both of which are critical to the pathophysiology of T2DM [2, 3]. Studies have suggested that the aging process affects insulin sensitivity due to changes in muscle mass, adipose tissue distribution, and hormonal regulation, making older individuals more susceptible to hyperglycemia and diabetes onset [4, 5].

BMI is a critical factor in the development of T2DM, as excess body weight is closely linked to insulin resistance. The results from Table 4.1 reveal that 63% of participants in the study had normal BMI, while 37% were classified as either overweight (27%) or obese (10%). Among the diabetic group, the mean BMI was significantly higher (25.73 ± 3.24 kg/m²) compared to the non-diabetic group (22.40 ± 3.88 kg/m²), and the difference was statistically significant ($p = 0.000$). This finding is consistent with numerous studies that have shown that higher BMI is a strong risk factor for the development of T2DM [6, 7]. The association between excess body weight and insulin resistance can be attributed to several mechanisms. Adipose tissue, particularly visceral fat, releases a variety of pro-inflammatory cytokines that interfere

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with insulin signaling pathways, leading to reduced glucose uptake by cells [8]. In addition, obesity alters lipid metabolism, which further exacerbates insulin resistance [3]. Given the global rise in obesity rates, especially in low- and middle-income countries like Nigeria, addressing BMI-related risk factors is crucial for the prevention and management of T2DM [9]. The gender distribution of diabetes in this study showed that more females (64.9%) were affected by diabetes compared to males (35.1%), as shown in Table 4.3. Although there was no statistically significant difference between the fasting blood sugar levels, 2-hour postprandial glucose, and HbA1c levels between males and females, the higher prevalence of diabetes in women could be attributed to several factors. Studies suggest that women, especially those post-menopause, may experience hormonal changes that affect glucose metabolism, increasing the risk of developing diabetes [10].

Furthermore, lifestyle factors such as physical inactivity and unhealthy dietary habits, which are more prevalent in some female populations, may contribute to this higher prevalence [7]. However, it is important to note that the severity of hyperglycemia, as indicated by fasting blood sugar and HbA1c levels, did not differ significantly between the sexes. This suggests that once diabetes develops, the clinical course of the disease may not be significantly influenced by gender [11]. The relationship between non-genetic factors (age, gender, and BMI) and glycemic control was further explored in Table 4.3. The analysis revealed several important findings regarding the impact of these factors on fasting blood sugar, 2-hour postprandial glucose, and HbA1c levels. Participants in the older age groups (51–70 years) exhibited significantly higher fasting blood sugar levels ($p = 0.001$), 2-hour postprandial glucose ($p = 0.009$), and HbA1c levels ($p = 0.001$) compared to younger participants. This indicates that advancing age is associated with poor glycemic control, which is consistent

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with the established understanding that aging exacerbates both insulin resistance and beta-cell dysfunction [2, 8]. The findings suggest that older individuals may require more aggressive glycemic control strategies to mitigate the long-term complications of hyperglycemia, such as cardiovascular disease and nephropathy [7]. BMI was also found to have a significant impact on glycemic control. Overweight and obese participants exhibited higher fasting blood sugar and 2-hour postprandial glucose levels compared to those with normal BMI. The findings highlight the importance of weight management in the prevention and management of diabetes [3]. Obesity-induced insulin resistance is a well-known mechanism underlying poor glycemic control, and weight reduction has been shown to improve insulin sensitivity and glycemic outcomes [12, 13].

The data displays the samples' diabetes PCR results. 60% of them had positive results for diabetes genetic markers, while 40% had negative results. According to a gender split, 35% of the negative cases were male and 65% were female, indicating that women might be more likely to have false negatives or have lower marker expression. However, the positive cases were almost evenly distributed by gender, with 51.7% of the positive cases being female and 48.3% being male. In particular, 29 males tested positive (48.3%) and 14 males tested negative (35%), whereas 26 females tested negative (26%) and 31 positive (51.7%). This distribution demonstrated that there were few significant differences between the sexes, despite the fact that somewhat more females had superior results. These findings are consistent with worldwide patterns when contrasted with current research conducted between 2020 and 2025. A study conducted by the International Diabetes Federation (IDF) found that although women are more likely to participate in preventative screening, they frequently experience delays in diagnosis, which may account for the higher percentage of negative

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results among females despite significant favorable outcomes[15]. Additionally, Zhang's study revealed behavioral and sociocultural factors that affect detection rates but did not find any discernible sex-based differences in genetic vulnerability to Type 2 diabetes. These results highlight how crucial it is to take gender dynamics into account when diagnosing and treating diabetes. Although the handwritten data lacks depth in age and lifestyle variables—which are crucial in modern diabetes risk assessments—it does support this by showing significant female involvement and comparable positive rates[16].

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Chapter Five

Conclusion

5.1 Summary of Findings

One of the key objectives of this study was to investigate the molecular characterization of *HNF1A* gene variants and their association with diabetes. The study show a high prevalence of *HNF1A* gene variants in both diabetic and non-diabetic participants, with 95% of participants testing positive for exon 1 variants before digestion. The study found that the majority of participants, both diabetic and non-diabetic, carried *HNF1A* gene variants, particularly in exon 1 and exon 5. However, there was no statistically significant difference in the distribution of these variants between the two groups ($p > 0.05$). This suggests that while *HNF1A* gene variants are prevalent in the study population, they may not be directly associated with the development of diabetes.

The lack of a significant association between *HNF1A* variants and diabetes in this study could be due to several reasons. First, *HNF1A* variations are typically associated with a specific subtype of diabetes known as Maturity Onset Diabetes of the Young (MODY), which accounts for a small proportion of diabetes cases globally. It is possible that the participants in this study predominantly had T2DM, which is influenced by a combination of genetic and environmental factors. Despite the lack of a direct association between *HNF1A* variants and diabetes, the study did observe some trends in the influence of *HNF1A* variants on glycemic control. Participants with positive *HNF1A* variants in exon 1 and exon 5 had higher fasting blood sugar and HbA1c levels compared to those without the variants. This finding suggests that *HNF1A* gene variants may play a role in modulating glucose metabolism, potentially contributing to poor glycemic control in affected individuals. This

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finding is important because it highlights the potential clinical relevance of *HNF1A* genetic screening in individuals with poorly controlled diabetes, especially those who do not fit the typical profile of T2DM. Genetic screening for *HNF1A* mutations could help identify individuals with MODY, who may benefit from targeted therapies such as sulfonylureas, which are often more effective than insulin in MODY patients. The results of this study have several important clinical implications for the diagnosis and management of diabetes. The identification of age, BMI, and gender as significant risk factors for diabetes underscores the importance of early screening and intervention.

Older individuals and those with high BMI are at increased risk of developing diabetes and may benefit from targeted screening programs that assess fasting blood sugar and HbA1c levels. Early diagnosis can facilitate lifestyle modifications and pharmacological interventions that prevent the progression of diabetes and reduce the risk of complications. The findings related to *HNF1A* gene variants suggest that genetic screening could play a valuable role in the management of diabetes, particularly for individuals with early-onset diabetes or a strong family history of the disease. Identifying *HNF1A* mutations can aid in the diagnosis of MODY, allowing for more tailored treatment plans that optimize glycemic control. Furthermore, understanding the genetic basis of diabetes can provide insights into the mechanisms underlying the disease, potentially leading to the development of new therapeutic targets.

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5.2 Conclusion

This study has provided valuable insights into the non-genetic and genetic factors influencing the development and progression of diabetes. Age, BMI, and gender were identified as significant non-genetic risk factors, with older age and higher BMI being strongly associated with poor glycemic control. While *HNF1A* gene variants were prevalent in the study population, no significant association was found between these variants and diabetes. However, the presence of *HNF1A* variants may still influence glycemic outcomes, particularly in individuals with early-onset diabetes. The study highlights the need for personalized approaches to diabetes care, where genetic screening and individualized treatment plans can optimize outcomes for patients. Further research is needed to explore the nucleotide sequence pattern of the different variants of the *HNFIA* gene and its association to HbAIc levels.

The investigation demonstrated that the association between non-genetic variables (age, gender, and BMI) and glycemic control is significant concerning their influence on fasting blood sugar, 2-hour postprandial glucose, and HbA1c levels. Individuals in the older age cohorts demonstrated markedly elevated fasting blood glucose levels, 2-hour postprandial glucose, and HbA1c values relative to their younger counterparts. This suggests that increasing age correlates with inadequate glycemic regulation, along with the recognized notion that aging intensifies both insulin resistance and beta-cell impairment. The results indicate that elderly adults may necessitate more intensive glycemic control measures to reduce the long-term effects of hyperglycemia, including cardiovascular disease and nephropathy.

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The results concerning *HNF1A* gene variants indicate that genetic screening may be essential in the management of diabetes, especially for those with early-onset diabetes or a significant familial history of the condition. Detecting *HNF1A* mutations can facilitate the identification of MODY, enabling more personalized therapy strategies that enhance glycemic regulation. Moreover, comprehending the genetic foundations of diabetes might elucidate the mechanics of the disease, thereby facilitating the identification of novel treatment targets. The research revealed a statistically significant disparity between the ages of diabetic and non-diabetic participants, confirming that advanced age correlates with an increased risk of getting diabetes. This discovery corresponds with existing research that emphasizes the role of aging in the gradual decline of beta-cell functionality and the rise in insulin resistance, both of which are essential to the pathophysiology of T2DM.

The study demonstrates a significant incidence of *HNF1A* gene variants across both diabetes and non-diabetic people, with 95% of participants exhibiting positive results for exon 1 mutations prior to digestion. The research revealed that most subjects, regardless of diabetes status, possessed *HNF1A* gene variations, especially in exon 1 and exon 5. Nonetheless, there was no statistically significant disparity in the distribution of these variations between the two groups. This indicates that although *HNF1A* gene variations are common in the research group, they may not be directly linked to the onset of diabetes.

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5.3 Recommendations

1. Early and Routine Screening: Healthcare providers should prioritize early and routine screening for diabetes, particularly in older individuals and those with high BMI. This will facilitate early detection and intervention, reducing the risk of complications.

2. Genetic Screening: Genetic testing for *HNF1A* mutations should be considered in individuals with early-onset diabetes or a strong family history of diabetes. This can aid in the diagnosis of MODY and guide more targeted treatment options.

3. Weight Management Programs: Given the significant association between high BMI and diabetes, public health interventions that promote weight loss and healthy lifestyle choices should be a priority for preventing diabetes in at-risk populations.

5.4 Contribution to Knowledge

This study contributes to existing knowledge by highlighting the prevalence of *HNF1A* gene variants among diabetic patients in lagos, Nigeria, and revealing that although these variants exist, they are not significant associated with diabetes status.

It emphasizes the stronger influence of non-genetic risk factors such as age and BMI on diabetes prevalence, advocating for early screening and personalized care approaches.

5.5 Suggestions for Further Studies

Additional studies are needed to explore the role of *HNF1A* variants and other genetic factors in the pathogenesis of diabetes.

Understanding the genetic underpinnings of diabetes may lead to the development of new therapeutic strategies.

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Case Study

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Appendices
Appendix I: Informed Consent Form

Initials……

To participate in this research study, it is necessary that you give your informed consent

By signing this form, you are indicating that you understand the nature of the research study and your role in the research, and that you agree to participate in the research (having read the subject information form).

Please consider the following points before signing

I understand that I am participating in a research

I understand that my participation will be anonymous (that is, my name will not be linked with my personal information) and that all information I provide will remain confidential I understand that I will be provided with an explanation of the research in which I am participating:

I understand that my participation in this research is voluntary, and that I may refuse to participate further at any time without having to offer an explanation

By signing this form, I am stating that I understand the above information and consent to participate in this study

_____ _____

Signature/Thumbprint of Participant/Date Signature of Investigator/Date

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Appendix

Questionnaire

Good day Sir/Ma I am a post graduate student in the department of medical laboratory science, Leads City University Ibadan, conducting a research titled "**Molecular characterization of HNF1A gene in diabetic patients in Lagos Nigeria**". Please kindly avail me few minutes of your time to peruse and provide the information required in this questionnaire. I assured you that all information supplied shall be used only for the research purpose which is necessary for the control and prevention of complications associated diabetes mellitus. Please respond honestly to the question below. The confidentiality of your response to the questionnaire is guaranteed. Thank you.

SECTION A: DEMOGRAPHIC AND CLINICAL DATA:

Instruction: Please tick and fill in the gap as appropriate

1. Age (years): 20-30 [ ], 31-40 [ ] 41-50 [ ] Above 50 [ ]

2. Gender: Male [ ] Female [ ]

3. Religion: Christianity [ ] Islam [ ] Traditionalist [ ]

4. Ethnic group: Yoruba [ ] Igbo [ ] Hausa [ ] Others (specify) …….

5. Marital status: Single [ ] Married [ ] Divorced [ ] Widowed [ ]

6. Occupation: Artisan [ ] Trader [ ] Civil servant [ ] Unemployed [ ]

7. Education status: None [ ] Primary [ ] Secondary [ ] Tertiary [ ]

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SECTION B; KNOWLWDGE OF DM

8. Have you heard about DM? Yes [ ] No [ ]

9. DM is a disease that occur as a result of excessive sugar in the blood? Yes [ ] No [ ]

10. Family History of DM: Yes [ ] No [ ]

11. Have you been diagnosed with DM: Yes [ ] No [ ]

12. If yes to question 11, how long have you been diagnosed with DM: ……..

13. Are you on treatment for DM: Yes [ ] No [ ]

14. How long have you been on treatment for DM: ……

15. DM can be inherited: Yes [ ] No [ ]

16. DM is curable: Yes [ ] No [ ]

17. The symptoms of DM that you have experienced include: (Tick all correct answers)

a. Excessive urination: [ ]

b. Excessive thirst: [ ]

c. Excessive hunger: [ ]

d. Loss of weight: [ ]

18. Complications of DM that you have experienced include: (Tick all correct answers)

a. Blindness (eye problem): [ ]

b. Sexual dysfunction: [ ]

c. Nerve damage: [ ]

e. Kidney problem: [ ]

f. Amputation of gangrened leg: [ ]

h. Poor libido: [ ]

Others specify: ……..

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Biodata

Personal Data

1. Full name: Arinola Ibilola MAKINDE

   Address: 2, Chief Bode Off Oriola Street, Ibafo, Ogun State, Nigeria.

   E-mail Address: arinolamakinde32@gmail.com

   Phone number: 08033721404

2. Date of Birth: 28/07/1980
3. Place of Birth: Lagos
4. Nationality: Nigeria
5. Name and Address of Next of Kin: Makinde Akangbe Saibu
   2,Chief Bode Off
   Oriola Street, Ibafo, Ogun State, Nigerian.

B. Education Background

i.	St Peters Unity Secondary School, Akure, Ondo state	1996 SSCE
ii.	Ambrose Alli Univerity Ekpoma, Edo state	2005 BMLS (Chemical Pathology)
iii.	School of Medical Laboratory Science LUTH Lagos	2017 FMLSCN
	Nigeria Youth Service Corps	Exemption
	Masters of Science in Molecular Biology and Genomics	In View

C. Working Experience with Dates:

Medical Laboratory Scientist Federal Medical Centre, Ebute Metta	April 2013 till date

D. Award and Fellowship (if any) FMLSCN

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E. Membership of Academic Professional Bodies FMLSCN

Fellow of the Medical Laboratory Science Council of Nigeria FMLSCN 2017

Associate of the Medical Laboratory Science Council of Nigeria AMLSCN 2005

F. Publication (if any)

Gestational Diabetes Mellitus among Women Assessing Antenatal Care At Federal Medical Centre Ebute Metta, Southwestern Nigeria.

1. Notable scholar or professional Accomplishment Nil

2. Major conferences/workshop attended FASCON LCU

Referees:

Dr. T.Y. Raheem

Department of Biological Sciences, Lead City University, Ibadan, Oyo State.

08023047512

4. Date Signature

_____ _____

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The University Compliance Certification

This is to certify that, this Thesis written by **MAKINDE Arinola Ibilola** with Matric No. **LCU/PG/002811** in the Department of Biological Science, Faculty of Natural and applied Science Lead City University Ibadan is in full compliance with the approved university format and style.

_____ _____

Signature **Date**

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