Cape Peninsula
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THE PREVALENCE OF MATURITY ONSET DIABETES OF THE YOUNG (MODY) IN A POPULATION FROM THE WESTERN CAPE

by

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DECLARATION

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07 November 2019

Signed **Date**

ABSTRACT

Background: Maturity Onset Diabetes of the Young (MODY) is a monogenic type of diabetes caused by a single gene mutation. Up to date 14 different MODY subtypes have been identified. Mutations in the glucokinase (MODY 3) and hepatic nuclear factor 1 alpha (HNF1A) (MODY 2) are the most frequent causes of MODY in all populations studied. Patients with MODY are misdiagnosed with type 1 or type 2 diabetes. Identifying patients with MODY is important as it affects treatment, for example, MODY 2 patients need no treatment, whereas those diagnosed with MODY 3 are very sensitive to low doses of sulphonylureas. To date, no data is available on the prevalence of MODY in populations from Africa. Thus, we aimed to investigate and report on the incidence of MODY, specifically mutations in the HNF1A gene in a population from the Western Cape.

Methods: In this study, we screened for HNF1A MODY (MODY 3) mutations (rs115080759, rs140491072 rs137853245, and rs142318174) in 1639 (males = 406) individuals using real-time PCR. Positive MODY samples were confirmed by subsequent sequencing. All individuals underwent an oral glucose tolerance test.

Results: The mean age of participants was 47.1±15.6 in males and 49.9±15.1 females. We identified 12 (0.73%) individuals with HNF1A gene polymorphisms; 12/1642 of rs115080759. Seven participants with a SNP in rs115080759 presented with normoglycemia, one with prediabetes, and four with diabetes. No polymorphism was detected in three SNPs; rs140491072, rs137853245 and rs142318174.

Discussion and conclusion: To our knowledge, this is the first African study on MODY, and the incidence is similar to that reported in other studies. The results suggest that MODY is misdiagnosed with other types of diabetes in Africa; therefore, our findings support the introduction of diagnostic genetic testing for MODY in South Africa.

Key words: Diabetes mellitus, HNF1A, MODY, Monogenic diabetes, Polymorphism

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DEDICATION

This thesis is dedicated to:

My mother, Azwinndini Tshivhase and brother, Rofhiwa Patrick Tshivhase

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GLOSSARY

LIST OF ABBREVIATIONS

2h-PG	2-hour post glucose
ABCC8	ATP binding cassette subfamily C member 8
ADP	Adenosine triphosphate
APPL1	Adaptor protein, phosphotyrosine interacting with PH domain, leucine Zipper 1
ATP	Adenosine triphosphate
BLK	BLK proto-oncogenes, Src family tyrosine kinase
BMI	Body mass index
CEL	Carboxyl ester lipase
Chol	Cholesterol
Cm	Centimetres
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
ExAC	Exome Aggregation Consortium
FBG	Fasting blood glucose
G6P	Glucose-6-phosphate
GAD	Glutamic acid decarboxylase
GCK	Glucokinase
GLUT2	Glucose transporter 2
GnomAD	Genome Aggregation Database
GPO-POD	Glycerol phosphate oxidase in the presence of peroxidase
GWAS	Genome wide association studies
HbA1c	Glycated haemoglobin
HDL	High density cholesterol
HipC	Hip circumferences
HNF1A	Hepatic nuclear factor 1 alpha
HNF1B	Hepatic nuclear factor 1 beta
HNF4A	Hepatic nuclear factor 4 alpha
HPLC	High performance liquid chromatography
HPLC-CE	High performance liquid chromatography-Capillary Electrophoresis
HPLC-MS	High performance liquid chromatography-Mass Spectrophotometry
hsCRP	High-sensitivity C-reactive protein

IA-2	Insulinoma antigen 2
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
INS	Insulin
IPF1	Insulin promoter factor 1
KAPT	Adenosine triphosphate sensitive potassium channel
KCNJ11	Potassium voltage-gated channel subfamily J member 11
Kg	Kilograms
KLF11	Krueppel-like factor 11
LDL	Low Density Lipoprotein
MDM	Monogenic diabetes mellitus
mIU/L	mill international units Per Litre
mm Hg	millimetres of the mercury
mmol/L	mill moles Per Litre
MODY	Maturity Onset Diabetes of the Young
NDM	Neonatal diabetes mellitus
NEUROD1	Neurogenic differentiation factor 1
NF-Kb	Nuclear factor Kappa-beta
ng/mL	Nano grams Per Millilitre
NR2A1	Nuclear receptor subfamily 2
OAD	Oral antidiabetic drugs
OGTT	Oral glucose tolerance test
PAX4	Paired Box 4
PDM	Polygenic diabetes mellitus
PDX1	Pancreatic and duodenal homeobox 1
PNDM	Permanent neonatal diabetes
RT-PCR	Real-time polymerase chain reaction
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SNPs	Single nucleotide polymorphism (s)
SOD	Superoxide dismutase
SUR1	Sulfonyl-urea receptor 1
T1D	Type 1 diabetes

T2D	Type 2 diabetes
TNDM	Transient neonatal diabetes
UCPCR	Urinary C-peptide to creatinine ratio
Waist C	Waist circumferences
WHO	World health organization
WHR	Waist hip ratio
β-cells	Beta cells

DEFINITIONS

Diabetes mellitus: is a chronic disease in which the body's ability to respond to insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood.

Hepatocyte nuclear factor 1 alpha (HNF1A): is a transcriptional activator which regulates the expression of multiple genes.

Maturity Onset Diabetes of the Young (MODY): is a monogenic form of diabetes mellitus caused by mutations in an autosomal dominant gene disrupting insulin production.

Monogenic diabetes: it is a rare form of diabetes which results from mutations or changes in a single gene.

Single nucleotide polymorphism (SNP): refers to a variation at a single gene position in a DNA sequence among individuals.

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Diabetes mellitus (DM) is a chronic disease affecting people worldwide. It is one of the leading causes of morbidity and mortality and is relatively prevalent worldwide (Firdous *et al.,* 2018). The International Diabetes Federation (IDF) estimated that in 2017, 451 million adults worldwide were affected with diabetes, with a projection of 693 million cases in 2045 (IDF, 2017). It is characterized by increased blood glucose levels as a result of defects of insulin action or insulin secretion (Skyler *et al*., 2017). This can result in characteristic symptoms such as thirst, polyuria, and weight loss. The long term-effects of DM are associated with microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiac, cerebrovascular, and peripheral vascular) complications (Danaei *et al*., 2011). The prevalence or incidence of DM increases daily in an alarming proportion. Globally, approximately 50% of diabetes cases are undiagnosed, with the majority of cases occurring in low-income and middle-class countries. In South Africa, the proportion of undiagnosed diabetes is 69.2% (Bertram *et al.,* 2013). As a result, it poses a great threat to human health (Gao *et al.,* 2017). Patients with DM have either type 1 (~10%) or type 2 diabetes (85%), both of which are polygenic diabetes mellitus (PDM) resulting from the interaction between genetic and environmental factors; less prevalent forms of DM have monogenic origins (Figure 1.1) (Heuvel-Borboom *et al*., 2016). Monogenic and polygenic diabetes are differentiated by clinical features (Table 1.1) (Nyunt *et al.,* 2009). Monogenic diabetes mellitus (MDM) is a term used to describe all subtypes of diabetes that are caused by mutations in a single gene; it is characterized by insufficient insulin secretion from pancreatic beta cells (Murphy *et al.,* 2008).

Neonatal diabetes mellitus (NDM) and Maturity Onset Diabetes of the Young (MODY) are two types of MDM, the most prevalent being MODY. MODY is caused by mutations in a single gene and has been linked with approximately 14 different genes (Shields *et al*., 2010). MODY is thought to account for 1-5% of all DM cases (Firdous *et al.,* 2018). Cross-sectional studies conducted on mixed-ancestry populations of the Western Cape have reported high prevalence of diabetes (28.2%), and significant cardiovascular disease (CVD) risk (Erasmus *et al.,* 2012; Matsha *et al.,* 2012). No data is available for MODY in mixed-ancestry populations in the Western Cape, the target area of the current study. The lack of available data forms the reason for this study.

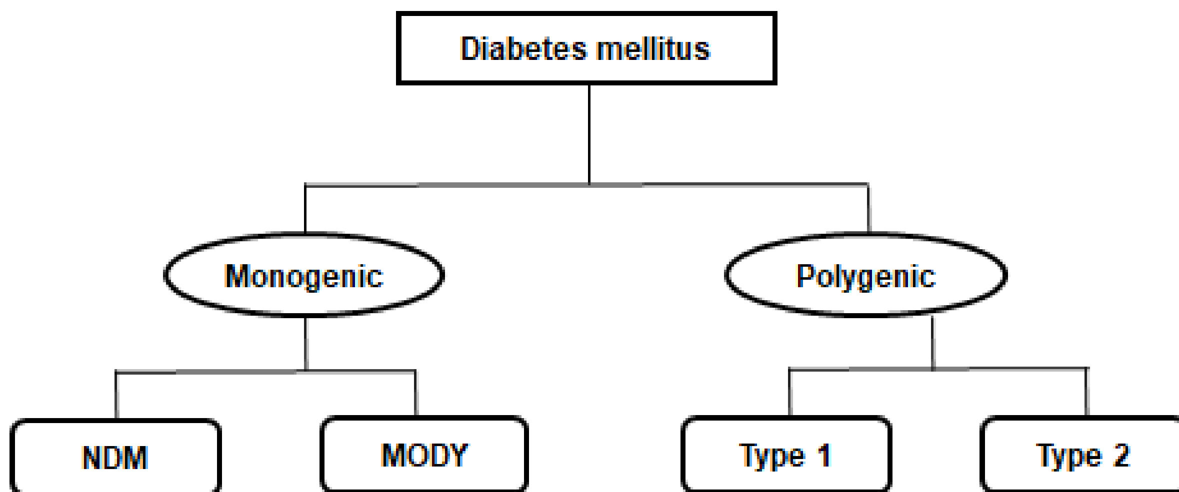

Figure 1.1: Flow diagram illustrating different types of Diabetes Mellitus
(NDM- Neonatal diabetes mellitus, MODY-Maturity Onset Diabetes of the Young)

Table 1.1: Clinical features that differentiate MODY from T1D and T2D (Nyunt *et al.,* 2009).

	MODY	**Type1**	**Type 2**
Genetics	Monogenic, autosomal dominant	Polygenic	Polygenic
Frequency	2-5%	Common	Most common
Family history	100%	<15%	>50%
Ethnicity	Different race	Different race	Asians, Polynesians
Age of Onset	<25 years old	Throughout childhood	Post-Puberty
Metabolic syndrome	Absent	Absent	Common
Severity of onset	Mild/asymptomatic	Acute and severe	Mild
Pathophysiology	β-cell dysfunction	β-cell dysfunction	Insulin resistance
Autoimmunity	Negative	Positive	Negative

1.2. Maturity Onset Diabetes of the Young (MODY)

MODY is a type of MDM, caused by mutations in a single gene, which has been reported in patients before the age of 25 years (Pihoker *et al.,* 2013). The genes involved are essential in beta cell development, function, and regulation, and can lead to disorders in glucose sensing and insulin secretion (Chambers *et al*., 2016; Naylor and Philipson, 2011). MODY is inherited in an autosomal dominant manner (var der Zwaag *et al*., 2014). The molecular genetic basis of MODY indicates that the mutations result in diabetes by inducing pancreatic beta cell dysfunction (Fajans and Bell, 2011). MODY is not a single entity, but represents genetic, metabolic, and clinical heterogeneity (Costa *et al*., 2000). It is characterized by mild Hyperglycemia, autosomal dominant inheritance, early onset of diabetes, insulin resistance, and preservation of endogenous insulin secretion (McDonald *et al.,* 2011 [a]; Owen *et al.,* 2003).

MODY is divided into several subtypes depending upon the gene involved and the clinical phenotypes. To date, 14 MODY subtypes have been identified, each resulting from a unique gene mutation (Table 1.2) (Kim, 2015). Of the 14 subtypes of MODY, there are three genes primarily responsible for MODY cases, namely GCK, HNF1A and HNF4A (Nyunt *et al.,* 2009). These mutations vary in prevalence, clinical features, severity of diabetes and related complications, as well as response to treatment. Each mutation encodes proteins involved in glucose homeostasis of pancreatic β-cells (Heuvel-Borboom *et al*., 2016; Barrio *et al*., 2002).

Table 1.2: Summary of MODY subtypes, gene name, locus and their clinical features

Type	Gene name	Locus	Clinical features	Reference
1	HNF4A	20q13.12	Mild - increased fasting and postprandial plasma glucose, sensitivity to sulfonylurea derivatives	Heuvel-Borboom *et al* 2016
2	GCK	7p13	Mild fasting hyperglycemia	Heuvel-Borboom *et al.,* 2016
3	HNF1A	12q24.31	Diminished renal threshold for glycosuria, sensitivity to sulfonylurea derivatives	Heuvel-Borboom *et al.,* 2016
4	IPF/PDXI	13q12.2	Pancreatic agenesis	Shields *et al.,* 2010
5	HNF1B	17q12	Characterized by renal disease and urogenital tract abnormalities in females	Anik *et al.,* 2015
6	NEUROD1	2q31.3	Characterized by obesity and insulin resistance	Thanabalasingham and Owen, 2011[a]
7	KLF11	2p25.1	Pancreatic malignancy	Thanabalasingham and Owen, 2011[a]
8	CEL	9q34.13	Associated with both endocrine and exocrine pancreatic dysfunction	Thanabalasingham and Owen, 2011[a]
9	PAX4	7q32.1	Important for the transcription for beta cell development	Thanabalasingham and Owen, 2011[a]
10	INS	11p15.5	Associated with neonatal diabetes	Anik *et al.,* 2015
11	BLK	8p23.1	Contribute to qualitative and quantitative control of beta signalling	Anik *et al.,* 2015
12	ABCC8	11p15.1	Associated with renal diabetes	Anik *et al.,* 2015
13	KCNJ11	11p15.1	Associated with renal diabetes	Anik *et al.,* 2015
14	APPL1	3p14.3	Associated with DIDMOAD syndrome	Anik *et al.,* 2015

GCK: Glucokinase, HNF1A, HNF4A, HNF1B: Hepatic nuclear factor alpha/beta, PDX1/IPF1: Pancreatic and duodenal homeobox 1/Insulin promoter factor 1, NEUROD1: Neurogenic differentiation factor 1, KLF11: Krueppel-like factor 11, CEL: Carboxyl ester lipase, PAX4: Paired Box 4, INS: Insulin, BLK: BLK proto-oncogenes, Src family tyrosine kinase, ABCC8: ATP binding cassette subfamily C member 8, KCNJ11: Potassium voltage-gated channel subfamily J member 11, APPL1: Adaptor protein, phosphotyrosine interacting with PH domain and leucine Zipper 1

1.3. History of MODY

Initial studies were carried out in different parts of the world suggesting a mild form of diabetes in young people, which was identified as pre-insulin. The symptomless diabetes was first

reported in non-obese children and teenagers in 1958, whose glucose level was maintained by sulfonylurea (Fajans and Conn, 1962). At this time MODY was classified as type 2 DM, of which could be managed by diet, insulin or oral drugs (Fajans and Conn, 1962). The term 'MODY' was first described in a case report published by Tattersall (1974). The authors discovered families with dominantly inherited forms of diabetes, which were being treated with sulfonylureas for 40 years. The clinical features of the three families –H, N and R- were similar. Tattersall and Fajans (1975), together described MODY as "fasting Hyperglycemia" diagnosed under the age of 25 years, which could be treated without insulin for more than two years. Fajans and Brown (1993) reported that patients may become unresponsive to sulfonylureas and would require treatment with insulin for normalization. In the 1970s, there was great interest in MODY due to its presentation as a genetic form of diabetes. In the 1980s and 1990s research groups in Europe and the United States of America reported multiple genetic entities responsible for various MODY subtypes (Fajans and Brown, 1993). To date 14 MODY subtypes have been identified (Table 1.3).

1.4. Prevalence

The prevalence of MODY remains unknown in different populations world-wide. It is estimated to account for approximately 1-5% cases of all DM (Shields *et al.,* 2010; Irgens *et al*., 2013). The prevalence of MODY depends on the country. As it is mostly misdiagnosed with type 1 or type 2 DM, inappropriate treatment often results. For example; In the UK, 80% of patients with MODY are misdiagnosed with T1D or T2D, which underestimates its prevalence (Shields *et al.,* 2010). The first study on MODY prevalence was performed in Germany; criteria were based on the young age of onset and the absence of insulin requirement for five years. They reported that 0.14% of the paediatric diabetes population had MODY (Panzram and Adolph, 1981). A study in Germany was based on the following inclusion criteria: subjects that were included in the study had to be diagnosed before the age of 25 years, they had to have had at least one first-degree relative diagnosed with diabetes, in addition, subjects had to have undergone diet or oral drug treatment for five years. The authors reported 1.8% of subjects diagnosed with diabetes are, in fact, individuals with MODY (Ledermann, 1995).

Recent studies have reported MODY incidence of 2-45/1,000,000 children and 100/1,000,000 adults (Shields *et al*., 2010; Pihoker *et al*., 2013). Pihoker and co-workers reported that the minimum prevalence of MODY has increased to 2.1/100,000 in individuals less than 20 years of age (Pihoker *et al.,* 2013). Many newer gene mutations and subgroups of MODY have been identified since its first characterization by Tattersall in 1974-75 (Nyunt *et al.,* 2009). The prevalence and incidence of MODY subtypes vary greatly (Table 1.3), with MODY 2 and

MODY 3 being the most prevalent (Table 1.4). The frequency varies due to differing recruitment data and ethnic variability (Firdous *et al*., 2018). MODY 2 is primarily diagnosed in countries where blood glucose screening is a routine procedure (such as Spain, Italy, France, Czech Republic), and MODY 3 is commonly diagnosed where blood glucose tests are seldom performed (such as Denmark, the Netherlands, Norway, and the United Kingdom) (Thanabalasingham and Owen, 2011[a]; Schober *et al*., 2009), (Table 1.4).

Table 1.3: Genes and single nucleotide variants associated with MODY, prevalence and the year of recognition

Gene	Single nucleotide variants	Allele frequency	Prevalence (%)	Year of Recognition	Reference
HNF4A			5.0	1991	Yamagata *et al*., 1996; Pruhova *et al.,* 2003
	rs145314165	0.0000106			Genome Aggregation Database (gnomAD)
	rs138221628	0.0001427			gnomAD
	rs148745312	0.000003979			gnomAD
	rs113725562	0.03485			gnomAD
	rs1401143857	0.00003186			gnomAD
GCK			15-25	1993	Froguel *et al.,* 1993; Barrio *et al.,* 2002
	rs117678382	0.004316			GnomAD
	rs148474161	0.0001345			GnomAD
	rs75267165	0.05335			GnomAD
	rs11372295	1.0			Exome Aggregation Consortium (ExAC)
	rs149412035	0.001273			GnomAD
HNF1A			30-50	1996	Horikawa *et al*., 1997; Pruhova et al., 2003
	rs1169288	0.3479			GnomAD
	rs142318174	0.0002745			GnomAD
	rs137853245	0.000007145			GnomAD
	rs140491072	0.0001450			GnomAD
	rs115080759	0.0006024			GnomAD
IPF/PDXI			<1.0	1997	Stoffers *et al.,* 1997 [b]; McCarthy *et al.,* 2008
	rs149275902	0.0004859			GnomAD
	rs148001995	0.00006883			GnomAD
	rs137852787	0.001139			GnomAD
	rs75498935	0.0002971			GnomAD
	rs192902098	0.0003169			GnomAD
HNF1B			5.0	1997	Edghill *et al.,* 2006; Kim, 2015
	rs139107479	0.004114			GnomAD
	rs140562402	0.0005831			gnomAD
	rs141809533	0.00003535			GnomAD

	rs148713761	0.0002899			gnomAD
	rs150712273	0.00006347			gnomAD
NEUROD1			<1.0	1999	Malecki *et al.,* 1999; Firdous et al., 2018
	rs148104444	0.00004950			gnomAD
	rs140129079	0.00001194			gnomAD
	rs146389992	0.00004245			gnomAD
	rs115189138	0.004744			gnomAD
	rs147634094	0.00005303			gnomAD
KLF11			<1.0	2005	Neve *et al.,* 2005: Kim, 2015
	rs143579586	0.00245592			ExAC
	rs151292984	0.0002334			gnomAD
	rs142970758	0.00001193			gnomAD
	rs11687357	0.8366			gnomAD
	rs117449189	0.00007431			gnomAD
CEL			<1.0	2006	Torsvick *et al.,* 2006; Gardner and Tai, 2012
	rs111893665	0.001780			gnomAD
	rs140299595	0.000003994			gnomAD
	rs115873608	0.002217			gnomAD
	rs140977366	0.003807			gnomAD
	rs115568146	0.001128			gnomAD
PAX4			<1.0	2007	Plengvidhya *et al.,* 2007; Firdous et al., 2018
	rs150578361	0.01047			gnomAD
	rs143084654	0.0001037			gnomAD
	rs142426878	0.0003020			gnomAD
	rs140070434	0.0005354			gnomAD
	rs114416157	0.00008534			gnomAD
INS			<1.0	2008	Molven *et al.,* 2008; Heuvel-Borboom et al., 2016
	rs143405115	0.00003567			gnomAD
	rs114936120	0.00003567			gnomAD
	rs146945950	0.00005290			gnomAD
	rs11564720	0.001873			gnomAD
	rs144093133	0.0002050			gnomAD
BLK			<1.0	2009	Borowiec *et al.*,2009; Gardne and Tai, 2012
	rs142772999	0.00007788			gnomAD
	rs113656715	0.00003895			gnomAD
	rs147022480	0.00009559			gnomAD
	rs145993253	0.000003989			gnomAD
	rs140188373	0.00004595			gnomAD
ABCC8			<1.0	2012	Bowman *et al.*, 2012; Kim, 2015
	rs115716690	0.00004605			gnomAD
	rs112070496	0.001494			gnomAD

	rs149028222	0.0003993			gnomAD
	rs145386421	0.0004174			gnomAD
	rs8192690	0.05412			gnomAD
KCNJ11			<1.0	2012	Bonnefond *et al.*, 2012; Firdous et al., 2018
	rs140599332	0.000007956			gnomAD
	rs142272833	0.0004033			gnomAD
	rs145386421	0.0004174			gnomAD
	rs139079635	0.0003345			gnomAD
	rs145429555	0.00001993			gnomAD
APPL1			<1.0	2015	Prudente *et al.*, 2015; Thanabasingham and Owen, 2011
	rs7643644	0.02504			gnomAD
	rs141978531	0.0007276			gnomAD
	rs147750872	0.0007276			gnomAD
	rs140443720	0.000007974			gnomAD
	rs11544593	0.1286			gnomAD

GnomAD: Genome Aggregation Database, ExAC: Exome Aggregation Consortium

Table 1.4: Comparison between MODY 2 and MODY 3 prevalence in different countries

Country	MODY 2 (%)	MODY 3 (%)	Reference
Czech Republic	31%	12%	Pruhova *et al.*, 2003
Denmark	10%	36%	Johansen *et al.*, 2005
France	40-60%	<25%	Frougel *et al.*, 1993; Vaxillaire *et al.*, 1997
Italy	40-50%	10-15%	Massa *et al.*, 2001; Gragnoli *et al.*, 1997
Spain	20-45%	35%	Costa *et al.,* 2000; Barrio *et al.*, 2002
United Kingdom	10-20%	73%	Thomson *et al.*, 2003; Frayling *et al.*, 1997

Table 1.5: Genes associated with diabetes, single-nucleotide variants and their allele frequency

Gene	Single nucleotides variant	Allele frequency	Reference
G6PC2	rs560887	0.7896	gnomAD
MTNR1B	rs10830963	0.2423	gnomAD
GCK	rs4607517	0.00009558	gnomAD
SLC30A8	rs1326634	0.02367	gnomAD
GLIS3	rs7034200	0.5347	gnomAD
PROX1	rs340874	0.3984	gnomAD
SLC2A2	rs11920090	0.1918	gnomAD
ADCY5	rs11708067	0.1778	gnomAD
CRY2	rs11605924	0.3881	gnomAD
TCF7L2	rs7903146	0.2564	gnomAD

IGF2BP2	rs4402960	0.3762	gnomAD
CDKAL1	rs7754840	0.4048	gnomAD
HHEX	rs1111875	0.3800	gnomAD

1.5. Pathophysiology

MODY is caused by mutations in a single gene resulting in pancreatic β-cell dysfunction, compromising the production or excretion of insulin. It is associated with insulin secretory defects in the absence of insulin resistance (Chambers *et al.,* 2016; Naylor and Philipson, 2011). The secretion of insulin involves a complex array factor (Figure 1.2), which starts with glucose being taken up by β-cells through type 2 glucose transporters (GLUT 2) on the plasma membrane. An enzyme, called glucokinase (GCK), converts glucose into glucose-6-phosphate, which then undergoes glycolysis in the mitochondria and produces adenosine triphosphate (ATP). The ATP-ADP ratio increases, resulting in the closure of ATP-sensitive potassium channels (KATP), which prevent potassium efflux, leading to plasma membrane depolarization and opening of voltage-dependent calcium channels. Cellular calcium influx stimulates exocytosis of insulin-containing granules (Naylor *et al.,* 2011; Heuvel-Borsboom *et al.,* 2016) (Figure 1.2).

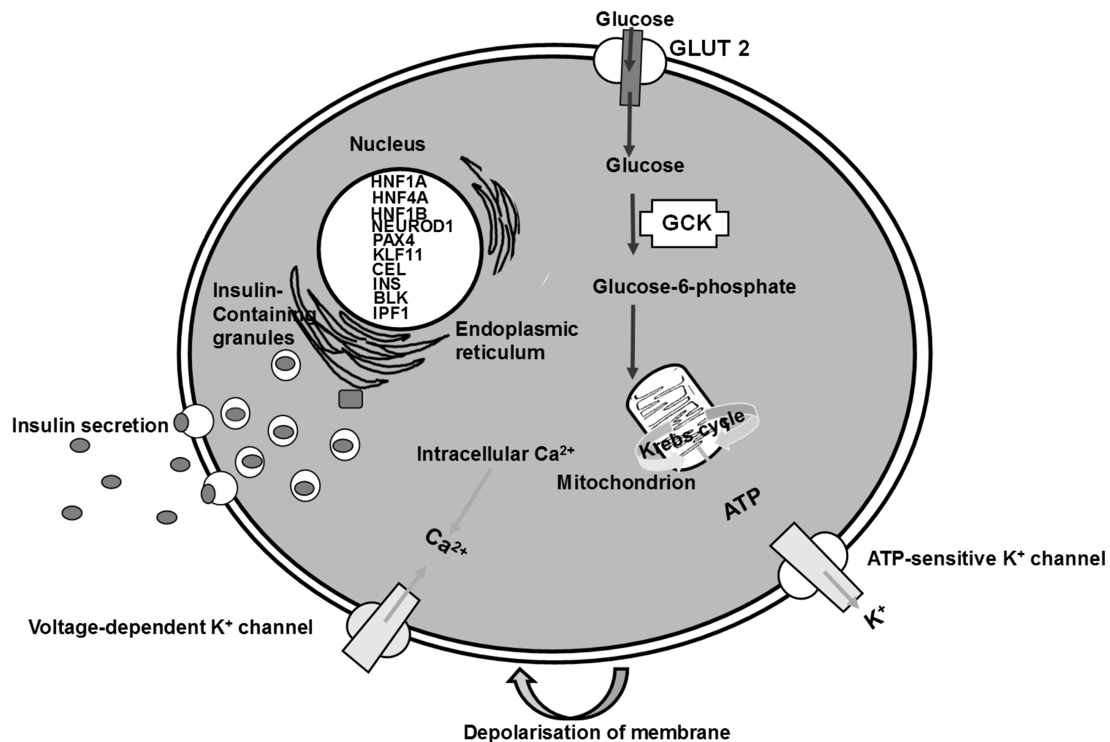

Figure 1.2: Schematic model of pancreatic β-cell and genes implicated in Maturity Onset Diabetes of the Young (MODY), (Adapted from McDonald and Ellard, 2013).

1.5.1. Description of different MODY subtypes

1.5.1.1. Hepatic nuclear factor 4 alpha - HNF4A (MODY 1)

MODY 1 is also known as NR2A1 (Nuclear receptor subfamily 2) and is caused by mutations in the HNF4A gene (Kanwal *et al.,* 2011; Yamagata *et al.*, 1996). HNF4A, located on chromosome 20q13.12, is 30kb in size with an estimated exon count of 13 (Bell *et al.,* 1991). It is a member of the nuclear receptor family that binds DNA as a homodimer. HNF4A is expressed in the liver, pancreas, and kidney and may be associated with changes in apolipoprotein AII, CIII, LP (a) and triglycerides (Anik *et al*., 2015). It is responsible for the regulation of gene expression in hepatic and pancreatic beta cells (Kuo *et al*., 1992). HNF4A plays a role in the functioning of Kir6.2 receptor and is therefore required in beta cell insulin secretion (Gupta *et al.,* 2005). HNF4A gene mutations result in the progressive decrease of insulin production and are associated with autosomal dominant non-insulin T1D (Yamagata *et al*., 1996). Insulin therapy is required for HNF4A-induced MODY. This leads to neonatal hyperinsulinemia, hypoglycemia, and is the cause of macrosomia in 50% of cases (Covantev *et al*., 2016). Vascular complications are often associated with HNF4A mutations. (Nyunt *et al*., 2009).

1.5.1.2. Glucokinase - GCK (MODY 2)

GCK, also known as Hexokinase IV or D, belongs to the hexokinase family, and is one of the most common forms of MODY. The GCK gene is located on chromosome 7p13 and is expressed in the pancreas and liver. The GCK gene plays an important role in glucose-stimulated insulin secretion in the pancreas, while it contributes to glucose uptake and glycogen conversion in the liver (Kanwal *et al.,* 2011; Matschinsky *et al.,* 1993). Mutated GCK genes cause MODY 2 (Froguel and Velho, 1994; Matschnisky, 1990). GCK plays a glucose sensing role in pancreatic beta cells, thus contributing to glucose homeostasis. GCK mutations are associated with an increase in the threshold concentration of glucose necessary to stimulate insulin secretion (Froguel *et al*., 1993). It expresses and catalyzes the initial step in the glycolytic pathway by ATP-dependent phosphorylated glucose to glucose-6-phosphate (Matschinsky *et al.,* 1993). GCK is characterized by hyperglycemia at birth and does not worsen at a later stage (Osbak *et al*., 2009). Mutations in the GCK gene upregulate insulin secretion, and reset the glucose threshold, resulting in an enhanced fasting glucose level (Byrne *et al.,* 1994). Approximately 600 different mutations have been reported in ten exons of the GCK gene (Naylor and Philipson, 2011; Obsbak *et al*., 2009). A mutation in this gene alters enzyme activities associated with multiple types of diabetes (e.g. neonatal diabetes) and hyperinsulinemic hypoglycemia (Nyunt *et al.,* 2009; Gloyn, 2003).

1.5.1.3. Hepatocyte nuclear factor 1 - HNF1A (MODY 3)

MODY 3 is caused by HNF1A gene mutations and is one of the most common forms of the disease (Kanwal *et al.,* 2011). The association was discovered when conducting linkage analysis of R-W pedigree by Fajans and Brown (1993*)*. The HNF1A gene is mapped on chromosome 12q24.31 and it encodes a transcriptional factor important in β-cell differentiation and function, and therefore affects cases with progressive β-cell defects (Velho *et al.,* 1996; Nyunt *et al.,* 2009). The HNF1A gene is expressed in the liver, kidney, intestine, and pancreatic β-cell (Dukes *et al.,* 1998; Kim, 2015). It plays a significant role in the expression of insulin genes in mature β-cells and glucose transport GLUT2 genes (Galan *et al.,* 2011). MODY 3 is characterized by a progressive reduction in insulin secretion (Velho *et al.,* 1996). A study conducted by Anuradha and co-workers reported high incidence of amino acid polymorphisms at codon ($Ala^{98} \rightarrow Val$), which is associated with MODY 3 (Anuradha *et al.,* 2005; Nyunt *et al.,* 2009). HNF1A mutation can lead to hyperglycemia, which can be controlled by sulfonylureas for several years (Fajans and Brown, 1993). HNF1A mutation increases β-cell apoptosis (Bacon *et al.,* 2012).

1.5.1.4. Pancreatic and duodenal homeobox 1 - PDX1 (MODY 4)

MODY 4 is caused by mutations in the PDX1 gene. PDXI is also known as insulin promoter factor 1 (IPF1) (Kanwal *et al.,* 2011). The PDXI gene is located on chromosome 13q12.2. PDXI is a homeodomain-containing transcriptional factor that acts in pancreas development, as well as insulin gene expression (Stoffers *et al.,* 1997[a]). It plays a major role in regulating genes coding for glucagon, insulin, glucose transporter 2 (GLUT2), and glucokinase (GCK) enzymes (Kim *et al.,* 2002). It serves as the master switch for maintenance of hormonal and enzymatic functions of the pancreas (Schwitzgebel *et al.,* 2003). Agenesis is a pancreatic anomaly, which can occur as a result of disrupted PDX1/IPF1 gene activity. Heterozygous PDX1 mutations can lead to defective insulin secretion, whereas homozygous mutations result in permanent neonatal diabetes (PND) and pancreatic exocrine insufficiency (Stoffers *et al.,* 1997 [b]; Jonsson *et al.,* 1994; Ahlgren *et al.,* 1998).

1.5.1.5. Hepatocyte nuclear factor 1 beta - HNF1B (MODY 5)

MODY 5 is a rare form of the disease and is caused by mutations in the HNF1B gene (Kanwal *et al.,* 2011). HNF1B is located on chromosome 17q12. HNF1B is also known as transcription factor-2 (TCF2) and is expressed in the liver, intestine, stomach, lung, and pancreatic islets (Kanwal *et al.,* 2011; Coffinier *et al.,* 1999). It plays an important role in developmental processes, such as nephron and embryonic pancreas development (Firdous *et al.,* 2018;

Lindner *et al.,* 1999). Patients with HNF1B-MODY usually exhibit notable histological anomalies, indicating *metanephrines* and renal cysts, referred to as renal cysts and diabetes syndrome (RCAD). Mutations in this gene are responsible for severe kidney disease, which may appear before the impairment of glucose tolerance (Bellanne-Chantelot *et al.,* 2004). MODY 5 can lead to complications such as vaginal aplasia, rudimentary uterus, Hyperglycemia, gout, and infant birth weight reductions (900g) (Lindner *et al.,* 1999; Bingham *et al.,* 2003; Edghill *et al.,* 2006).

1.5.1.6. Neurogenic differentiation factor 1 - NEUROD1 (MODY 6)

MODY 6 is caused by heterozygous mutations in the NEUROD1 gene (Kanwal *et al.,* 2011). NEUROD1 is located on chromosome 2q31.3 and is expressed in pancreatic and neuronal cells (Malecki *et al.,* 1999). It is a basic-loop-helix transcription factor, which plays a crucial role in pancreatic and neuronal development, affecting pancreas morphology and neuronal differentiation (Firdous *et al.,* 2018).

1.5.1.7. Krueppel-like factor 11 - KLF11 (MODY 7)

MODY 7 is caused by mutations in the KLF11 gene (Kanwal *et al.,* 2011). KLF11 is located on chromosome 2p25.1 and is expressed in β-cells. KLF11 plays a major role in pancreatic β-cell functioning by modulating the expression of certain free radical scavengers, such as catalase and superoxide dismutase (SOD) (Kanwal *et al.,* 2011; Scohy *et al.,* 2000; Firdous *et al.,* 2018). KLF11 behaves as a tumor suppressor for pancreatic malignancy (Neve *et al.,* 2005).

1.5.1.8. Carboxyl ester lipase - CEL (MODY 8)

MODY 8 is caused by mutations in the CEL gene (Kanwal *et al.,* 2011). CEL is mapped on chromosome 9q34.13. This gene controls both exocrine and endocrine functions of the pancreas; it is usually expressed in mammary glands and pancreatic acinar tissue (Kanwal *et al*., 2011; Johansson *et al.,* 2011). CEL plays a significant role in infants, by aiding in milk digestion and hydrolysis of dietary esters in the duodenum (Hui *et al.,* 2002).

1.5.1.9. Paired Box 4 - PAX4 (MODY 9)

MODY 9 is caused by heterozygous mutations in the PAX4 gene (Kanwal *et al.,* 2011). PAX4 is a transcription factor and belongs to the PAX family (Firdous *et al.,* 2018). It is located on chromosome 7q32.1 (Firdous *et al.,* 2018). PAX4 is initially expressed in endocrine promoter cells at the early stage of embryonic development and at β-cells at the later stage (Habener *et al.,* 2005). It plays an important role during fetal development and the progression of cancer (Kanwal et al., 2011). PAX4 is required for B-cell regeneration in adults; however, mutations in

this gene inhibit β-cell proliferation (Biason *et al.,* 2005). Mutation of the PAX4 gene can lead to ketosis-prone diabetes (Mauvais-Jarvis *et al.,* 2004).

1.5.1.10. Insulin - INS (MODY 10)

MODY 10 is caused by heterozygous mutations in the INS gene. This gene is located on chromosome 11p15.5 (Kanwal *et al* 2011). Insulin is responsible for regulating energy and glucose metabolism in the body (Kanwal *et al.,* 2011). This gene encodes for proinsulin, a precursor of insulin, and its mutation can lead to primary defects in nuclear factor Kappa-light-chain-enhancer of activated B cells NF-κb (Dandona *et al.,* 2001; Kanwal *et al.,* 2011). Mutations in the INS gene can lead to neonatal diabetes (Edghill *et al.,* 2008).

1.5.1.11. BLK proto-oncogenes, Src family tyrosine kinase - BLK (MODY 11)

MODY 11 is caused by heterozygous mutations in the BLK gene (Kanwal *et al.,* 2011). The BLK gene, also known as B lymphocyte kinase, plays an important role in thymopoiesis in immature T cells (Kanwal *et al.,* 2011; Islam *et al.,* 1995). It is located on chromosome 8p23.1. The BLK gene encodes for tyrosine receptor protein and is a member of the SRC family of proto-oncogenes, which stimulate β-cells to synthesise and secrete insulin (Borowiec *et al.,* 2009). The BLK gene is only expressed in β-cells and functions in signal transduction pathways (Firdous *et al.,* 2018; Dymecki *et al.,* 1990). BLK gene mutations affect MIN6-B-cells (a highly differentiated B cell line) (Borowiec *et al.,* 2009).

1.5.1.12. ATP binding cassette subfamily C member 8 - ABCC8 (MODY 12)

MODY 12 is caused by heterozygous mutations in the ABCC8 gene (Kanwal *et al.,* 2011). ABCC8 is located on chromosome 11p15.1 and is responsible for insulin secretion, which regulates blood sugar levels (Kapoor *et al.,* 2009). This gene encodes for sulfonyl-urea receptor 1 (SUR1), which is a subunit of ATP-sensitive potassium (K-ATP) channels found across β-cell membranes (Firdous *et al.,* 2018). Mutations in the ABCC8 gene can cause congenital hyperinsulinism, which can occur as a result of dominantly inherited inactivating mutations. These mutations in the ABCC8 gene can also cause permanent or transient neonatal diabetes (TNDM or PNDM) as a result of activating mutations or recessive loss-of-function mutations (Kapoor *et al.,* 2009).

1.5.1.13. Potassium voltage-gated channel subfamily J member 11 - KCNJ11 (MODY 13)

MODY 13 is caused by heterozygous mutations in the KCNJ11 gene (Gloyn *et al.,* 2004). The KCNJ11 gene was thought to encode for human BIR (beta cell inward rectifier) and Kir6.2, subunits of K-ATP channels (Inagaki *et al*., 1995; Kim, 2015). The KCNJ11 gene is mapped on chromosome 11p15.1. Mutations in this gene result in severe conditions, such as channel

inactivation due to disrupted subunit interaction. This disruption was found to be associated with Arg^{301} mutations, usually resulting in hyperinsulinism and possibly neonatal diabetes (Lin *et al.*, 2008; Firdous *et al.*, 2018).

1.5.1.14. Adaptor protein, phosphotyrosine interacting with PH domain and leucine Zipper 1 - APPL1 (MODY 14)

MODY 14 is caused by mutations in the APPL1 gene (Prundate *et al.*, 2015). APPL1 is located on chromosome 3p14.3. This gene is involved in the regulation of cell proliferation and the crosstalk between adiponectin and insulin signalling pathways. Mutations in APPL1 can result in apoptosis in highly expressing tissues; overexpression induces dysmorphic phenotypes and developmental delays (Schenck *et al.*, 2008).

1.6. Diagnosis and biomarkers of MODY

1.6.1. Diagnosis

Direct sequencing can be used to diagnose MODY with sensitivity close to 100% (Shields *et al.*, 2010). The next generation sequencing (NGS) method can be employed successfully to identify MODY gene mutations using gene targeted and whole exon-sequencing (Ellard *et al.,* 2013; Johansson *et al.*, 2012). In specialized laboratories, genetic testing can be used for MODY diagnoses (Bennett *et al.*, 2015). T1D treatment involves administration of exogenous insulin, which is not always the case for MODY (Kavvoura and Owen, 2013). Specific criteria are required before administering genetic tests to ensure accurate diagnosis (Anik *et al.*, 2015). The diagnostic guidelines for MODY indicate that genetic testing should be carried out on individuals diagnosed with diabetes at a young age (<25 years), and those with familial history of diabetes, evidence of endogenous insulin secretion, detectable c-peptide levels, and negative antibody results (Ellard *et al.*, 2008). According to the model, which was developed by Shields *et al* (2010) a diagnosis before the age of 30 years is a useful discriminator between MODY and T2D, whereas parental history of diabetes increased the possibility of a patient initially diagnosed with T1D later being diagnosed with MODY by 23-fold. Clinical and laboratory parameters have been established to define which candidates are put forward for molecular diagnosis (Thanabalasingham *et al.*, 2012).

1.6.2. Importance

Although MODY represents 1% to 5% of all diabetes, it has important implications (Gardner and Tai, 2012). The diagnosis of MODY is essential for patients and their families. It is crucial to characterize different types of MODY and differentiate them from other types of DM, as most patients with MODY are misdiagnosed with type 1 or type 2 DM, resulting in patients

receiving inappropriate treatment. Treatment protocols tend to follow the glycemic control plans for either type 1 or type 2 DM, including administration of exogenous insulin (Thanabalasingham and Owen, 2011[a]). This may be the result of overlapping clinical features, which are more common in diabetes, the high cost of genetic testing, as well as low levels of awareness among clinicians (Mughal *et al.,* 2013). Appropriate diagnosis would allow for optimal therapeutic management and treatment strategies that differ significantly compared to those for type 1 or type 2 DM. Additionally, it is important to create awareness of the disease through educational lectures and public seminars (Sukumar *et al*., 2011; Kim, 2015). Patients who have been following treatment strategies based on a type 1 DM diagnosis can switch to oral agents (i.e. sulfonylureas), which will improve their quality of life and glycemic control (Pearson *et al.,* 2003). Appropriate diagnosis will in turn affect prognosis; if an individual is found to have mild hyperglycemia in late teenage life, it will require a different treatment strategy (Steele *et al.,* 2011). MODY diagnosis is the key to provide accurate counselling regarding the predicted clinical outcome, genetic counselling, and identification of affected family members. Accurate diagnosis will further serve to inform families of the carriers and this can prevent misdiagnosis in other family members (Kavvoura and Owen, 2013).

1.6.3. Biomarkers of MODY subtypes

1.6.3.1. Characteristics of an ideal biomarker

Biomarkers are tools used to help clinicians predict a diagnosis, aid in disease monitoring, as well as screen for specific diseases. A biomarker is considered clinically useful if it demonstrates specificity and high sensitivity, is cheap and locally available, is not operator or assay dependent, and can discriminate between subjects with and without the disease. The receiver operating characteristic (ROC) curve analysis is a plot of sensitivity versus 1-specificity. The C-statistic, which is the area under the ROC curve, reflects the discriminatory potential of the diagnostic test. A C-statistic of 0.5 indicates that the biomarker provides no discrimination between the two disease states and a value of 1.0 indicates perfect discrimination (Mughal *et al.,* 2013).

1.6.3.1.1. High-sensitivity C-reactive protein (hsCRP)

To date, hsCRP is the most promising biomarker for HNF1A-MODY. It has been found to be useful in discriminating HNF1A-MODY from other types of diabetes (Mughal *et al.,* 2013). Three independent genome wide association studies (GWAS) in 2008-2009, showed that the common variation near HNF1A was reproducibly associated with modest difference in C-reactive protein (CRP) levels in healthy adults (Reiner *et al.,* 2008; Ridker *et al.,* 2008; Elliott *et al.,* 2009). Findings of the genome-wide association study are supported by the fact that the

CRP promoter contains binding sites for HNF1A (Toniatti *et al.,* 1990; Nishikawa *et al.,* 2008) and the expression of CRP is downregulated in Hnf1a knockout mice (Shih *et al.,* 2001). This led to the hypothesis that loss-of-function HNF1A mutations are associated with lower hsCRP levels (McDonald *et al.,* 2011[b]). This was also confirmed in a pilot study showing that HNF1A-MODY patients exhibited significantly lower levels of hsCRP compared with patients with autoimmune diabetes, type 2 diabetes and GCK-MODY, as well as healthy controls (Owen *et al.,* 2010). These results were then replicated in two large independent studies investigating MODY associated with HNF4A and HNF1A mutations (Figure 1.3).

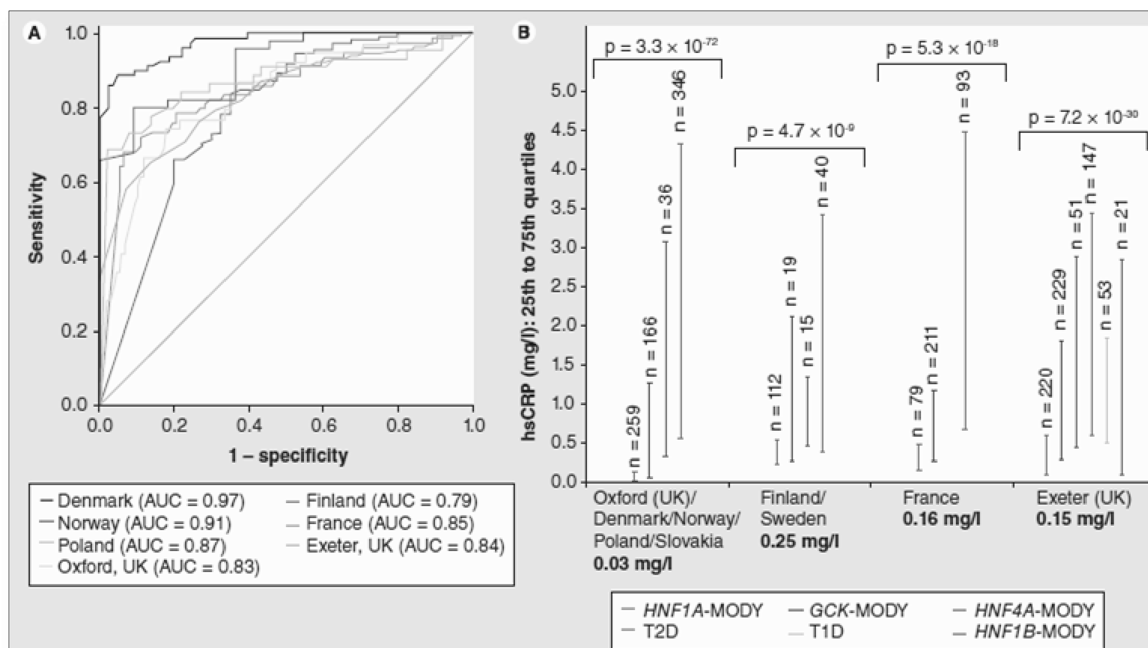

Figure 1.3: Graphs indicating combined results of two large independent studies examining hsCRP in different subtypes of diabetes. (A) Receiver operating characteristic (ROC) curves indicating the performance of hsCRP distinguishing between HNF1A-MODY and type 2 diabetes across European centres. (B) Levels of hsCRP in different forms of diabetes (*Adapted from: Thanabalasingham et al., 2011[b]; Mughal et al., 2013).*

Given the good discriminative power and common use in clinical practice, hsCRP has the potential to be used as a biomarker to select patients with young-onset diabetes for molecular diagnostic testing. Limitations of hsCRP include the fact that CRP is an acute-phase protein and is used to diagnose and monitor infection as well as inflammation. High hsCRP levels can be misleading in someone with clinical suspicion of HNF1A-MODY, but with a concurrent infection. It is advisable to repeat hsCRP testing several weeks after the initial test in patients with high clinical suspicion of MODY (Mughal *et al.,* 2013).

1.6.3.1.2. C-peptides

C-peptide is co-secreted with insulin from beta cells and is commonly used to measure beta-cell function (Mughal *et al.,* 2013). As a result of autoimmune destruction, C-peptide levels gradually decline in patients with T1D, whereas patients with MODY retain their endogenous beta-cell function. In a cross-sectional study, subjects with clinically labelled T1D with detectable c-peptide levels underwent genetic testing for MODY. Of all sequenced subjects, only two (10%) were found to have HNF1A-MODY (Thanabalasingham *et al.,* 2012). In another study, urinary C-peptide to creatinine ratio (UCPCR) was found to be lower in long-standing T1D compared with HNF1A/4A MODY with a C-statistic of 0.98 UCP (Besser *et al.,* 2011).

1.6.3.1.3. Islet autoantibodies

The majority of patients with T1D (85%-90%) have one or more pancreatic islet autoantibodies present during diagnosis, namely, glutamic acid decarboxylase (GAD) or insulinoma antigen 2 (IA-2) antibodies (American Diabetes Association, 2012). However, these antibodies are not expected in patients with MODY, diagnostic guidelines for MODY suggest testing those who are antibody negative (Ellard *et al.,* 2008). A study conducted by the UK Diagnostic Testing Centres reported < 1% prevalence of GAD and IA-2 antibodies in MODY (McDonald *et al.,* 2011[a]). However, a study conducted in a registry-based German pediatric cohort reported a slightly higher number of patients (17%) with confirmed MODY mutations presented with pancreatic islet autoantibodies (Schober *et al*., 2009). In Swedish and British studies, 4.8% and 21% of patients with MODY tested positive for GAD antibodies, respectively (Thanabalasingham *et al.,* 2012; Lehto *et al.,* 1999), but IA-2 antibodies were not detected (Thanabalasingham *et al.,* 2012). This suggests that in the case of strong clinical suspicion, the presence of islet autoantibodies should not preclude genetic testing (Mughal *et al.,* 2013).

1.7. Complications of MODY

MODY presents several complications similar to those present in other types of diabetes mellitus. Complications of MODY depend on which gene is affected, and could include heart and blood vessel disease, nerve damage, kidney damage, eye damage, foot damage, skin conditions, osteoporosis, Alzheimer's disease, and hearing difficulties (Kanwal *et al*., 2011). Microvascular and macrovascular complications of patients with GCK-MODY seem to be limited, whereas these issues are very common in patients with HNF1A-MODY (Steele *et al*., 2014; Heuvel-Borboom *et al*., 2016). Early detection and treatment of MODY may lead to

reduced incidence of diabetic complications (Sagen *et al.*, 2002). Microvascular complications appear rare in HNF1B-MODY (Faguer *et al.*, 2011).

1.8. Treatment and management of MODY

Accurate diagnosis of MODY allows for initiation of the most suitable treatment and prevents inappropriate treatment (Amed and Oram, 2016). Different treatment options have been recommended depending on the severity and type of MODY (Table 1.6) (Kanwal *et al.*, 2011). GCK-MODY is asymptomatic and presents as mild, stable hypoglycemia, therefore patients with confirmed GCK-MODY do not require treatment other than dietary alterations (Stride *et al.*, 2014; Hattersley *et al.,* 2009; Kanwal *et al.*, 2011). However, a pregnant woman with GCK-MODY requires insulin administration for fetal development and to prevent obstetric complications caused by maternal hyperglycemia (Anik *et al.*, 2015; Spyer *et al.*, 2009). Patients with HNF1A and HNF4A MODY can initially be treated with diet and lifestyle modifications, but they may still experience high postprandial blood sugar levels. As beta cells deteriorates over time, pharmacologic therapy becomes necessary to prevent diabetes-related complications (Amed and Oram, 2016). Patients with HNF4A and HNF1A-MODY are sensitive to sulfonylureas, which are the most effective therapy (Becker *et al.,* 2014; Covantev *et al.*, 2016). A comparison of patients with HNF1A-MODY treated with insulin alone and those treated with sulfonylureas or meglitinides (non-sulfonylurea insulin secretagogues) indicates increased mean A1C levels in those treated with insulin (7.5%) as opposed to those treated with oral agents (6.7%) (Raile *et al.,* 2015). Patients with HNF1B-MODY do not respond well to sulfonylurea derivatives therefore these patients require insulin therapy (Pearson *et al.,* 2004).

Table 1.6: Summaries of MODY gene names and their treatment

Gene name	Pathophysiology	Treatment	Reference
HNF4A	β-cell dysfunction	Sulfonylureas	Pearson *et al.*, 2005
GCK	Glucose-sensing defects	Diet, except possibly during-pregnancy	Chakera *et al.,* 2015
HNF1A	β-cell dysfunction	Sulfonylureas	Shepherd *et al.,* 2009
IPF/PDX1	β-cell dysfunction	Insulin or Diet	Fajans *et al.*, 2010
HNF1B	β-cell dysfunction	Insulin	Dubois-Laforgue *et al.,* 2017
NEUROD1	β-cell dysfunction	OAD or Insulin*	Kristinsson *et al.,* 2001
KLF11	Decreased glucose sensitivity of β-cells	OAD or Insulin	Neve *et al.,* 2005
CEL	Pancreatic endocrine and exocrine dysfunction	OAD or Insulin	Raeder *et al.,* 2006
PAX4	β-cell dysfunction	Diet, OAD or Insulin	Plengvidhya *et al.*, 2007
INS	β-cell dysfunction	OAD OR Insulin	Boesgaard *et al.*, 2010

BLK	β-cell dysfunction	Diet, OAD or Insulin	Borowiec *et al.*, 2009
ABCC8	Insulin secretion defects	Diet, OAD or Insulin	Bowman *et al.*, 2012
KCNJ11	ATP-sensitive potassium channel dysfunction	Diet, OAD or Insulin	Liu *et al.*, 2013
APPL1	Dysmorphic phenotypes, development delay	OAD or Insulin	Firdous *et al.*, 2018

*OAD: Oral antidiabetic drugs

1.9. Problem statement and rationale

The prevalence of MODY appears to be steadily increasing world-wide. Data is lacking in many parts of the world and further research is required in order to determine the actual prevalence of MODY. MODY is a rare type of diabetes. Patients are often misdiagnosed with type 1 or 2 diabetes mellitus, as it is difficult to distinguish between these forms of diabetes due to similarity in clinical features, hence the true prevalence of MODY is probably underestimated (Shields *et al.*, 2012). Due to this reason, thousands of patients are not receiving appropriate treatment. Further, MODY is a genetic form of diabetes which is often passed from generation to generation; relatives are often unaware of their increased risk for the condition. The actual prevalence of MODY in populations by country remains unknown, as few studies have been conducted since 1974.

Previous research indicated that the South African Indian population presents with a high prevalence of MODY compared to populations in other countries. It was reported that 10% of South African Indians diagnosed with diabetes present with MODY (Bhatia E, 1994). Current studies show that MODY is distributed unequally world-wide, therefore each country should perform its own population study in order to determine the prevalence of MODY (Irgens *et al.*, 2013; Shields *et al.,* 2010). The prevalence of specific MODY mutations differs considerably among various ethnic groups (Hattersley, 1998). Therefore, the current study aims to address the scarcity of information in South Africa with regards to the prevalence of MODY, specifically in the targeted mixed-ancestry population where no data is available. The significance of the study lies in investigating and reporting on the prevalence of MODY, as well as assessing the clinical characteristics of MODY in a mixed-ancestry population (a population of mixed European (“white”) and African (“black”) or Asian ancestry). This is especially important in the mixed-ancestry population of the Western Cape, South Africa, where a high prevalence of diabetes (28.2%) and high 30-year CVD risk have been reported (Erasmus *et al.*, 2012; Matsha *et al.*, 2012). This study would provide baseline information on MODY prevalence, specifically MODY 3, as well as increasing awareness, and enhancing service provision. The identification of individuals who are at risk of developing MODY could facilitate strategies to delay or prevent disease progression.

1.10. Research question

What is the prevalence and characteristics of MODY in the mixed-ancestry population?

1.11. Aim and Objectives

1.11.1 Aim

To determine the prevalence of MODY in a population from the Western Cape, South Africa, and assess the clinical characteristics of MODY.

1.11.2. Objectives

- ✓ To measure glucose levels and to determine the prevalence of MODY mutations in a mixed-ancestry population aged 20 years and older from Bellville South, Western Cape, South Africa.
- ✓ To determine anthropometric measurements and lipid profiles (total cholesterol, triglycerides, high density lipoprotein, and low-density lipoprotein) in a mixed ancestry-population aged 20 years and older
- ✓ Screening and measuring frequency of HNF1A-MODY in a mixed-ancestry population aged 20 years and older of the Western Cape using Real-time PCR (qPCR) and subsequent sequencing.

1.12. Hypothesis

It is hypothesized that a percentage of adults with diabetes or prediabetes residing in Bellville South, Western Cape, could suffer from undiagnosed MODY.

CHAPTER TWO
METHODOLOGY

2.1. Ethics statement

Ethics approval was granted for the primary study, the Vascular Metabolic Health (VMH) Study of which Professor TE Matsha is the principal investigator (CPUT/HW-REC 2015/H01 (renewal)). The research proposal for the current study was submitted to the Health and Wellness Sciences Research Ethics Committee (HWS-REC) at Cape Peninsula University of Technology for ethical approval, which was also granted (CPUT/HW-REC 2019/H8). The study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki 2013). Participants were asked to sign a consent form after the study principles had been fully explained to them in the language of their choice. Thereafter, they were free to ask any questions pertaining to the study and their involvement.

2.2. Study design

This is a cross-sectional descriptive study. Quantitative methods were used to determine the prevalence of Maturity Onset Diabetes of the Young (MODY) in the mixed-ancestry population from Bellville South, Western Cape, South Africa.

2.3. Study settings

Bellville South is a mixed-ancestry population formed in the late 1950s; it is located within the Northern suburbs of Cape Town, Western Cape, South Africa. According to the 2011 census data, this population is comprised of 76.0% mixed-ancestry, 18.5% African black, 1.0% Asian, 0.5% Caucasian, and 4.0% individuals from other ethnicities. The area is inhabited by approximately 29301 individuals, consisting of 6048 households, with an average of 4.84 individuals per household. Individuals residing in this township are mostly of lower- or middle-income class, with 37% of households earning a monthly income of R3 200 or less (Statistics South Africa, 2011). Recent studies in this population have shown an increase in the number of individuals with diabetes (Erasmus *et al.,* 2012). Erasmus and co-workers reported a high prevalence of T2DM (28.2%), hence this community presents an ideal study setting to investigate the prevalence of MODY.

2.4. Study population

The study population was comprised of individuals who voluntarily participated in the VMH study conducted between 2014 and 2016. A door to door campaign was conducted in Bellville-South. A total of 1657 individuals (diabetic and non-diabetic) were recruited. All participants (20 years and older) were screened for the presence of MODY irrespective of age as it is possible that some may have been long misdiagnosed with T1D orT2D.

2.4.1. Inclusion criteria

The participants had to be of mixed-ancestry, 20 years and older, both males and females (diabetic and non-diabetic) enrolled in the study on a voluntary basis and gave informed consent in a language of their choice.

2.4.2. Exclusion criteria

Pregnant women, severely ill individuals, and those who declined to participate were excluded from the study.

2.5. Sample size

The prevalence of MODY world-wide has been estimated to account for 1-5% of all diabetes mellitus cases (Shields *et al*., 2010; Irgens *et al*., 2013). The prevalence of MODY is unknown in the South African population, therefore the prevalence of diabetes in the South African population, which has been reported as 28% by Erasmus *et al.* (2012), has been used for the sample size calculation.

The formulae used to calculate the sample size needed for this study is as follows:

$\mathbf{n = z^2(pq)/e^2}$

Where:

n = the sample size

z = standard error associated with the chosen level of confidence (1.96)

p = estimated population percentage

q = 100-p

e = acceptable sample error (5%)

Calculation:

$\mathbf{n = z^2(pq)/e^2}$

$n = \{1.96^2(28.2 * (100-28.2)\}/5^2$

n = 311

Minimum sample size required was 311 participants. Final sample size used was 1657 (413 males and 1244 females), which was the available sample size from the original study population.

2.6. Data collection

Data collection included anthropometric measurements, blood pressure, and biochemical analysis

2.6.1. Anthropometric measurements

Anthropometric measurements included height, weight, and hip (HipC) and waist (WaistC) circumferences . These measurements were performed three times and the average was used for final analysis. The body mass index (BMI) was calculated by dividing the weight by the height squared: weight/height^2 (kg/m^2). All the anthropometric measurements were conducted by trained, registered nursing sisters.

2.6.1.1. Height

Both height and weight measurements of the participants were measured using a portable stadiometer, the Omron Body Composition Monitor (BF511: Omron, Japan). Wheelchair-bound participants were excluded from both height and weight measurements. The surface area of the scale was cleaned with a disinfectant and wiped dry. The participant was requested to stand on the flat surface of the stadiometer, with his/her shoes off. The back of the participant was aligned to the measuring pole, with the participant standing in a standard anatomical position. The height was recorded in centimetres (cm).

2.6.1.2. Weight

The weight of the participants was recorded in kilograms (kg). Participants were weighed fully clothed but with shoes, socks, and heavy overcoats removed. The surface area was disinfected, and the scale zeroed, after which participants were asked to stand on the scale with their hands placed at their sides. The participant was asked to stand face up, in the correct anatomical position during weight reading.

2.6.1.3. Waist circumference

The participants were fully clothed but asked to remove thick jackets. They were asked to stand with feet approximately 15 cm apart and in an anatomical position. WaistC was measured using a stretch-resistant tape measure, which was placed horizontally around the narrowest part of the torso, halfway between the iliac crest and the lowest rib.

2.6.1.4. Hip circumference

The HipC (cm) was measured using a non-elastic tape, at the maximal circumference over the buttocks. The participants were fully clothed but asked to remove thick jackets. The participants were asked to stand in an anatomical position while the HipC measurement was recorded.

2.6.2. Blood pressure measurements

Blood pressure is expressed as systolic (SBP) over diastolic (DBP) millimetres of mercury (mm Hg) (Pickering *et al.,* 2005). The measurements of blood pressure were performed by a registered nurse according to WHO guidelines (WHO, 1999). The Omron M6 Comfort-preformed Cuff Blood Pressure Monitor (Omron, Japan) was used to measure blood pressure. Participants were asked to remove thick jackets and sit down in a comfortable position during the blood pressure reading. The cuff of the Blood Pressure Monitor was placed on the left arm of the participant, two fingers above the elbow joint. The readings were taken 3 times with 3-minute intervals between each reading. The lowest SBP and corresponding DBP readings were used in the current study.

2.6.3. Blood collection

Collection of blood was conducted by trained, registered nursing sisters. Participants were requested to fast overnight according to WHO guidelines (WHO, 1999), in order to enable the collection of fasting samples and samples collected after the 2-hour oral glucose tolerance test (OGTT). Self-reported T2DM participants and/or those on medication had only the fasting blood sample taken. All other participants underwent the 2-hour OGTT.

2.6.3.1. The oral glucose tolerance test

1. Participants were given 75 grams of anhydrous glucose dissolved in 250-300 ml of water after the overnight fasting samples had been taken.
2. Participants were asked to drink the mixture within 3-5 minutes
3. The time was recorded, and the second blood sample was taken after 2 hours.

2.6.3.2. Blood samples required

Fasting and 2-hour postprandial blood samples were each collected in grey capped-tubes (sodium fluoride), two plain top tubes (no anti-coagulant) and two purple capped-tubes (EDTA). The grey tubes were used to measure blood glucose concentrations, while the plain top tubes were used to obtain serum for serological tests, such as serum cotinine, insulin, and the lipid profile. The purple capped tubes were used to measure HbA1c levels and for DNA extraction. Collected blood samples were transported in an icebox to PathCare Reference Laboratory (Cape Town, South Africa) for further processing and biochemical measurements. Serum and full blood samples were processed and stored at -80˚C for future use.

2.6.3.3. Biochemical measurements

Plasma glucose (mmol/L) concentrations were measured using the hexokinase method (Beckman AU or DXC). The glycated haemoglobin HbA1c (%) was measured using high performance liquid chromatography (HPLC) (BioRad Variant Turbo, USA). Insulin (mIU/L) was measured using a Paramagnetic particle assay (Chemiluminescence). LDL-chol (mmol/L) was measured using an Enzymatic Selective Protection–Endpoint assay (Beckman AU, UK), HDL-chol (mmol/L) using an Enzymatic Immuno-inhibition-Endpoint assay (Beckman AU) and the triglycerides (mmol/L) were estimated using a glycerol phosphate oxidase in the presence of peroxidase (GPO-POD) Endpoint assay (Beckman AU).

2.6.3.3.1. Type 2 diabetes (T2DM) classified according to WHO (2016)

T2DM: if fasting blood glucose (FBG) was ≥7.0 mmol/L or post 2 hour blood glucose (2HR BG) ≥11.1 mmol/L; Impaired fasting glucose (IFG): if FBG is between 6.1 and 6.9 mmol/L and if measured, post 2HR BG is <7.8 mmol/L; Impaired glucose tolerance (IGT): if FBG is <7.0 mmol/L and post 2HR BG is between ≥7.8 mmol/L and <11.1 mmol/L.

2.7. Molecular methods

2.7.1. Isolation of genomic DNA

Genomic DNA was isolated from whole blood collected in EDTA and stored at -20°C. A salt extraction method was used to isolate DNA from 1 - 2 mL of blood.

Day 1: Briefly, after thawing the frozen blood samples, blood cells were lysed by adding five times the volume (~5 - 10 mL) of nucleic lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM

EDTA; pH 7.4). The tubes were vigorously vortexed for 10 - 15 seconds, then placed on ice for 5 minutes. This step was repeated three times followed by centrifugation (1,500 rpm, 10 minutes) using a Beckman GP centrifuge (Beckman Coulter Inc., UK). The supernatant was discarded, pellets were washed with 10 mL phosphate buffered saline (PBS; 2.68 mM KCl, 136 mM NaCl, 1.8 mM KH_2PO_4, and 10 mM Na_2HPO_4, pH 7.4) and centrifuged twice (1,500 rpm, 10 minutes). Thereafter, nucleic lysis buffer (3 mL, 10 mM Tris, 400 mM NaCl, 2 mM EDTA; pH 8.2), proteinase K (30µL, 10mg/mL), and 10 % (w/v) sodium dodecyl sulfate (300µL, SDS) was added and the pellet was dissolved by vortex agitation. The tubes were incubated at 55 °C overnight to digest proteins.

Day 2: A saturated solution of NaCl (1 mL, 6 M) was added; the tubes were then vortexed and centrifuged (2,500 rpm, 30 minutes). The supernatant was transferred into clean 15 mL polypropylene tubes where DNA was precipitated by adding two volumes (~ 9 mL) of 100 % ice cold ethanol. As a result of DNA being insoluble in ethanol, a string-like precipitate was formed. Tubes were then centrifuged at 2,500 rpm for 30 minutes. The supernatant was discarded, and 70 % ethanol (1 mL) was added to the DNA pellet to remove any residual salt solution. The tubes were centrifuged in a microcentrifuge (Beckman Coulter, Germany) at 13,800 rpm for 30 minutes. The supernatant was discarded, and the DNA pellet was dried by inverting the tube on a paper towel and air drying for 15 minutes. The DNA pellet was dissolved in nuclease free water (100 - 200 µL), depending on the size of the pellet. Tubes were placed on a mixer overnight to resuspend the pellet.

Day 3: The optical density of the samples was read on a Nanodrop One (Thermo Fisher Scientific, USA), which recorded the DNA concentrations, as well as the 260/280 ratios. A 260/280 ratio between 1.8 and 2.2 was indicative of pure DNA. A lower ratio is suggestive of the presence of protein, phenol, or other contaminants which absorb strongly at or near 280 nm and 230 nm, respectively. Extracted DNA was then stored at -80ºC.

2.7.2. Real-time Polymerase Chain Reaction (RT-PCR) Single-Nucleotide Polymorphism (SNP) Genotyping

In this study, genotypes of four different SNPs (rs140491072, rs115080759, rs142318174, and rs137853245; Table 2.1) were determined using PCR techniques performed in two different laboratories; results were confirmed by sequencing. Firstly, real-time PCR was carried out locally at CPUT, using Applied Biosystems™ QuantStudio™ 7 Flex (Thermo Fisher Scientific, US). Thereafter, samples were sent to Inqaba Biotechnical Industries (Pretoria, South Africa) for PCR confirmation and sequencing. The reagents and primers used in this study (Taqman

SNP Genotyping Assays) were designed by Thermo Fisher Scientifics and used to amplify and detect specific SNP alleles in the purified genomic DNA samples.

Table 2.1: SNPs genotyping used

SNP ID	Polymorphism	Context Sequence
rs140491072	A/G, Transition Substitution	CTGCTTCCCTCTCCAGGTGTGCGCT**[A/G]** TGGACAGCCTGCGACCAGTGAGACT
rs115080759	G/T, Transversion Substitution	CAGCACCCTGACAGCACTGCACAGC**[G/T]** TGGAGCAGACATCCCCAGGCCTCAA
rs142318174	C/G, Transversion Substitution	CTGGACAAGGGGGAGTCCTGCGGCG**[C/G]** CGGTCGAGGGGAGCTGGCTGAGCTG
rs137853245	C/G, Transversion Substitution	TTTGCCAACCGGCGCAAAGAAGAAG**[C/G]** CTTCCGGCACAAGCTGGCCATGGA

2.7.3.1. Real-time PCR performed locally

A master mix (TaqMan® Master Mix, SNP genotyping and nuclease-free water; Table 2.2) was prepared for 96 samples. Master mix (8 µL) was added to each well of a 96-well plate, followed by the addition of genomic DNA (2 µL, 5 ng/mL). Nuclease-free water was plated in triplicate to serve as a negative control (NTC). An optical adhesive film was used to completely cover the wells of the reaction plate; thereafter the plate was briefly centrifuged (500 rpm for 1 minute).

The reaction plate was placed in the QuantStudio™ 7 flex Real time PCR System. The run conditions for the TaqMan® SNP Genotyping assays were optimized on the instrument and ran for 2 hr and 15 minutes (Table 2.4). The results were obtained from the QuantStudio™ 7 Flex Real-Time PCR System.

Table 2.2: Master mix for PCR per 96-well plate

Component	Volume per sample	Total volume for 96 samples
TaqMan® Master Mix	5.00 µL	500 µL
Nuclease-Free Water	2.75 µL	275 µL
SNPs Genotyping	0.25 µL	25 µL

Table 2.3: Master mix and DNA aliquots per well

Components	Samples (93-wells)	Negative controls (3-wells)
Mater mix	8 μL	8 μL
Genomic DNA	2 μL	-
Nuclease free water	-	2 μL
Total volume per well	10 μL	10 μL

Table 2.4: Cycling conditions for PCR

TaqMan® DME Genotyping assay			
Steps	**Temperature**	**Time**	**Cycles**
Initiation	95°C	10 minutes	HOLD
Denaturation	95°C	15 seconds	
Annealing/Extension	60°C	90 seconds	50

2.7.3.2. Real-time PCR and sequencing by Inqaba Laboratory

1642 of our genomic DNA samples were sent to Inqaba Biotechnical Industries for SNP genotyping and sequencing. The SNP Genotyping assay was performed using the Inqaba protocol, which includes an initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators of an oligonucleotide primer.

2.8. Statistical analysis

Data was captured on an Excel spread sheet and analysed using the software program TIBCO Statistica 13.5 (Statsoft, http://www.statsoft.com). ANOVA was used to calculate mean and standard deviation between variables (*R* and *P*-values). Breakdown and one-way ANOVA was used to calculate median (categorical variable) and Kruskal-Wallis test for *P*-value. SNPs were tested using the Hardy-Weindberg Equilibrium (HWE) expectation via a chi square goodness of fit test. The prevalence of HNF1A-MODY was calculated using percentage. A *P*-value of <0.05 was considered statistically significant.

CHAPTER THREE
RESULTS

3.1. Characteristics of the study population

1657 DNA samples were available for genetic testing. A total of 1642 samples were used (15 did not amplify), of these samples 408 were male and 1234 were female. Females were significantly older than males (*P*=0.0012). The anthropometric measurements, including BMI, WaistC (cm), and HipC (cm) were significantly higher in females than in males (*P*<0.0001). Similarly, the glycemic measurements were significantly increased in females as well: FBG (mmol/L) mean±SD in males: 5.39±2.18 and in females: 5.85±2.91, *P*=0.0035; HbA1c (%) in males: 5.92±1.25 and in females: 6.27±1.60, *P*=0.0001; Fasting BI (mIU/L) in males: 7.6±9.7 and in females: 10.2±12.3, *P*=0.0002. Additionally, lipid measurements were increased in females: LDL Cholesterol (mmol/L) mean±SD in males: 2.95±0.97 and in females 3.32±1.03, *P*<0.0001; HDL Cholesterol (mmol/L) in males: 1.25±0.37 and in females: 1.35±0.36, *P*<0.0001. The prevalence of pre-DM (10.8%), screen-detected DM (4.7%) and known DM (10.3%) were high in male subjects and significantly higher in female subjects: pre-DM (15.8%), screen-detected DM (6.6%) and known DM (13.5%), *P*=0.0064 (Figure 3.1.).

Table 3.1: Characteristics of the study population, per gender

	Total, N1642	**Males, N408**	**Females, N1234**	
		Mean±SD		***P*-value**
Age (years)	49.2±15.3	47.1±15.6	49.9±15.1	**0.0012**
BMI	29.3±8.1	24.7±6.6	30.8±8.0	**<0.0001**
WaistC (cm)	92.9±17.3	86.3±16.8	95.0±16.9	**<0.0001**
HipC (cm)	104.6±16.6	94.5±12.5	107.9±16.4	**<0.0001**
WHR	0.89±0.09	0.91±0.09	0.88±0.09	**<0.0001**
SBP (mmHg)	127.2±23.8	126.7±24.2	127.4±23.6	0.6179
DBP (mmHg)	81.9±13.8	80.9±15.3	82.2±13.2	0.0993
FBG (mmol/L)	5.74±2.76	5.39±2.18	5.85±2.91	**0.0035**
Post 2 HRs BG (mmol/L)	6.62±2.93	5.76±2.99	6.91±2.85	**<0.0001**
HbA1c (%)	6.19±1.53	5.92±1.25	6.27±1.60	**0.0001**
Fasting BI (mIU/L)	9.6±11.8	7.6±9.7	10.2±12.3	**0.0002**
Post 2 HRs BI (mIU/L)	55.6±53.7	33.8±38.4	63.2±56.1	**<0.0001**
Glucose/Insulin ratio	1.00±0.88	1.32±1.06	0.90±0.79	**<0.0001**
Triglycerides-S (mmol/L)	1.47±1.08	1.55±1.58	1.44±0.85	0.0804
LDL Cholesterol (mmol/L)	3.23±1.03	2.95±0.97	3.32±1.03	**<0.0001**
HDL Cholesterol (mmol/`L)	1.32±0.36	1.25±0.37	1.35±0.36	**<0.0001**
Cholesterol (mmol/L)	5.18±1.17	4.83±1.12	5.29±1.17	**<0.0001**
Cholesterol/HDL ratio	4.11±1.20	4.07±1.33	4.13±1.15	0.4181
CRP (mg/L)	8.5±15.7	8.2±17.3	8.6±15.2	0.6663
Cotinine (ng/mL)*	13.0 (10.0; 267.0)	140.0 (10.0; 269.0)	10.0 (10.0; 265.0)	**0.0015**
Gamma GT-S (IU/L)	46.3±70.8	54.1±97.4	43.8±59.3	**0.0106**

*Median (Q25; Q75)

3.2. Genotypes

The distribution of genotypes from the two different laboratories was similar with the exception of rs115080759, in which two heterozygous genotypes were identified by one of the laboratories. Following direct sequencing the results were confirmed and a consensus was reached.

HNF1A SNPs (rs142318174, rs115080759, rs137853245, and rs140491072) were tested for Hardy-Weinberg equilibrium (HWE) expectations. Table 3.2 shows the allele and genotype distributions of all SNPs used in the present study. The genotype distribution of one SNP rs115080759 agreed with HWE (*P*=0.8818). In three SNPs; rs140491072, rs142318174, and rs137853245, the HWE was not applicable because only one allele frequency was presented. Therefore, these SNPs were excluded from further statistical analysis. SNP rs140491072 was also excluded from further analysis due to insufficient participants.

The genotype frequencies for SNP rs115080759 were: T/T: 99.3%, G/T: 0.7%, G/G: 0%, G: 0.4%,for rs140491072: A/A: 100%, A/G: 0%, G/G: 0% and G: 0.1%, for rs142318174: G/G: 100%, GC: 0%, C/C: 0% and C: 0%, and for rs137853245: C/C: 100%, C/G: 0%, G/G: 0% and G:0% (Table 3.2)

Table 3.2: Genotype distributions, minor allele frequencies

HNF1A, rs142318174		HNF1A rs115080759		HNF1A rs137853245		HNF1A rs140491072	
G/G, n (%)	1638 (100)	T/T, n (%)	1628 (99.3)	C/C, n (%)	1642 (100)	A/A, n (%)	1641 (100)
G/C, n (%)	0	G/T, n (%)	12 (0.7)	C/G, n (%)	0	A/G, n (%)	0
C/C, n (%)	0	G/G, n (%)	0	G/G, n (%)	0	G/G, n (%)	0
C, n (%)	0	G, n (%)	12 (0.4)	G, n (%)	0	G, n (%)	0
HWE (*P*-value)	NA	HWE (*P*-value)	0.8818	HWE (*P*-value)	N/A	HWE (*P*-value)	N/A

3.3. General characteristics of the study population compared to SNP (rs115080759) genotypes

The characteristics of study population according to genotypes of rs115080759 polymorphism of HNF1A gene was summarized in Table 3.3. Regarding the participants' characteristics, a comparison was performed between T/T and G/T alleles as no G/G allele was found in all screened participants. There was no observed age difference between participants with T/T

and G/T alleles. There were no significant differences observed between the genotypes (T/T and G/T) in all anthropometric measurements (BMI, WaistC (cm), and HipC (cm)). The glycemic measurements were significantly increased in participants with the G/T allele compared to those with the T/T allele: FBG (mmol/L) mean ± SD in G/T: 7.92±4.99 and in T/T 5.72±2.74, *P*=0.0061; HbA1c (%) in G/T: 7.30±2.73 and in T/T: 6.18±1.52, *P*=0.0116; Glucose/Insulin ratio in G/T: 1.56±1.76 and in T/T: 1.00±0.87, *P*=0.0284. Participants with the T/T allele exhibited a slight increase in cotinine levels (ng/mL), median (Q25; Q75) were: 14.4 (10.0; 267.0) in T/T and 10.0 (10.0; 95.4) in G/T, P=0.0773. There was no significant difference in CRP (mg/L) across the genotypes, *P*=0.5902.

Table 3.3: SNPs allele comparison with participants' characteristics

	Total group HNF1A, rs115080759					
	T/T N1628	**G/T N**12	**GG N0**	**Gender**	**SNP**	**Gender*SNP**
	Mean±SD			***P*-value**		
Diabetes, Yes, N (%)	305 (18.7)	4 (33.3)	0			0.4078*
Pre-DM, Yes, N (%)	238 (14.6)	1 (8.3)	0			
Age (years)	49.2±15.3	49.0±13.9		0.8061	0.9609	0.8061
BMI	29.3±8.1	31.1±6.9		**0.0287**	0.4520	0.8760
WaistC (cm)	92.8±17.3	97.4±16.3		**0.0421**	0.3676	0.4897
HipC (cm)	104.5±16.6	108.6±13.4		**0.0129**	0.3962	0.7844
WHR	0.89±0.09	0.90±0.08		0.9765	0.8036	0.4571
SBP (mmHg)	127.2±23.9	126.9±22.0		0.8026	0.9619	0.7319
DBP (mmHg)	81.9±13.8	84.3±9.5		0.4625	0.5512	0.3169
FBG (mmol/L)	5.72±2.74	7.92±4.99		**0.0339**	**0.0061**	**0.0098**
Post 2 HR BG (mmol/L)	6.62±2.93	5.47±0.85		0.5317	0.2705	0.8996
HbA1c (%)	6.18±1.52	7.30±2.73		0.0615	**0.0116**	**0.0127**
Fasting BI (mIU/L)	9.6±11.8	7.8±3.6		0.6059	0.6073	0.9581
Post 2 HRs BI (mIU/L)	55.5±53.7	52.7±39.5		0.4714	0.8822	0.7352
Glucose/Insulin ratio	1.00±0.87	1.56±1.76		**0.0004**	**0.0284**	**0.0213**
Triglycerides (mmol/L)	1.47±1.08	1.24±0.53		0.6385	0.4518	0.4686
LDL Chol (mmol/L)	3.23±1.03	3.33±1.00		0.4978	0.7311	0.7849
HDL Chol (mmol/L)	1.32±0.36	1.27±0.40		0.4065	0.5868	0.8687
Chol (mmol/L)	5.18±1.17	5.17±1.27		0.4942	1.0000	0.7262
Chol/HDL ratio	4.11±1.20	4.27±1.08		0.8717	0.6423	0.7716
CRP (mg/L)	8.5±15.8	6.0±7.3		0.6849	0.5902	0.7371
Cotinine (ng/mL)**	14.4 (10.0; 267.0)	10.0 (10.0; 95.4)			0.0773	
Gamma GT-S (IU/L)	46.2±70.7	49.1±68.2		0.7800	0.8897	0.5058

* Chi-square
**Median (Q25; Q75)

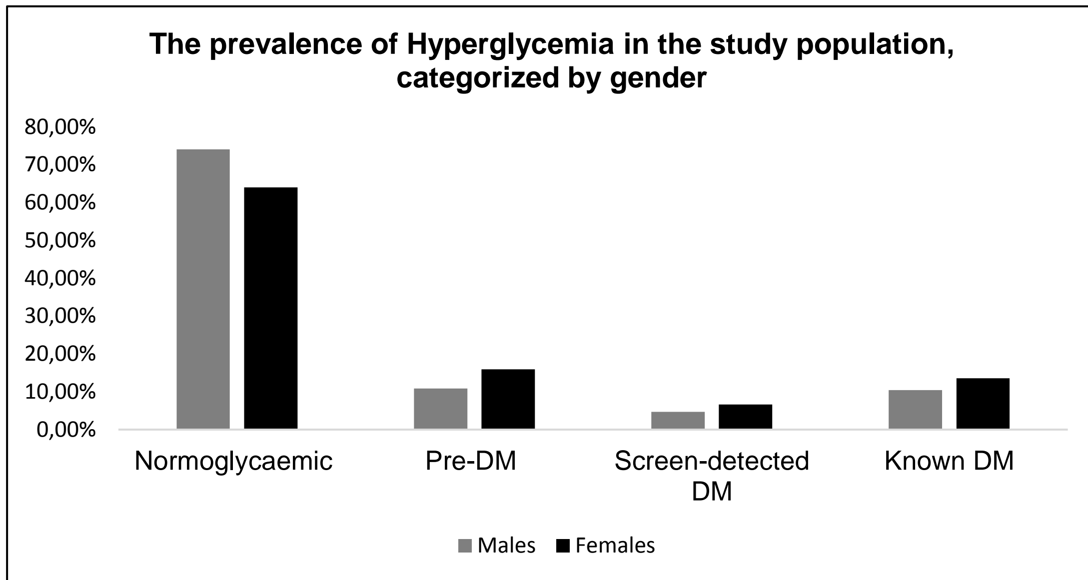

Figure 3.1: The prevalence of Hyperglycemia in the study population. In males (N408) there were 303 normoglycemic (74.3%), 44 pre-DM (10.8%), 19 screen-detected DM (4.7%), and 42 known DM (10.3%) subjects. In females (N1234) there were 790 normoglycemic (64.0%), 195 pre-DM (15.8%), 82 screen-detected DM (6.6%), and 167 known DM (13.5%), *P*=0.0064.

CHAPTER FOUR
DISCUSSION

The aim of this study was to investigate the prevalence of MODY, specifically HNF1A-MODY (MODY3), in a population from the Western Cape, South Africa, where high prevalence of diabetes (28.2%) and high significance of cardiovascular disease (CVD) risk have been reported (Erasmus *et al.,* 2012; Matsha *et al.,* 2012). Our results revealed that 12 participants (10 females and 2 males) out of 1639 (0, 73%) expressed HNF1A gene polymorphisms.

MODY is a monogenic form of diabetes caused by single-gene defects (Shields *et al.,* 2010). Up to date 14 different MODY subtypes have been identified, and their incidence varies greatly due to differing recruitment data strategies and ethnic variability (Firdous *et al.,* 2018: Kim, 2015). MODY is considered a rare form of diabetes, with both GCK and HNF1A genes accounting for up to 85% of cases in Europe. Currently, the prevalence estimates of MODY subtypes generally vary between 1 and 5%. In many instances, patients with MODY are commonly misdiagnosed with other forms of diabetes (Shields *et al.,* 2010). Correct diagnosis of MODY requires genetic analysis, with up to 100% sensitivity (Kim, 2015). However, genetic testing is expensive. Diagnosing patients with MODY is very challenging due to the high cost of genetic testing, low awareness amongst clinicians and the general population world-wide, as well as the fact that several MODY clinical features overlap with other forms of diabetes (Shields *et al.,* 2010; Mughal *et al.,* 2013). Genetic testing is crucial for prediction, prognosis, progression, and treatment of the disease; it also presents high sensitivity and accuracy. Therefore, several clinical criteria have been developed in order to select appropriate candidates for genetic testing. The clinical diagnosis of MODY includes autosomal dominant inheritance, insulin-dependence, and onset below the age of 25 years (Pihoker *et al.,* 2013).

MODY 3 is caused by heterozygous mutations in the HNF1A gene, characterized by a transcription factor essential for β-cell differentiation and function, and therefore affects cases with progressive β-cell defects (Nyunt *et al.,* 2009). Shepherd *et al* (2001) and Shields *et al* (2010) reported that the variant alleles that cause HNF1A-MODY manifest high penetrance, with 63% of variant carriers developing diabetes before the age of 25 years, and 96% before the age of 55 years. HNF1A-MODY is considered a severe form of diabetes, due to the high occurrence of diabetic complications. Most MODY cases (63%) in the UK have been reportedly caused by mutations of the HNF1A gene (Frayling *et al*., 2001). Studies have shown that HNF1A-MODY is most prevalent to European countries (Lehto *et al.,* 1999, Lindner *et al.,* 1999; Bjørkhaug *et al.,* 2003).

In the present study, we investigated the prevalence of HNF1A-MODY (MODY 3) using molecular genetic methods and all positive subjects were confirmed by sequencing. MODY 3 is more prevalent in countries where blood glucose tests are frequently performed (Thanabalasingham and Owen, 2011[a]; Schober *et al.*, 2009), (Table 1.4). We analysed four HNF1A gene SNPs (rs115080759, rs140491072, rs137853245, and rs142318174) located on chromosome 12q24.31 in a total of 1642 individuals aged ≥ 20 years (1093 normal glucose tolerance, 239 pre-diabetes, 101 screen-detected and 209 known-diabetes (Figure 3.1). However, in our study no polymorphisms were detected in three SNPs (rs140491072, rs137853245, and rs142318174) in all subjects, these SNPs were therefore excluded from further analysis. Only one SNP (rs115080759) followed the Hardy-Weinberg Equilibrium (*P*=0.8818), this SNP was then used for further statistical analysis. From the subjects identified with the disease-causing allele variant of HNF1A, ten were females (0.61%) and two were males (0.12%). This might be due to that there were more females (N1234) than males (N408) participants in the study.

To our knowledge, this is the first study to report on the prevalence of HNF1A-MODY in South Africa/Africa. Our aim was to determine relevant information on MODY, a rare form of diabetes, and to enhance awareness about this type of diabetes in the South African population, specifically a population with a reportedly high incidence of diabetes. We identified 12 subjects with HNF1A gene polymorphisms in one SNP (rs115080759). The allele frequency of these SNPs has been previously reported by The Exome Aggregation Consortium (ExAC) and the Genome Aggregation Database (gnomAD) (Table 1.3). Seven subjects identified with SNP rs115080759 presented as normoglycemic, one with pre-diabetes, and four with known diabetes. We found no significant difference across the rs115080759 genotypes (T/T and G/T) in the anthropometric measurements (BMI, WaistC, and HipC). We also observed increased glycemic measurements (FBG (mmol/L), HbA1c (%), and Glucose/Insulin ratio) across rs115080759 genotypes (*P*=0.0061, *P*=0.0116, and *P*=0.0284, respectively). No difference was observed in other glycemic measurements across rs115080759 genotypes. Mughal *et al* (2013) reported that to date hsCRP is one of the useful biomarker that discriminate HNF1A-MODY from other types of diabetes. Surprisingly, in our study we did not observe any difference in CRP (mg/L) levels across rs115080759 genotypes (*P*=0.5902).

Our results contrast with a study conducted in Croatia which found a higher prevalence of HNF1A-MODY (1.7%) (Pavic *et al.*, 2018). We believe that this may be due to differing data recruitment and ethnicity. Pavic *et al* (2018) investigated HNF1A-MODY in 477 young adults diagnosed with diabetes before the age of 45 years. They identified 13 subjects with 9 different, rare HNF1A allelic variants. From these, 8 subjects were confirmed with HNF1A-

MODY. Subjects with HNF1A allelic variants were predominantly females (*P*=0.02), this agrees with our study. Pavic *et al* (2018) found no difference in age between subjects with HNF1A-MODY and those without. Those with HNF1A allelic variants had lower BMI values compared to the ones without HNF1A- allelic variants (*P*=0.003). However, results indicate increased HDL-cholesterol (1.4 vs. 1.2 mmol/L, *P*=0.024) and decreased median triglyceride levels (1.1 vs. 1.6 mmol/L, *P*=0.015) in subjects with HNF1A variants compared to those without.

A study conducted in Norway reported a lower prevalence of HNF1A-MODY compared to our results. This can also be due to recruitment strategy and ethnicity. This study investigated 1972 diabetic HUNT2 subjects; based on clinical criteria (family history, disease onset, and anti-glutamic acid decarboxylase autoantibody status), a subgroup of 43 subjects (2.2%) suspected to have MODY was identified (Eide *et al.*, 2008). Out of 1850 diabetic HUNT subjects, eight subjects with HNF1A-MODY was identified. The minimum prevalence of HNF1A-MODY was therefore 0.4%. However, study population characteristics were not reported (Eide *et al.,* 2008).

Although these studies differ in recruitment strategies and investigate different polymorphisms or mutations of HNF1A-MODY, the results from our study are comparable. In our study we did not follow the clinical criteria of HNF1A-MODY, which includes family history of diabetes, onset diagnosed before the age of 25 years, and negative autoantibodies. We hypothesise that a percentage of adults with diabetes or prediabetes residing in Bellville South, Western Cape, could suffer from undiagnosed MODY. This may be due to the lack of awareness regarding MODY. Further, it is very likely that diabetic patients are actually living with undiagnosed MODY. A recent study in a French monogenic diabetes group reported an HNF1A prevalence of 39% in their cohort. Subjects identified with MODY 3 (40%) were diagnosed after the age of 25 years, and only half of these subjects exhibited three generations of familial diabetes (Bellanné-Chantelot *et al.,* 2011). Similarly, a study reporting on an Irish MODY cohort described a 40% prevalence, with the subjects diagnosed with MODY after 25 years of age (Kyithar *et al.,* 2011). This indicates that many cases of MODY 3 are being overlooked due to strict clinical criteria. Studies showed that age of diagnosis and parental diabetes are poor discriminators of HNF1A-MODY (Lambert *et al.,* 2003; Owen *et al.*, 2002). Family history of diabetes can often be overlooked or unknown, leading to misdiagnosis with other forms of diabetes, ultimately resulting in inappropriate treatment for patients. The family history of diabetes was not included in our study. Interestingly, a study conducted in Norway involving diabetic subjects included three clinical criteria, namely strong family history, disease onset,

and anti-glutamic acid decarboxylase autoantibody status. A total of 1850 individuals participated in the study, and eight were found to test positive for HNF1A-MODY. In our study we included normal subjects (N=1093), unlike the study conducted in Croatia and Norway. Estalella *et al* (2007) reported that patients with HNF1A-MODY manifest the disease at an older age; this was confirmed in our study. Certain subjects (0.4%) identified with HNF1A-MODY in our study were above 45 years of age and yet, were still classified as normal because the disease had not yet manifested.

The advantage of our study is that we included subjects from 20 years of age and above, normal or diabetic, preventing the possibility of excluding any positive cases. All subjects underwent genetic testing regardless of antibody status, family history, or endogenous insulin production. Our findings are very important to the population of the Western Cape, and South Africa as a whole. In this study we found four participants who were previously diagnosed with diabetes, who in fact, tested positive for MODY, this indicates a lack of awareness regarding this type of diabetes in the general population. This study will bring awareness to clinicians and health care professionals, as well as the population of the Western Cape.

CHAPTER FIVE
CONCLUSION AND LIMITATIONS

5.1. Conclusion

In conclusion, our study provides evidence that certain individuals in the Western Cape suffer from HNF1A-MODY.We calculated a minimum incidence of 0.73% HNF1A-MODY in a population of the Western Cape. Currently, many individuals with HNF1A-MODY in the Western Cape and South Africa remain undiagnosed, and are therefore, receiving inappropriate treatment, which can further exacerbate any disease complications. We can conclude that identification of individuals with MODY could be helpful in terms of therapeutic decisions and prognosis prediction. The low incidence of HNF1A-MODY in this population could be due to the relatively high incidence of other MODY subtypes. Furthermore, our findings suggest that MODY is misdiagnosed as other type of diabetes in Africa; therefore, our findings support the introduction of diagnostic genetic testing for MODY in South Africa

5.2. Limitations of the study

Although this study presented interesting findings about the incidence of HNF1A-MODY in a population of the Western Cape, South Africa, there are several limitations. MODY is a rare form of diabetes, a larger sample size could lead to the discovery of more individuals with HNF1A-MODY. However, the study showed the incidence of HN1A-MODY in the population. Firstly, in the present study we investigated only four SNPs of HNF1A. Second, we have investigated the incidence HNF1A-MODY only, the literature shows that the incidence of MODY subtypes differs from one country to another and it might be that in this population more people would test positive for GCK or HNF4A MODY. The incidence of other MODY subtypes such as GCK, HNF4A, HNF1B, and others remains unknown in a population of the Western Cape. Third, biomarkers with the potential to determine MODY, such as c-peptides and autoantibodies, could not be determined due to financial constraints.

5.3. Recommendation for future studies

Although this study could not report on the incidence of all MODY subtypes, our results provide the first report of MODY prevalence in a population from the Western Cape. Future studies could include an investigation into the incidence of this type of MODY using larger sample sizes. Further, it would be useful to follow up on families of participants who tested positive for HNF1A-MODY and conduct genetic tests on these relatives. Future work should include additional HNF1A-MODY variants using an increased number of SNPs and other

subtypes of MODY, not only in a population of the Western Cape, but South Africa as a whole. It would be interesting to determine the prevalence or incidence of all MODY subtypes in South Africa. This is necessary for increasing awareness and enhancing service provision. The identification of individuals with MODY, and those who are at risk of developing it, could facilitate strategies to delay or prevent disease progression.

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APPENDICES

APPENDIX A: PARTICIPANT INFORMATION AND CONSENT FORM

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVINGGENETIC STUDIES

TITLE OF RESEARCH PROJECT: PROGRESSIVE RESEARCH ON RISK FACTORS OF TYPE 2 DIABETES AND CARDIOVASCULAR DISEASES IN SOUTH AFRICA

REFERENCE NUMBER:

PRINCIPAL INVESTIGATORS:	**Professor Tandi Matsha (Cape Peninsula University of Technology)** **Professor Rajiv Erasmus (Stellenbosch University)** **Professor Andre Kengne (SA Medical Research Council)**
Project manager:	**Dr Gloudina Maria Hon (Cape Peninsula University of Technology)**
ADDRESS:	**Obesity and chronic diseases of lifestyle** **Department of Biomedical Sciences** **Faculty of Health & Wellness Sciences** **Cape Peninsula University of Technology,**
Bellville CONTACT NUMBER:	**Prof T Matsha 021 959 6366 or email: matshat@cput.ac.za**
Ethics approval:	**Cape Peninsula University of Technology** **Ethics Reference number: CPUT/SW-REC**

2015/H01 University of Stellenbosch

Ethics Reference number: N14/01/003

We would like to invite you to participate in a research study that involves genetic analysis and possible long-term storage of blood or tissue specimens. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is ***entirely voluntary and*** you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. ***You are also free to withdraw from the study at any point, even if you do agree to take part initially.***

This research study has been approved by the ethics **Faculty of Health & Wellness Sciences of the Cape Peninsula University of Technology** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki, and the SA Department of Health's 2004 Guidelines: *Ethics in Health Research: Principles, Structures and Processes.*

1. What is Genetic research?

Genetic material, also called DNA or RNA, is usually obtained from a small blood sample. Occasionally genetic material is obtained from other sources such as saliva or biopsy specimens. (A biopsy is a tiny piece of tissue that is cut out e.g. from the skin or from a lump, to help your doctor make a diagnosis.) Genes are found in every cell in the human body. Our genes determine what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

2. What does this particular research study involve?

This research study seeks to address the increasing problem of diabetes and cardiovascular diseases such as heart attack and stroke amongst the mixed-ancestry or coloured population of South Africa. In this study we shall identify people with diabetes

and those at high risk of diabetes as well as investigate the environmental and genetic risk factors that predispose some individuals to the development of diabetes and cardiovascular diseases. Examples of environmental factors include body weight, diet, and physical activity. Additionally, this project aims to investigate whether oral health is a risk factor for diabetes and cardiovascular diseases. In this study we shall investigate whether some individuals have early cardiovascular diseases by using an ultrasound machine. This project also aims to collect genetic material (blood) to analyze for certain variants and to store excess material for future research. When a large group of patients with similar diseases has been collected, meaningful research into the disease processes may become possible.

3. Why have you been invited to participate?

Our research team has previously conducted a similar research study involving the coloured community and found out that more that 18 out of 100 individuals had diabetes but did not know. We also found that some of the risk factors associated with diabetes in other populations were not necessary the same as those affecting the coloured population of South Africa. You have therefore been invited to take part in this research study to assist in establishing the risk factors for diabetes and cardiovascular diseases affecting the coloured people of South Africa.

4. What procedures will be involved in this research?

A. You will be requested to provide information about your medical history, family history and information on eating, drinking and smoking habits. Completion of the questionnaire will take no longer than 30 minutes.

B. You shall be requested to provide a record of the medication you are currently taking, therefore if you are taking chronic medication, you shall be requested to provide this to the research team to record the medication.

C. Measurement such as weight, height, waist and hip will be done.

D. Fasting Venous Blood (20ml) will be collected thereafter you will be asked to drink a glucose solution (glucose content 75g). After two hours another venous blood (10ml) will be collected. The blood will be used to determine whether you have diabetes or you are at high risk for developing diabetes.

E. The other tests that will be determined from your blood sample are: Cholesterol, triglycerides, creatine levels to assess your kidney function, liver enzymes to assess your liver, and biochemical markers for inflammation.

F. A finger prick blood sample (a drop of blood), to be taken at the same time of the first venous blood sample, may also be required from you. The finger prick blood sample will be used to test for diabetes or the risk of developing diabetes on a point-of-care test instrument. Researchers will compare the finger prick point-of-care diabetes test with that of the send away venous blood laboratory test and would be able to establish whether the point-of-care test provides the same accurate results as that of the laboratory. Point-of-care testing may in the future be used to provide fast and accurate results without the need to send blood away to a laboratory for processing. This may be of benefit to people undergoing testing for diabetes as results would be available within a few minutes.

G. The remainder of the blood sample will be used for genetic and future research studies. The serum and DNA may be stored for several years until the technology for meaningful analysis becomes available. No pharmaceutical agent (medication) will be tested in the study.

H. For oral health, research study personnel will extract wooden toothpick, flocked brush, and mouthwash saliva samples from you to test for the presence of Porphyromonas gingivalis as an indicator for periodontal disease. Flocked brush and wood toothpick sampling will involve inserting devices in the subgingival crevice between the last upper premolar and the first upper molar. The device will sweep down the anterior surface of the first upper molar with the direction of motion away from the gum to minimize any potential discomfort. Mouthwash sampling will involve rinsing with 10 ml sterile saline solution for 20 seconds.

I. Early cardiovascular diseases will be performed by means of an ultrasound machine.

J. The research team will follow up on you on a yearly basis and some of these test may be repeated. The investigators wish to follow you up for your entire life. In the unfortunate event that you are deceased during the study period. The study team will review stats SA data and/or medical records to ascertain whether the cause of death was due to diabetes or cardiovascular diseases. **. *If you do not wish to be followed up on a yearly basis and your Statistics SA and/or medical records not to be accessed in the unfortunate event that you are deceased whilst being a participant in the study, you will have an opportunity to request that it be not accessed when you sign the consent form.***

K. Radio imaging techniques will be done on consenting subjects. These include **(i)** ultra sound to assess whether you have signs of early cardiovascular diseases, **(ii)** computed tomography scan (CT-scan) to accurately assess the fat content that is dangerous for cardiovascular diseases **(iii)** Dual-energy X-ray absorptiometry (DXA) devices will be used to study the morphology of the liver. These radio imaging techniques involve

radiation which can be harmful if one is exposed excessively. For this study a low dose radiation will be used for acquisition of the images thereby minimizing radiation exposure to the participant. ***. If you do not wish to undergo any of these radio imaging techniques, you will have an opportunity to decline when you sign the consent form.***

L. An eye examination will be done to test your eye vision and any other abnormalities inthe eye. For this examination, drops placed in your eyes widen (dilate) your pupils to allow the doctor to better view inside your eyes. The drops may cause your close vision to blur for a short while.

5. Are there any risks involved in genetic research?

A slight bruising might occur after blood has been drawn from the arm but this will heal quickly. After the administration of the glucose solution, you may feel nauseous and dizzy in which case you must notify the medical personnel. A medical nurse or doctor will be present on all occasions. You may also learn that you have diabetes, in which case you will be referred to your health care giver with the results for further treatment and management. If during the study it is discovered that you have changes in your genes that may lead to a serious disease, a genetic counsellor at the expense of the principal investigators will counsel you. Radio imaging techniques such as the CT-scan involves radiation which can be harmful if one is exposed excessively. For this study a low dose radiation will be used for acquisition of the images thereby minimizing radiation exposure to the participant.

6. Are there any benefits to your taking part in this study and will you get told your results?

Your personal results will be made known to you ***only if they indicate*** that you may:

- Have diabetes, thereafter, you will be referred to your local health centre or general practitioner for further investigations and treatment.
- Have a condition or predisposition to developing diabetes that is treatable or avoidable e.g. by a lifestyle modification.
- Need genetic counselling.

- **However, participants with normal results who wish to know their results are free to contact the research team and their results will be given upon written request.**

7. How long will your blood be stored and where will it be stored?

The blood samples may be stored ***indefinitely*** to accommodate new technologies that may develop. In the event that a technology is not available in South Africa to analyse your blood sample, your blood specimen may be sent to another country with the technology either now or at a later date. However, if your specimen is to be sent to another country, permission to do so will be sought from relevant bodies. Your blood specimen will be stored at the Cape Peninsula University of Technology.

8. If your blood is to be stored is there a chance that it will be used for other research?

Your blood will only be used for genetic research that is directly related to Diabetes and cardiovascular diseases. Also if the researchers wish to use your stored blood for ***additional research in this field*** they will be required to apply for permission to do so from the ethics ***Faculty of Health & Wellness Sciences of the Cape Peninsula University of Technology. If you do not wish your blood specimen to be stored after this research study is completed, you will have an opportunity to request that it be discarded when you sign the consent form.***

9. **How will your confidentiality be protected?**

Your identity will be recorded once and kept confidential throughout. This is to allow the principal investigators to convey information that may be beneficial to you. Access will be limited to the principal investigators by assigning a special study code to all your data and blood samples. This means that your sample will be identified with a special study code that will remain linked to your name and contact details. However, during the entire research study, your blood specimens will be anonymised and the research staff won't be able to associate it with your name and contact details. You shall also be supplied this code so that if at any time the investigators need to contact you, you may only identify yourself using your special code. Any scientific publications, lectures or reports resulting from the study will not identify you.

Some insurance companies may mistakenly assume that taking part in research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies.

10. Will you or the researchers benefit financially from this research?

You will not be paid to take part in this study ***although your out-of-pocket expenses may be reimbursed.*** The expenses that will be covered by the research team are those that include transportation to a hospital radiography department should you consent to radio imaging.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties, but profits will be reinvested into supporting the cause of further research which may bring benefits to you or your family and to the community, such as health screening, medical treatment, educational promotions, etc.

11. Is there anything else you should know or do?

You should inform your family practitioner or usual doctor that you are taking part in a research study. You can contact

Prof T Matsha at 021 959 6366 or matshat@cput.ac.za,

If you have any further queries or encounter any problems, you can also contact the Cape Peninsula University of Technology Health and Wellness Sciences Research Ethics Committee,

Chairperson: Prof Engel-hills at 0219596570 or EngelhillsP@cput.ac.za or

You will receive a copy of this information and consent form for your own records if it is requested.

12. Declaration by participant

By signing below, I...agree to take part in a research project that includes genetic research study entitled *(***PROGRESSIVE RESEARCH ON RISK FACTORS OF TYPE 2 DIABETES AND CARDIOVASCULAR DISEASES IN SOUTH AFRICA***).*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.

- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.
- I have received a signed duplicate copy of this consent form for my records.

13. Tick the option you choose:

☐ I agree that my blood or tissue sample can be stored ***indefinitely*** after the project is completed but that it is anonymized with all possible links to my identity removed, and that the researchers may then use it for additional research in this or a related field. Once my sample is anonymized, my rights to that sample are waivered. My sample may be shipped to another laboratory in SA or abroad to be used in other research projects in this or a related field

OR

☐ I agree that my blood or tissue sample can be stored ***indefinitely,*** but I can choose
to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out.

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

14. Tick the option you choose:

☐ I ***consent*** that the research team may follow me up for yearly check-up ***AND*** in the unfortunate event that I am deceased whilst still part of the study, I ***consent*** that the team may access Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

OR

☐ I ***do not consent*** to follow me up for yearly check-up ***BUT*** in the unfortunate event that I am deceased whilst still part of the study, I ***consent*** that the team may access Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

OR

☐ I **do not consent** to follow me up for yearly check-up ***AND*** in the unfortunate event that I am deceased whilst still part of the study, I ***do not consent*** that the team accessing Statistics SA and/or my medical records to ascertain whether the cause of my death was due to diabetes or cardiovascular diseases.

15. Tick the option you choose: Radio Imaging

☐ I ***consent*** to ultra sound techniques to assess if I have early cardiovascular diseases

☐ I ***do not consent*** to ultra sound techniques that assess if I have early cardiovascular diseases

AND

☐ I ***consent*** computed tomography scan (CT-scan) to accurately assess the fat content that is dangerous for cardiovascular diseases

☐ I ***do not consent*** to computed tomography scan (CT-scan) that accurately assess the fat content that is dangerous for cardiovascular diseases

AND

☐ I ***consent*** to Dual-energy X-ray absorptiometry (DXA) used to study body composition.

☐ I ***do not consent*** Dual-energy X-ray absorptiometry (DXA) used to study body composition

CPUT ethics reference: CPUT/HW-REC 2015/HO1

Signed at (*place*) .... on (*date*) ....

Finger print

....

....

APPENDIX B: ETHICS APPROVAL

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

12 February 2019
REC Approval Reference No: CPUT/HW-REC 2019/H8

Dear Ms Abigail Mukhetwa Tshivhase

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to Ms Tshivase for ethical clearance on 15 November 2018. This approval is for research activities related to student research in the Department of Biomedical Sciences at this Institution.

TITLE: **The prevalence of Maturity Onset Diabetes of the Young (MODY) in population from the Western Cape.**

Supervisor: Prof T Matsha and Dr S Hector

Comment:

Approval will not extend beyond 13 February 2020. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Dr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences