Master Thesis

Monogenetic diabetes mellitus: precision diagnosis in clinical practice

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 6. Juni 2023 Gerlies Treiber m.p.

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Zusammenfassung

Einführung: Monogenetischer Diabetes mellitus umfasst erblich bedingte Diabetesformen, wie den neonatalen Diabetes, MODY (maturity onset diabetes of the young) und verschiedene syndromale Diabetesformen. Am bekanntesten ist der MODY-Diabetes, welcher ungefähr in 0,5-5% aller Patienten mit nicht-autoimmun bedingtem Diabetes vorliegt. Monogenetische Diabetes-formen sind meistens unterdiagnostiziert, vor allem durch mangelndes Wissen und fehlende genetische Abklärung. Eine korrekte Diagnosestellung ist für eine entsprechende personalisierte Behandlung notwendig.

Methode: Das Ziel dieser retrospektiven Untersuchung war es, den diagnostischen Ablauf in einer tertiären Diabetesambulanz für Erwachsene in der Abklärung für monogenetischem Diabetes zu evaluieren. Weiters wurde eine Literatursuche durchgeführt, für eine aktuelle Übersicht der verschiedenen Subtypen des monogenetischen Diabetes.

Ergebnis: Eine genetische Abklärung erfolgte bei 105 Personen (Alter: 37 (29-48) Jahre) und bei 35 Patient:innen wurde eine Variante in einem diabetes-assoziierten Gen gefunden. Am häufigsten betroffen waren *GCK* (49%), *HNF1A* (17%) und *HNF1B* (14%). Die genetischen Methoden haben sich weiterentwickelt von Einzelgenanalysen zu Multigenpanelanalysen und Whole Exome Sequencing, mit dem Potential auch sehr seltene monogenetische Diabetesformen zu finden.

Schlußfolgerung: Für die Diagnosestellung von monogenetischen Diabetesformen ist eine umfassende Anamnese zu den Patient:innen und dessen Familie, sowie Bestimmung von C-Peptid und Autoantikörper für Typ 1 Diabetes notwendig. Die Reevaluierung einer bestehenden Diabetesdiagnose sowie retrospektive Erhebung von weiteren klinischen Merkmalen ist hilfreich, um zu einer korrekten Diagnose für die Patient:innen und ihren Familien zu kommen.

Abstract

Introduction: Monogenic diabetes encompasses a spectrum of clinical disorders characterized by the onset of diabetes at early age, which include subtypes like neonatal diabetes, maturity onset diabetes of the young (MODY), and diverse syndromes associated with diabetes. MODY represents the predominant manifestation of monogenic diabetes, with estimated prevalence ranging from 0.5% to 5% among individuals diagnosed with non-autoimmune diabetes. However, MODY is currently underdiagnosed within the affected population due to lack of clinical recognition and to insufficient genetic testing. A correct molecular diagnosis is crucial for personalized treatment of these patients.

Methods: The aim of this retrospective study was, to evaluate the diagnostic approach used in the diagnosis of monogenetic diabetes in an adult diabetes outpatient clinic. Furthermore, a comprehensive literature search was conducted for an updated overview of several subtypes of monogenic diabetes.

Results: Genetic testing was performed in 105 adult patients (age: 37 (29-48)) and variants in diabetes-associated genes were identified in 35 patients (33%). *GCK* (49%), *HNF1A* (17%) and *HNF1B* (14%) accounted for most of the cases. Over the last decade genetic testing methods switched from single gene testing to multi-gene panel analysis and whole exome sequencing, with the potential to discover also rarer forms of monogenic diabetes.

Conclusion:
In the diagnosis of monogenic diabetes clinical assessment of detailed patients and family history, physical features, and biochemical measures, including C-peptide levels and type 1 diabetes antibodies is essential before referral for genetic testing. To provide the correct diagnosis for patients and their family retrospectively screening for presence of additional features and reevaluation of current diabetes form is recommended. The recognition of these subtypes will enhance the efficacy of the physician-based diagnostic approach for monogenic diabetes.

Table of content

Abbreviations

BMI	body mass index
ER	endoplasmic reticulum
GCK	glucokinase
GDM	gestational diabetes mellitus
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter
GRS	genetic risk score
GWAS	genome-wide association study
HbA1c	glycated hemoglobin
HNF1A	hepatocyte nuclear factor 1 homeobox A
HNF1B	hepatocyte nuclear factor 1 homeobox B
HNF4A	hepatocyte nuclear factor 4 alpha
MAF	minor allele frequency
MIDD	maternally inherited diabetes and deafness
MODY	maturity onset diabetes of the young
NDM	neonatal diabetes mellitus
NGS	next generation sequencing
PNDM	permanent neonatal diabetes mellitus
SGLT2	sodium glucose cotransporter 2
SNP	single nucleotide polymorphism
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
TNDM	transient neonatal diabetes mellitus
WES	whole exome sequencing

Genetic glossary

de novo mutation	newly arising mutation is found in an offspring
Deletion	loss of a segment of the genetic material from a chromosome
Exome	around 1-2% of the human genome that are protein-coding exons
Expressivity	variable degree to which individuals harboring pathogenic mutations exhibit all the characteristic features of the disease
Genome	DNA sequence of all genes from all chromosomes
Mendelian disease	monogenic disease inherited in line with the patterns first described by Gregor Mendel (autosomal dominant, autosomal recessive)
Monogenic	alteration of a single gene
Mutation	a variant that changes gene function
NGS	a technology that can sequence multiple small DNA fragments in parallel, allowing rapid sequencing of parts or complete exome or genome.
Penetrance	proportion of individuals with a pathogenic described mutation who eventually will have symptoms of the disease.
Polygenic	multiple genetic loci have an effect on a trait
Sanger sequencing	DNA sequencing technique introduced by Fred Sanger
Variant	alteration from the DNA sequence of the human reference genome

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1 INTRODUCTION

1.1 Diabetes mellitus

Diabetes mellitus is a genetic and phenotypic heterogeneous group of metabolic diseases characterized by elevated blood glucose levels. Diabetes mellitus or diabetes include couple of categories, namely type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes (GDM), and diabetes due to other causation (monogenic diabetes, pancreatic diseases, or medication-induced diabetes) and new subtypes within categories are described (1) (Figure 1.0). Most diabetes cases are related to T2D (90–95%) or T1D (5–10%) (2). In monogenic forms of diabetes, a mutation in a single gene is causal. This category accounts only for a small number of all diabetes cases and is reported between 1% to 5% in children and young-adult populations (3). In Austria the estimated diabetes prevalence is 5-7% with estimated 368,000 to 515,000 people affected by diabetes (4). Monogenic forms of diabetes could result for as many as 3,680-5,150 patients in Austria.

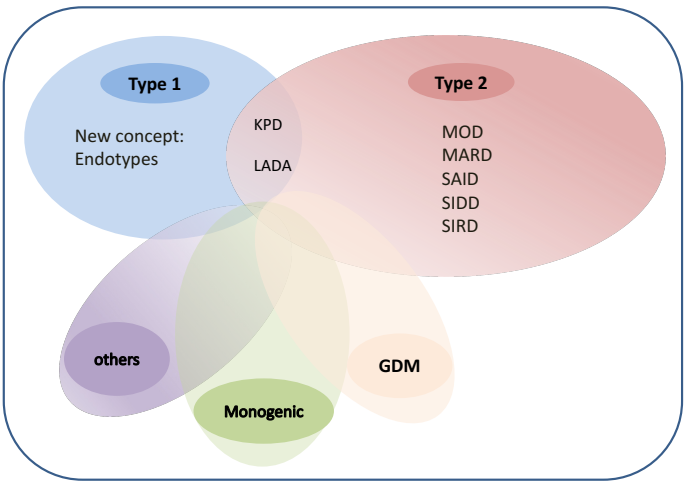

Figure 1.0 Diabetes subtypes.

Overlap exists between categories. Subtypes between T1D and T2D include ketosis-prone diabetes (KPD) and LADA. Further subtypes within T1D (endotypes) and within T2D are proposed. MOD (Mild Obesity related Diabetes), MARD (Mild Age Related Diabetes), SAID (Severe AutoImmune Diabetes), SIDD (Severe Insulin Deficient Diabetes), SIRD (Severe Insulin Resistant Diabetes). Adapted from (1)

1.1.1 Diagnosis of diabetes mellitus

Diabetes can be diagnosed with a fasting plasma glucose (FPG), HbA1c and/or the 2-hour oral glucose tolerance test (OGTT). The OGTT also helps with the detection of prediabetes states like impaired fasting hyperglycemia (IFG) and/or impaired glucose tolerance (IGT). A FPG >126mg/dl, a 2-hours glucose at OGTT or a random

blood glucose of more than 200mg/dl and/or a HbA1c >46 mmol/mol (6,5%) are indicative of diabetes (2).

1.2 Type 1 diabetes

Type 1 diabetes (T1D) is depicted by destruction of the pancreatic insulin-producing beta-cells, mainly because of an auto-immune process, leading to insulin deficiency. T1D is confirmed by testing for decreased or inadequate levels of C- peptide at diagnosis and the presence of autoimmune markers like auto-antibodies to insulin (IAA), to glutamic acid decarboxylase (GAD), to islet antigen-2 (IA-2A) and to zinc transporter autoantibodies (ZnT8) (5,6). Of note, a beta-cell specific autoimmune process destroys only the beta-cells of the pancreatic islets in the pathogenesis of T1D. T1D progression has been grouped into distinct stages prior to onset of symptoms. Stage 1 is characterized by the detection of two or more islet autoantibodies and still normal blood glucose. Stage 2 is defined by the detection of two or more islet autoantibodies with elevation of blood glucose. Stage 3 is typically the clinical diagnosis of T1D with symptoms of hyperglycemia like thirst, polyuria, weakness or in worst case diabetic ketoacidosis. (7). So fare lifelong replacement of insulin is required in order to regulate blood glucose levels. The development of a cure for T1D can only be successful, when the self-destructive autoimmune process can be prevented and insulin secretion from beta-cells can be restored. The triggers of this specific destructive process of beta-cells in T1D are still unclear, but genetic susceptibility as well as environmental factors both contribute to the development of T1D (8,9). (Figure 1.1) Incidence and prevalence in T1D are increasing worldwide but varies between countries. However, Europe and North America have the largest group of children with T1D. The highest reported incidence in individuals below 20 years is from Finland (10,11). This diabetes type is common in children and adolescents but T1D can develop in any adult age group (12).

1.2.1 Genetic in T1D

T1D is a polygenic disorder with well-known gene polymorphisms associated with disease. Major genetic risk factors include loci in the class II HLA region but more than 60 non-HLA-loci also affect disease susceptibility (13). The well-known major susceptibility loci of the HLA region impact mainly recognition as well as tolerance of T cells to autologous and foreign molecules and certain allele combinations

increase the genetic risk in T1D. Genome-wide association studies (GWAS) reported that HLA genes account for up to 50% of the genetic risk for T1D (14). However, several other loci also regulate specific immune paths and change the susceptibility of beta-cells to inflammatory signals. Insulin (*INS*) gene and *PTPN22 gene,* which encodes a lymphocyte protein tyrosine phosphatase, are other loci with strong effects on T1D risk. Genetic susceptibility might also influence responses to environmental factors or physiological pathways (9,15). One of the largest and most diverse genetic study in T1D with 61,427 participants yielded 78 genome-wide-significant regions. T1D-associated variants enriched particularly in CD4+ effector T cells were further investigated by functional GWAS. Intronic variant rs72928038 in *BACH2* was predicted as a candidate causal T1D variant, which leads to decreased enhancer accessibility and decreased *BACH2* expression in T cells (16).

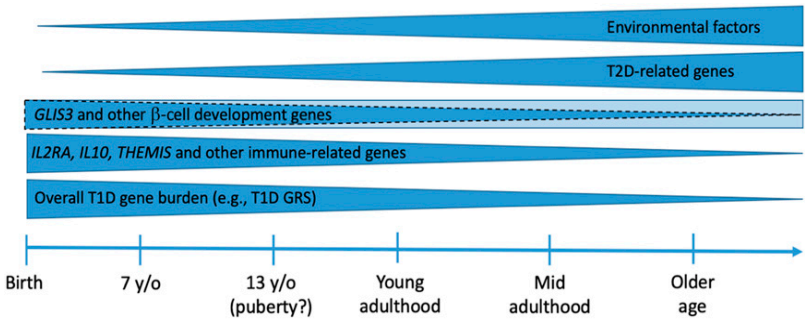

Figure 1.1: Genetic and environmental factors interact in the patho-genesis of T1D. Genes related to immune function are associated with very early-onset T1D. adapted from (17)

Publicly available genetic data for T1D are increasing and decrease in expenses of genotyping helps to use genetic risk scores for type 1 diabetes (T1D GRS) for prediction and classification of T1D (18,19). In T1D genetics consortium dataset Winkler et al (19) generated a genetic risk score based on 2 HLA SNPs and 9 non-HLA SNPs (DR3/4 state in addition *INS, BACH2, IL2RA, PTPN22, ERBB3, IL27*, *ORMDL3, RNLS* and *GLIS3*) This T1D GRS is superior to HLA genotyping alone in the general population and can be used to identify children at birth who have a 10% risk of future T1D (20). Oram et al demonstrated the use of GRS to distinguish between T1D und T2D in young adults (21). Thomas et al showed that a T1D GRS can be used to assess incidence of T1D in older life (12).

Polygenic predisposition can cause clustering of autoimmune endocrinopathies including T1D. For example in Autoimmune Polyendocrine Syndrome type 2 (APS2)

or also known as Schmidt`s syndrome a polygenic clustering of autoimmune endocrinopathies is observed Most affected are middle-aged women, who suffer from autoimmune adrenal insufficiency and either T1D or autoimmune thyroid disease or both (22). T1D can be also part of rare monogenic autoimmune endocrine diseases caused by mutations in genes like "*AIRE, FOXP3, CTLA4, ITCH, IL2RA, LRBA, STAT1*, *STAT3 and STAT5B"*. Further details are described in chapter 1.5.3.3. GRS can also be useful for distinguishing clustering of childhood T1D with autoimmunity from monogenic autoimmune disease. This T1D GRS was decreased in patients with monogenic caused autoimmunity than T1D individuals (23).

1.3 Type 2 diabetes

Type 2 diabetes (T2D) is also a metabolic disease with progression caused by insulin resistance in tissues with insulin receptors such as liver, muscles and adipose tissue, and exhausted beta-cell compensation (24). A normal beta-cell can adapt to decrease in insulin action but in patients with prediabetes and T2D beta-cell function is inadequate for the decrease in insulin sensitivity. This insulin resistance contributes to reduced peripheral uptake of plasma-glucose by muscle cells and adipose tissue and reduced suppression of endogenous glucose production in the liver. Many mechanisms or insulin resistance have been published, like the concepts of glucotoxicity and lipotoxicity with increased free fatty acids, elevated adipokines and activation of inflammatory cytokines as well as mitochondrial dysfunction and amyloid formation leading to beta-cell dysfunction (25). Risk factors for T2D include age, obesity, unhealthy lifestyle with intake of sugar-sweetened soft drinks and highly processed solid food and lack of physical activity, and prior gestational diabetes (GDM) (25,26). Over 90% of diabetes cases are T2D and many cases of T2D could be prevented by eating a healthy diet and exercising daily for at least 30 minutes (27). T2D is the most common diabetes type in adulthood, but is increasing in children and youth (28).

1.3.1 Genetic in T2D

Genetic elements are involved in the pathogenesis of T2D. A positive family history increases the risk for diagnosis of T2D by 2-4 times. (29). T2D is also a polygenic trait and over 560 genetic loci are related with T2D based on data by large genome-

wide association studies (GWAS). These data from GWASs revealed that variants in or close to following genes: "*FTO, TCF7L2, PPARG, KCNJ11, CDKAL1, CDKN2A/2B, HHEX, GCKR, IGF2BP2* and *SLC30A8"* have an association with T2D (30–32). Many of the common described susceptibility loci show small effect sizes and contribute only for a small part of heritability (33). Pancreatic islets have been identified as a key tissue involved in mediating GWAS signals in T2D risk (34,35). Many identified risk variants for T2D are related to islet transcription factors, pancreatic islet enhancers Disruption in islet enhancer activity and dysfunction in the interaction with the epigenome in the islet, may be a relevant element for the genetic susceptibility of T2D (36).

1.4 Gestational diabetes

Gestational diabetes (GDM) is characterized with "glucose intolerance with first onset during pregnancy" and develops if the beta-cell works inadequately to compensate for the insulin resistance during second half of gestation (2). This insulin resistance in pregnancy is caused by the hormone secretion of the placenta and metabolic changes. Corticotropin-releasing hormone, growth hormone, placental lactogen, prolactin, and progesterone have diabetogenic effects but are mandatory to ensure the supply of nutrients for the fetus. Women with GDM have increased risks of gestosis, accelerated intrauterine growth with large for gestational age (LGA) babies, and cesarean birth, and their related morbidities. Later in life women with prior GDM have higher risk of developing T2D, since the mechanism of inadequate insulin compensation of the insulin resistance during pregnancy also underlies the mechanism of metabolic dysfunction in T2D (37,38). GWAS data in GDM show similar candidate genes as for T2D (39). A GWAS for glycemic traits in pregnancy in a multi-ethnic population identified two novel loci (*HKDC1*, *BACE2*) both are related with postprandial glucose levels and C-peptide in fasting state in pregnant women. There is ongoing research to use polygenic risk scores, for risk prediction of GDM and T2D later in life (40). The global prevalence of GDM has been estimated to be 17%, using the diagnostic criteria in pregnancy recommended by the IADPSG (International Association of Diabetes and Pregnancy Study Groups) (41). In Austria all pregnant women undergo screening for hyperglycemia with a 75g OGTT between 24th and 28th week of gestation, with exception of women with preexisting GDM or diabetes.

1.5 Monogenetic diabetes

Monogenic diabetes arises from the heredity transmission of a mutation or spontaneous mutations in a gene associated with diabetes. Monogenic diabetes can be hereditary within families as a dominant, recessive, or non-Mendelian trait or can be a *de novo* mutation in a spontaneous case. To date more than 40 varying subtypes of monogenic diabetes are described with variable phenotype. Most cases of monogenetic diabetes are related to mutations in genes that affects beta-cell function (Figure 1.2). Neonatal diabetes mellitus (NDM) as well as maturity-onset diabetes of the young (MODY) are the more commonly known types of monogenic diabetes. Further subtypes of monogenic diabetes are syndromic diabetes and rare monogenic causes of severe insulin resistance without obesity, and lipodystrophy (3,42).

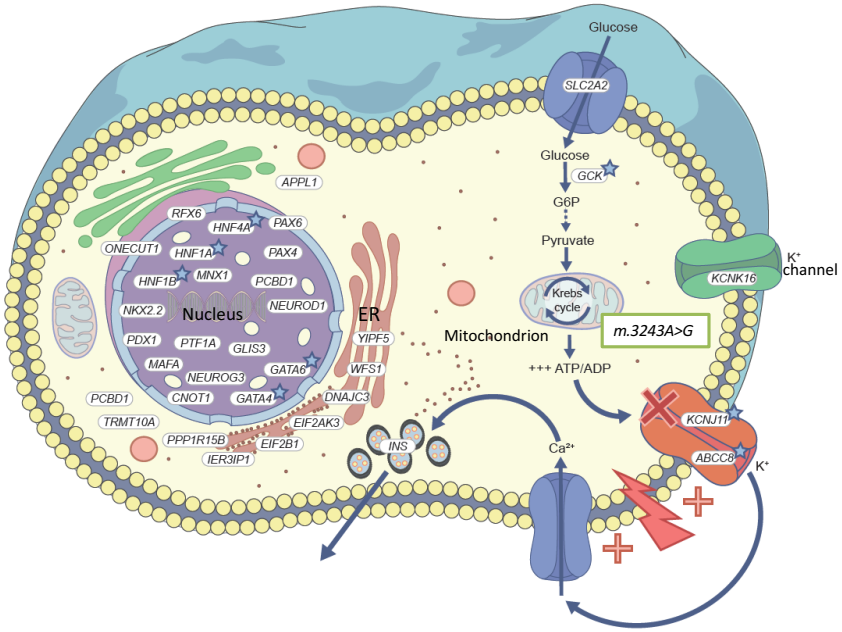

Figure 1.2: Genes linked with monogenic diabetes. Most of these genes encode for proteins that have important functions in beta-cells of the pancreas. Adapted from Bonnefond et al (43)

1.5.1 Neonatal Diabetes

Neonatal Diabetes (NDM) is typically recognised within first 6 to 12 months after birth and can occur in two way as transient (TNDM) or permanent (PNDM), that reflects severe beta-cell dysfunction. Clinical signs or symptoms of NDM are intrauterine growth retardation (IUGR) during gestation and after birth failure to thrive and polyuria leading to dehydration. In addition, neurological abnormalities may be present in some individuals. NDM will affect 1 in 90,000 to 260,000 live born

babies. Most cases of NDM (~2/3) cases are related either to variants in a specific imprinted region of chromosome 6q24 or are related with so called activating mutations in genes of the 2 subunits of the ATP-sensitive potassium (K_{ATP}) channel located in the membrane of the beta-cell. A smaller part of individuals with TNDM is related to variants in genes like *INS* and *HNF1B*. In children of related parents Wolcott-Rallison syndrome or also homozygous mutations in the *GCK* gene are the most frequently cause of NDM (3,44).

1.5.1.1 TNDM from imprinting anomalies on 6q24

The single most common cause of NDM are anomalies at the 6q24 locus, mainly in genes *HYMAI* and *PLAGL1*, which always result in TNDM (45). Normally this 6q24 locus is maternally imprinted, therefore simply the paternal allele is expressed. However, in TNDM these imprinted genes are overexpressed, and to date three specific molecular mechanisms are described: 1) About 50% of cases with sporadic TNDM are caused by paternal uniparental disomy of the chromosome 6. 2) Most of the familial cases of TNDM are related to unbalanced paternal duplication of 6q24. 3) In some sporadic cases of TNDM an abnormal methylation of the maternal allele is described (46). Babies with 6q24 abnormalities are usually born with marked IUGR and develop early on during the first week of life severe non-ketotic hyperglycemia. Despite the initial severe hyperglycemia, reduction of insulin-treatment will be possible and many patients do not require any diabetes therapy after 3 months. However, diabetes relapses in 50% - up to 85% around puberty and early adulthood (3,47).

1.5.1.2 NDM from mutations in K_{ATP} channel genes

The pancreatic K_{ATP} channel is formed by 4 pore-forming Kir6.2 subunits, which are encoded by the gene *KCNJ11* and by 4 regulatory SUR1 subunits, which are encoded by the gene *ABCC8*. Activating (gain-of function) mutations in *ABCC8* or *KCNJ11* are the main reason for PNDM and the second main cause of TNDM and subsequently prevent the closure of these K_{ATP} channels with failure to secret insulin in response to rising glucose (Figure 1.3) (48). *ABCC8* mutations cause TNDM in up to 80% of patient-cases and PNDM in 20%; the inverse pattern is described with *KCNJ11* mutations, which up to 90% are related with PNDM (49). Babies with K_{ATP} channel mutations present with milder IUGR and diabetes manifestation is slightly

later than in patients with 6q24 abnormalities. In KATP-TNDM children, diabetes remission is in general later and diabetes relapse in younger age than 6q24-TNDM. Children with KATP-NDM presents with very low C-peptide and diabetic ketoacidosis is mostly present at time of diagnosis (3). Of note, in about 20% of patients with mutations in *KCNJ11* present with neurological symptoms. Cases with severe damaging mutations in *KCNJ11* are related to DEND syndrome, including neurodevelopment delay and early-onset epilepsy and NDM (50). Most mutant channels can be closed by sulfonylurea drugs, such as glibenclamide (glyburide). Up to 90% of patients can be switched from insulin injections to oral diabetes therapy. Sulfonylurea are efficient for glycemic control and can mitigate some of the neurological features (3,51).

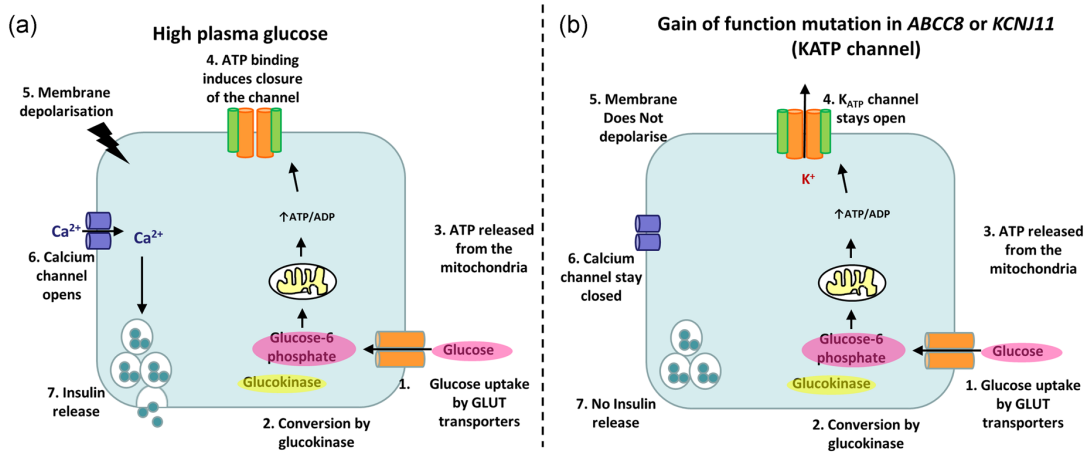

Figure 1.3: Insulin secretion in normal beta-cell and KATP-NDM;
a: Glucose-stimulated insulin secretion in normal beta-cell;
b: Beta-cell with defective insulin secretion due to activating KATP channel mutation; Adapted from DeFranco et al (48).

1.5.1.3 NDM from mutations in insulin gene

The second leading cause of PNDM next to mutations in K_{ATP} channel genes are related to heterozygous coding mutations in preproinsulin gene (*INS*). These mutations mostly result in a misfolded structure of the proinsulin that accumulate within endoplasmic reticulum (ER), followed by ER-stress and destruction of the beta-cell (52). Babies with heterozygous *INS* mutations have similar IUGR but diabetes manifests slightly later. Most of these mutation in *INS* are sporadic *de novo* mutations but approximately one-fifth of infants are related with a positive family history of NDM with dominant inheritance (53). In addition, ten specific recessive

INS mutations are described that cause NDM by decreased insulin biosynthesis. Babies with recessive *INS* mutations have an earlier diabetes diagnosis (median, 7 days vs 2,5 months). Also, the birth weight was lower than in babies with dominant heterozygous *INS* mutations. Two distinct disease mechanisms are involved in pathogenesis (54).

1.5.1.4 NDM due to *GCK* mutations

A total deficiency of the enzyme glucokinase due to mutations in both alleles of *GCK*, either homozygous or as compound heterozygous leads to PNDM (2-3% of all PNDM), due to lack of insulin secretion in response to hyperglycemia. The enzyme glucokinase is mandatory in the glucose metabolism in order to generate glucose-6-phosphate from glucose and works like a glucose sensor in the regulation of insulin secretion. Babies are born with severe IUGR, diabetes is diagnosed during the first week after birth and lifelong insulin therapy is required (55).

1.5.1.5 Wolcott-Rallison syndrome

This rare autosomal recessive syndrome is related to biallelic mutations in *EIF2AK3*, which leads to neonatal diabetes, spondyloepiphyseal dysplasia, and dysfunction in liver and/or kidneys. *EIF2AK3* encodes an important protein for regulation of ER stress response. Misfolded protein accumulates within ER and induce beta-cell apoptosis. Diabetes usually presents during infancy but also can present later in childhood (56). Wolcott-Rallison syndrome needs to be considered in offspring with NDM of related parents (57).

1.5.1.6 Further causes of NDM

To date more than 30 genetic forms of NDM are published. In table 1.0 and 1.1 clinical characteristics of the more known mutations of neonatal as well as early-onset diabetes are summarized (3). Patients with KATP-NDM and some patients with *SLC19A2* mutations will have good glucose control with oral sulfonylureas, all other causes require insulin therapy (3). Among babies with early onset diabetes and features of immune deficiency and/or life-threating infections, mutations in *FOXP3, LRBA and STAT3* need to be considered (22,58). More details on monogenic autoimmune diabetes are provided in chapter 1.5.3.3.

Table 1.0: Monogenic subtypes of neonatal diabetes, part 1 adapted by (3).

Gene	Locus	Heredity	Phenotype
Aberrant development of pancreas:			
CNOT1	16q21	spontaneous	PNDM +pancreatic agenesis + CNS abnormalities
GATA4	8p23.1	AD	PNDM +pancreatic agenesis+congenital heart defects
GATA6	18q11.1-q11.2	AD	PNDM +pancreatic agenesis+congenital heart defects and biliary abnormalities
GLIS3	9p24.3-p23	AR	PNDM + congenital hypothyroidism + glaucoma + hepatic fibrosis and renal cysts
HNF1B	17q21.3	AD	TNDM +pancreatic hypoplasia and renal cysts
MNX1	7q36,3	AR	PNDM +developmental delay+sacral agenesis and anus abnormalities
NEUROD1	2q32	AR	PNDM +cerebellar hypoplasia+deafness and visual impairment
NEUROG3	10q21.3	AR	PNDM +malabsorptive diarrhea
NKX2-2	20p11.22	AR	PNDM +developmental delay + hypotonia + constipation + short stature and deafness
ONECUT1	15q21.3	AR	PNDM + pancreatic hypoplasia and gall bladder hypoplasia
PAX6	11.p13	AR	PNDM + brain malformations and microphthalmia
PDX1	13q12.1	AR	PNDM + pancreatic agenesis
PLAGL1/HYMAI	6q24	Variable (imprinting)	TNDM +/- macroglossia +/- umbilical hernia
PTF1A	10p12.2	AR	PNDM + pancreatic agenesis+ central respiratory dysfunction and cerebellar hypoplasia/aplasia
PTF1A enhancer	10p12.2	AR	PNDM + pancreatic agenesis without CNS features
RFX6	6q22.1	AR	Mitchell-Riley syndrome: PNDM+intestinal atresia+gallblader agenesis
ZFP57	6p22.1	AR	TNDM (hypomethylation syndrome) +/- congenital heart disease +/- macroglossia +/- developmental delay +/- umbilical defects
Aberrant beta-cell function:			
ABCC8	11p15.1	spontaneous, AR or AD	TNDM/PNDM +/- DEND
GCK	7p15-p13	AR	Only PNDM
INS	11p15.1	AR	Only PNDM or TNDM
KCNJ11	11p15.1	spontaneous or AD	PNDM/TNDM +/- DEND
KCNMA1	10q22.3	spontaneous	PNDM (some cases) + developmental delay + intestinal malformations + cardiac malformations + dysmorphic features and bone dysplasia
SLC19A2	1q23.3	AR	Roger´s syndrome: PNDM + sensorineural deafness + thiamine-responsive megaloblastic anemia
SLC2A2 (GLUT2)	3q26.1-q26.3	AR	Fanconi-Bickel syndrome: PNDM + hypergalactosemia, liver dysfunction

Table 1.1 Monogenic subtypes of neonatal diabetes, part 2

Gene	Locus	Heredity	Phenotype
Reduction/apoptosis of beta-cells:			
EIF2AK3	2p11.2	AR	Wolcott-Rallison syndrome: PNDM + skeletal dysplasia + short statue and acute liver failure
EIF2B1	12q24.31	spontaneous	PNDM + recurrent dysfunction liver
IER3IP1	18q21.2	AR	PNDM + microcephaly +epileptic encephalopahty and lissencephaly
INS	11p15.5	spontaneous or AD	Only PNDM
WFS 1	4p16.1	AR	Wolfram syndrome: PNDM or early-onset diabetes + optic atrophy +/- deafness +/- diabetes insipidus
WFS 1	4p16.1	AD	PNDM or early onset diabetes+ deafness and congenital cataracts
YIPF5	5q31.3	AR	PNDM + epilepsy and severe microcephaly
Reduction/apoptosis of beta-cells + autoimmunity:			
CTLA4	2q33.2	spontaneous	Lymphoproliferative syndrome + enteropathy + cytopenias + thyroiditis and diabetes
FOXP3	Xp11.23-p13.3	XR	IPEX syndrome (autoimmune enteropathy, eczema, autoimmune hypothyroidism, elevated IgE) and diabetes
ITCH	20q11.22	AR	PNDM + multi-system autoimmunity+ facial dysmorphism
IL2RA	10p15.1	AR	Lymphoproliferation + multi-system autoimmunity and diabetes
STAT3	17q21.2	spontaneous	PNDM + enteropathy + other autoimmunity (cytopenias)

1.5.2 Autosomal dominant familial mild hyperglycemia or diabetes (MODY)

MODY are also a subtype of monogenic diabetes with impairment of insulin secretion, having one or more variations in a single gene. This type of monogenic diabetes usually will have autosomal dominant heredity but de novo mutations have been published. Based on the Online Mendelian Inheritance in Man (OMIM) database, MODY is currently categorized into 14 subtypes each caused by mutations in different genes (Figure 1.4). However, the term MODY is a confusing term for these forms of early onset diabetes, in Table 1.2 highly penetrant genes that causes monogenic diabetes are described (3).

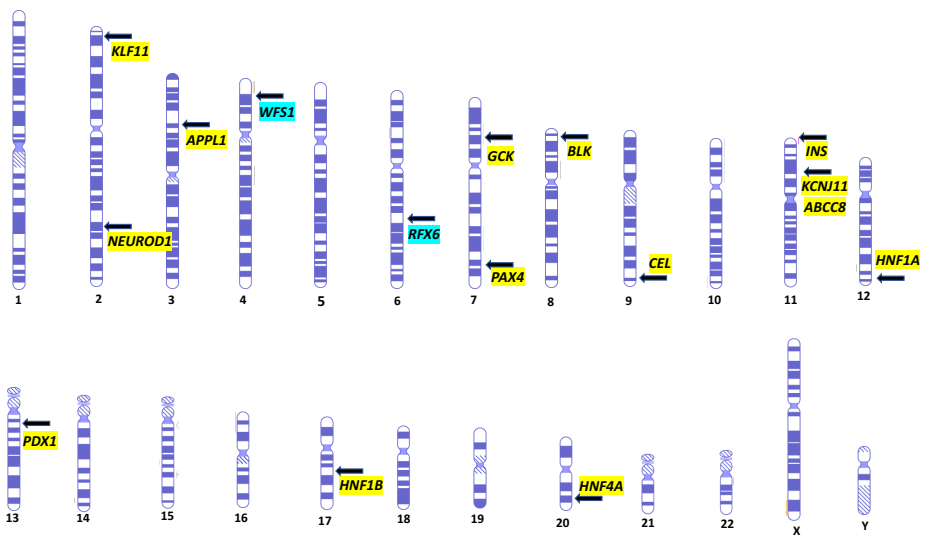

Figure 1.4: Karyogramm of common and rarer MODY forms and additional MODY-like mutations in *RFX6* and *WFS1* gene. Adapted from (95).

The prevalence for MODY is estimated 1 per 23,000 in children and 1 per 10,000 in adults based on European populations (59). The distinguished MODY subtypes have partly different age of disease onset, dynamics of blood glucose level and therapy response. Management of MODY is based on the subtype and entails oral antidiabetic drugs or insulin therapy and nutrition recommendations. The majority of cases with the so called MODY (80%) are based on mutations in following genes: *GCK, HNF1A, HNF4A* und *HNF1B*.

1.5.2.1 Well-established subtypes of MODY

1.5.2.1.1 *HNF1A*-(MODY3)

Heterozygous mutations in *HNF1A* gene can lead to progressive dysfunctional beta-cells with diminished glucose-depended secretion of insulin and early onset of glycosuria. *HNF1A* is an important transcription factor expressed in tissue of liver, pancreas and renal cells and intestine, and its function is the regulation of the gene-expression for insulin, glucose transporters (GLUT) 1/2 as well as sodium/glucose cotransporter 2 (SGLT2). Pathogenic variants of *HNF1A* have a negative effect on protein expression, which are important for glucose transport and affect enzymes related glucose metabolism in the mitochondria (59). *HNF1A* gene has three functional domains, and 10 exons are transcribed in three isoforms by alternative splicing, with different transcriptional properties and tissue expression pattern. The location of the mutation in *HNF1A* gene has an impact on the age of diabetes diagnosis. Individuals with mutations in the terminal exons 8 - 10 present with

diabetes up to eight years later than people with pathogenic variants in exons 1 - 6 (60). A fetus with maternal inherited mutation will be exposed to maternal diabetes *in utero* ,which will result in an earlier diabetes diagnosis by up to 12 years (61). *HNF1A* mutations show high penetrance for diabetes because 63% of mutation carriers will have diabetes onset before 25 years of age and up to 96% before 55 years (62). The risk for microvascular complications (nephropathy and neuropathy) is similar in comparison with T1D and T2D. But importantly *HNF1A* MODY has an increased risk for cardiovascular disease and retinopathy (63).

HNF1A-MODY is a very common subtype of monogenic diabetes, and this subtype is highly prevalent in populations with European ancestry (>70%) (64). *HNF1A* mutations result in a defect in impaired glucose stimulated insulin secretion, but the K_{ATP} channel act to close with sulfonylureas. Therefore, these patients are sensitive to sulfonylurea treatment (65). Individuals with *HNF1A* mutations specific have a lower threshold for the renal glucose reabsorption and postprandial glucosuria can be present before developing significant elevated blood glucose. The use of SGLT2-inhibitors induced higher glucosuria in patients with *HNF1A* and GLP1R (glucagon-like peptide-1 receptor) agonists are also effective (64). Since *HNF1A* is also expressed in extra-pancreatic tissues, liver adenomatosis occurred up to 7% in *HNF1A*-MODY patients due to biallelic inactivation of *HNF1A* gene (66).

1.5.2.1.2 *GCK* - (MODY 2)

GCK-MODY is related with heterozygous inactivating mutation in the glucokinase gene (*GCK*). Patients with *GCK*-MODY show non-progressive mild hyperglycemia, because secretion of insulin is adequate but increases at a higher set point than with a normal functioning enzyme (67). Therefore the risk for microvascular or macrovascular complications of diabetes is low (68). Most individuals with GCK-MODY won´t need diabetes specific treatment (69) except during pregnancy, when a mother with GCK mutation has an fetus with accelerated *in utero* growth (70). However, the presence of a *GCK* mutation does not exclude the development of T2D, which have similar prevalence than in the general population (71). Of note, population prevalence for GCK-MODY is estimated at 1 in 1,000 (DIP-Cohort) to ~1:2,000 (clinically unselected cohorts from USA and UK) (72). The penetrance of mild hyperglycemia is high between 89-97% for *GCK*-MODY carrier (73).

Table 1.2: Highly penetrant genes cause of monogenic diabetes in children and adults (adapted from (3).

Gene	Heredity	Phenotype
Genetic disorders of glucose metabolism:		
GCK	AD	MODY 2: nonprogressive increased fasting glucose levels
Genetic diseases regarding gene expression		
HNF1A	AD	MODY 3: insulin secretory dysfunction with progression, glucosuria; treatment with sulfonylureas
HNF4A	AD	MODY 1: insulin secretory defect- progressive, macrosomia at birth +/- neonatal temporary hypoglycemia; treatment with sulfonylureas
HNF1B	AD	MODY 5: multisystemic disease: renal cysts, malformations of genitourinary tract, atrophy of pancreas, hypomagnesia. Insulin therapy
PDX1	AD AR	MODY 4: rare, mild diabetes NDM: + SGA, diarrhea, malnutrition due to pancreas agenesis
NEUROD1	AD AR	MODY 6: rare NDM: + deafness and hypoplasia of cerebellum
KLF11	AD	MODY 7: rare – low genetic evidence
PAX4	AD	MODY 9: rare - low genetic evidence
BLK	AD	MODY 11: rare- low genetic evidence
PCBD1	AR	MODY-like: rare, with worsening dysfunction of insulin secretion
RFX6	AD AR	MODY-like: rare NDM+ SGA, intestinal atresias, gall bladder hypoplasia/aplasia
Genetic diseases regarding ion channels		
ABCC8	AD AR/AD	MODY 12: rare, +/- history of TNDM +/- neuropsychological difficulties; sensitive to high dose sulfonylureas NDM
KCNJ11	AD AD	MODY 13: rare, +/- history of TNDM +/- neuropsychological difficulties; sensitive to high dose sulfonylureas NDM
Genetic diseases regarding insulin synthesis		
INS	AD AD/AR	MODY 10: rare NDM + IUGR
Genetic diseases regarding ER stress/cell death:		
WFS1	AD AR	MODY-like: rare Wolfram syndrome
TRMT10A	AR	MODY-like: rare, + growth restricted, microcephaly
Disease of the exocrine pancreas		
CEL	AD	MODY 8: rare; atrophy of pancreas, exocrine pancreatic dysfunction
CFTR	AR	Cystic fibrosis, variable age of diabetes diagnosis
Mitochondrial disorders:		
MT-TL1, MT-TK, MT-TE	Maternally inherited	MIDD: maternally inherited diabetes and deafness
Genetic disease with iron overload of the beta-cells:		
HFE	AR	Hemochromatosis: variable diabetes onset, cardiomegaly, hepatic manifestations, “bronze” skin, arthropathy, hypogonadism

1.5.2.1.3 HNF4A - (MODY 1)

HNF4A is also a transcription factor mainly expressed in liver cells and secondary in beta-cells and kidney. *HNFA4* gene is located on 20q13.12 and composed of 13 exons and contains 2 promotors, which can result in many splice variants. Heterozygous *HNF4A* mutations account for 3-5% of all MODY cases and are characterized by progressive beta-cell dysfunction like *HNF1A*-MODY. *HNF4A* mutations carriers show variable penetrance and usually diabetes onset is before the age of 25 years. Treatment with sulfonylureas is also a characteristic of *HNF4A*-MODY and low dose sulfonylurea therapy in order to avoid hypoglycemia is the first line treatment (74). If the patients are free of hypoglycemia, low-dose sulfonylureas (daily 20-40mg gliclazide) can be sufficient for decades similar to HNF1A MODY (3).

As a result of increased insulin secretion seen in early life, up to 50% of individuals with *HNF4A* mutation have macrosomia at birth and up to 15% will develop neonatal hyperinsulinemic hypoglycemia (75). Hyperinsulinemia usually resolves during infancy and patients develop diabetes in adolescence due to decreased insulin secretion. In addition a mutation-specific phenotype with increased birthweight, neonatal hyperinsulinaemic hypoglycaemia, which may progress to diabetes and “atypical Fanconi Syndrome” and features of nephrocalcinosis is described in carriers of the p.R76W mutation in *HNF4A* (76). Another subtype with different specific phenotype is the p.R114W mutation (found in ∼15% of HNF4A-MODY) These mutation carries have a lower response rate to sulfonylureas but show a reduced penetrance and they have normal birthweight (77). *HNF4A*-MODY is associated with lower levels of HDL- cholesterol, triglycerides and apolipoprotein A1 and A2 but have elevated LDL-cholesterol levels (74).

1.5.2.1.4 HNF1B - (MODY 5)

Pathogenic variants in the gene encoding for the transcription factor HNF1B, which is expressed in cells of the pancreas, kidneys, genital tract, liver and gut, account 2% (78) - 10% (79) of all MODY cases. *HNF1B* gen is composed of 9 exons within the 3 functional domains and the “Intron 2 splice site” appears to be a mutation hotspot (80). In up to 50% of *HNF1B* mutations are deletions of the whole gene (81,82). Individuals with whole-gene deletions exhibit similar phenotype to those individuals with a coding or a splice site mutation, indicating that dysfunction is

because of a gene dosage effect, i.e., haploinsufficiency (82). *HNF1B* - MODY is considered to exhibit autosomal dominant inheritance, but de novo mutations are frequent (one-third to two-thirds of cases) (78,83).

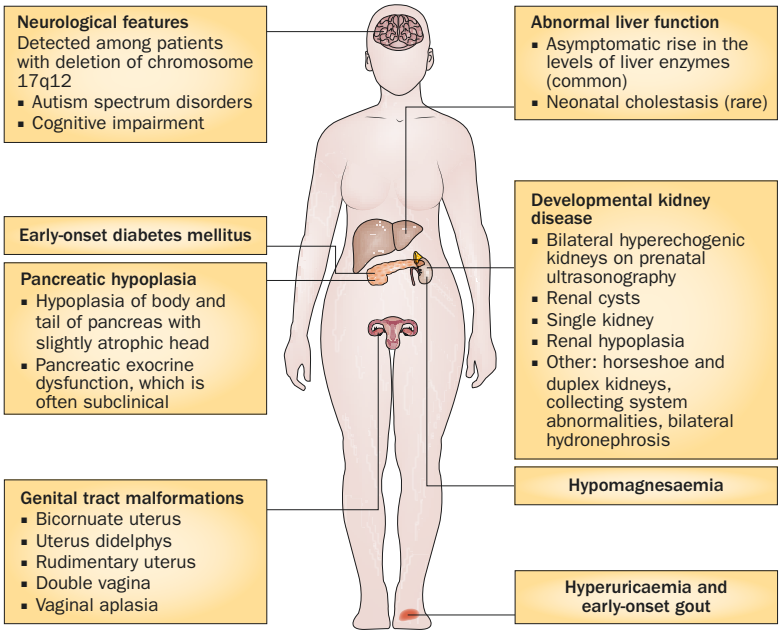

Figure 1.5 Extra-pancreatic phenotypes frequently observed among patients with *HNF1B*-MODY or *HNF1B* associated disease. Adapted from Clissold et al. (78).

HNF1B-associated diseases are a multi-system disorder. Figure 1.5 illustrates the multiple clinical features with extra-pancreatic phenotypes. Patients with heterozygous *HNF1B* mutation can present with early onset diabetes and features that affect the kidney leading to RCAD syndrome (Renal Cysts and Diabetes). Renal cysts, renal tract dysplasia (renal agenesis, horseshoe kidney) and hypoplastic glomerulocystic renal diseases are also related with this mutation. Some reports indicate that up to 50% of these patients will develop end-stage renal failure < 45 years of age (80,84). Renal cysts and anomalies of the pancreas, abnormal fecal elastase, malformations of the genital tract, liver dysfunction, low levels of magnesium, hyperuricemia with early-onset gout are indicative of *HNF1B* mutations (78,85). A fetus with a *HNF1B* mutation (de novo or paternal inherited) can have a reduced birth weight, which indicates decreased insulin secretion by fetal beta-cells in the uterus (86). The phenotype of *HNF1B* mutation carriers is very variable and there is no clear genotype-phenotype correlation (83). Also, the penetrance of diabetes in *HNF1B* carrier is very variable, with mean age of diabetes onset 26 (range von 0-61) years and diabetes develops in about half of *HNF1B* mutation

carriers due to insulin deficiency related pancreatic hypoplasia, altered glucose-sensing mechanisms and hepatic insulin resistance. Usually patients with *HNF1B* related diabetes show low response to sulphonylureas and therefore early insulin therapy is necessary (78,84).

HNF1B deletion is associated with 17q12 deletion syndrome (87). The 17q12 microdeletion syndrome with deletion of a 1.4 Mb area on the long arm of the chromosome 17 can also lead to renal or urinary tract malformations, diabetes and neurodevelopmental or neuropsychiatric features (87,88). All patients with *HNF1B* MODY should be assessed for clinical features of 17q12 deletion syndrome and in addition specific microarray testing of the 1.4-Mb deletion on chromosome 17q12 should be performed, because the most common cause of *HNF1B* MODY is a whole *HNF1B* gene deletion (81,88).

1.5.2.1.5 INS - (MODY 10)

The insulin gene is only a small gene on chromosome 11p15.5 with 1,425 base pairs and consists of 3 exons separated by 2 introns, interesting only exons 2 and 3 are coding for proteins. More than 25 different homozygous or compound heterozygous mutations in the *INS* gene have been reported in NDM and in individuals with diabetes diagnosed outside of early childhood (89). Most insulin gene mutations lead to misfolded proteins and this proteins cause ER stress and lead to beta-cell death (90,91) or in rare cases of deletions to reduced insulin biosynthesis (92). A deep intronic homozygous mutation was described, which result in failure of translation to insulin protein in still viable beta-cells (93). Dominant and gain-of-function mutations in the *INS* gene are the 2nd most common genetic cause for PNDM with variable age of onset of diabetes (94). Heterozygous *INS* missense mutations have been reported in 25 families across the world that co-segregate with MODY (95,96). The mean age of diabetes onset reported was 13.7 years, clinical presentation ranged from mild diabetes to DKA at diagnosis and diabetes complication have been reported. Patients with *INS*-MODY responded to diet only, OHA as well as insulin therapy since diagnosis (95). The mutation c.163C>T (p.R55C) is associated with DKA, requiring early insulin therapy (90).

1.5.2.1.6 *ABCC8* - (MODY 12)

ABCC8 gene is located on chromosome 11p15.1 and encompass 39 exons that encode for all 1,582 amino acids of the SUR1 subunit of the K_{ATP} channel. At the 5′ end of exon 17 there is an alternatively spliced recognition site, which has led to inconsistency with the correct nomenclature of variants in exons 17 to 39 (48). Several mutations of the *ABCC8* gene are reported in individuals with NDM as summarized in chapter 1.5.1.2. Recent published reports have also shown *ABCC8* mutations in patients with later-onset diabetes and are a cause for MODY (97,98). Of note, a genetic analysis of 152 individuals with clinically diagnosed MODY reported that *ABCC8* mutations were the 2nd most common cause in this reported cohort in South India (99). In summary, different gain-of-function mutations in *ABCC8* result in various phenotypes and can be related with dominant, recessive, or compound heterozygous mutations (49). In the same family with an identical mutation time of diabetes onset ranged from baby to adulthood and with mild hyperglycemia to insulin dependent diabetes (100). In addition a publication of diabetes related to a loss-of function mutation in *ABCC8* previously associated with congenital hyperinsulinism highlights the variable clinical expression of beta-cell dysfunction related to *ABCC8* (101). Individuals with diabetes due to mutations in *ABCC8* show good response to therapy with high dose sulfonylureas.

1.5.2.1.7 *KCNJ11* - (MODY 13)

KCNJ11 is also located on chromosome 11p15.1, 4.5Kb away from *ABCC8* and has only one single exon encoding a 390-amino acid protein for the Kir6.2 subunit of the K_{ATP} channel. As already described in chapter 1.5.1.2 heterozygous or homozygous mutations in this gene can cause transient or permanent NDM. However, Yorifuji et al identified already 2005 a heterozygote mutation in *KCNJ11* gene in a 4-generation Japanese family with diabetes mellitus. One individual had childhood-onset diabetes, two had adult-onset of diabetes and one had transient neonatal diabetes (102). In 2012 Bonnefond et al (103) described *KCNJ11* as the 13th MODY gene. Heterozygous mutations in *KCNJ11* have also been associated with various phenotypes of diabetes in a French family with 12 affected individuals. Age at diagnosis varied from adolescent (youngest 13 years) to late adulthood (oldest 59 years), and diabetes treatment varied from diet to oral diabetes medication to insulin

injections (103). Heterozygous *KCNJ11* mutations also were reported in 6 families in China with early onset T2D. Some of these individuals were switched from insulin therapy to sulfonylurea (104).

1.5.2.2 Rare subtypes of MODY with reasonable genetic evidence

1.5.2.2.1 PDX1 - (MODY4)

Pancreatic and duodenal homeobox 1(*PDX1*) prior known as insulin promotor factor 1 (IPF1) encodes a transcriptional activator of couple of gene related to beta-cell function like insulin, glucokinase, GLUT 2 and islet amyloid peptide. This pancreatic transcription factor, like other HNF-related transcription factors regulates the gene expression of insulin and the development of the beta-cell. PDX1 gene consists of two exons with a size of 6kb. Homozygous mutations of *PDX1* result in pancreatic agenesis with NDM and heterozygous loss-of-function mutations can cause *PDX1*-MODY4 (105). To date 14 families are reported with *PDX1*-MODY (95,106).The age of diabetes onset varied between 2 and 35 years and in general these patients showed mild diabetes with obesity reported in two families. Most of the patients are treated with insulin therapy and microvascular complications are rarely reported (95). A recent report of a novel heterozygous frameshift mutation in *PDX1* in family in Japan showed that this *PDX-1* MODY has a reduced GIP (gastric inhibitory polypeptide) secretion and the successful use of DPP-4 inhibitors was described (106).

1.5.2.2.2 NEUROD1 - (MODY 6)

A very rare cause of MODY are heterozygous mutations in *NEUROD1*, which encodes also a transcription factor (Neurogenic Differentiation 1). The *NEUROD1* gene is important for endocrine cell development, transcription of GCK and GLUT2 and is necessary for insulin synthesis and secretion. *NEUROD1* also plays an important role in formation and function of retina, inner ear, cerebellum, and hippocampus (107). To date up to 16 families have been described worldwide with heterozygous mutations in *NEUROD1* causing MODY. This form shows incomplete penetrance of diabetes in Europeans, since 21% of mutation carriers are still glucose tolerant. All the Japanese patients developed overt diabetes before the age of 15 years and diabetic ketoacidosis at diabetes presentation are described. Insulin

therapy is commonly used in these patients, but oral glucose-lowering agents or diet were successfully used in some patients. Cases with severe diabetic microangiopathy with proliferative retinopathy and renal failure are reported (95,107). Three cases with homozygous mutations in *NEUROD1* are described which resulted in PNDM and neurological abnormalities (108).

1.5.2.2.3 *CEL*- (MODY8)

CEL gene encodes for an enzyme “carboxyl ester lipase”, which is secreted from the exocrine pancreas. The *CEL* gene contains 11 exons and in the coding region of exon 11 there is a variable number of tandem repeats (VNTR) (109). So fare damaging mutations of *CEL* are single-bp deletions in the proximal VNTR segments, which cause exocrine and endocrine pancreatic dysfunction (110). Patients with CEL-MODY develop pancreatic exocrine function (low fecal elastase levels) in early childhood and develop diabetes and clinical malabsorption in their fourth decade of life. Pancreatic expression of *CEL* is primarily located in acinar cells. Imaging of the pancreas show pancreatic atrophy as well as increased pancreatic fat content (pancreatic lipomatosis) or pancreatic cysts later in life (111). *CEL*-MODY has been reported in only few families worldwide. Insulin therapy is the treatment of choice among these patients with this rare form of monogenetic diabetes (95).

1.5.2.3 Genes reported as causal for MODY

Genetic evidence is not compelling: KLF11-(MODY7), PAX4-(MODY9) and BLK-(MODY11)

1.5.2.3.1 *KLF11*- (MODY 7)

KLF11 (Kruppel-like factor 11) is found on chromosome 2p25.1 and encodes for an insulin gene regulator, which regulates PDX1 transcription in beta cells. Neve et al. reported 2005 two variants in *KLF11* gene in 3 families in a sequencing study of 190 individuals of families with early-onset T2D. Neither variant was found in 313 patients with late-onset T2D or in 313 normoglycemic individuals. A likely mechanism of action was indicated for variants by gain of function increasing *KLF11* repression activity (112). In the clinical TODAY trial among overweight youth in US another variant in *KLF11* was reported in a 16-year old hispanic male, with an HbA1c value of 6.4% and dyslipidemia (113). However, recent data also indicate that

published variants in *KLF11* have poor cosegregation with diabetes mellitus and are too common in population cohorts to cause MODY (114).

1.5.2.3.2 PAX4 - (MODY9)

PAX4 gene is located at chromosome 7q32.1 and encodes for a homedomain transcription factor with transcription repressor function. This process has a critical role in development of beta-cells and beta-cell function. Two variants in the *PAX4* gene were first reported from Thailand in 2007 from 46 Thai individuals with MODY like diabetes. Neither mutation was found in 344 controls of Thai origin (115). Few more families from Asia with *PAX4*-MODY were reported. The age of onset varied between 6 and 44 years (mean 24.2 years) and was reported to be more common in men. Insulin injections was the treatment modality (95,116). However, no other numerous MODY family with cosegregation for a disease causing variant in *PAX4* has been published since the initial reports.

1.5.2.3.3 BLK - (MODY 11)

The *BLK* gene location in on chromosome 8p23.1 and encodes a B-lymphocyte kinase of the SRC family of proto-oncogenes. This kinase can be found in several tissues including the beta-cells of the pancreas. This protein also enhances the synthesis of insulin, the glucose-dependent insulin secretion and the expression of couple of beta-cell transcriptions factors. Five rare mutations in *BKL* gene were published to segregate with diabetes in MODY families, first described 2009 (117). Obesity is reported among these three US families with the *BLK* mutations. Around 60% of the patients were treated with insulin injections (117). No other large MODY family with cosegregation have been published for *BLK* since this first report. The role of *BLK* in MODY was investigated in two European cohorts and the reassessment demonstrated that the *BLK* variants are very unlikely to cause MODY and they found that this variant is too common for a rare disease in the general population (118).

1.5.2.4 Recent established genes causing MODY

APPL1- (MODY14), *RFX6 - MODY*

1.5.2.4.1 APPL1- (MODY14)

The *APPL1* (adaptor protein, phosphotyrosine interaction, PH domain, and leucine zipper containing 1) gene is found on the chromosome 3p14 and contains 23 exons. *APPL1* is a newly discovered MODY-related gene with the initial report in 2015. *APPL1* encodes for a protein which interacts with adiponectin and has a role in the insulin-signaling pathway. 2 loss-of-function mutations in *APPL1* gene have been found in two families with whole-exome sequencing study in 60 families from US and Italy. Functional studies were supportive that these variants caused loss of function and they were absent from population databases (119). It is yet to be determined if there will be any reports of *APPL1* variants in other MODY pedigrees (79).

1.5.2.4.2 RFX6 - MODY

RFX6 (regulatory factor X6) is located on chromosome 6q22.1, has 20 exons and encodes for the RFX6 transcription factor, which is highly expressed only in pancreas cells. This gene is required for the islet cell differentiation for insulin production and has also a role in the regulation of the involved transcription factors in beta-cell maturation and beta-cell function (120). Homozygous mutations in *RFX6* cause the very rare Mitchell–Riley syndrome, which is an autosomal recessive syndrome with NDM, pancreas anomalies (hypoplastic or annular form), intestinal atresia as well as gall bladder anomalies (121).

RFX6 heterozygous mutations cause a mild MODY like phenotype with reduced penetrance but with normal pancreas development. *RFX6* protein truncating variants were initially identified in UK cases with a clinical diagnosis of MODY and the findings were replicated in a Finnish cohort (7.5% of Finnish cases had *RFX6* variants). 27% developed diabetes by age of 25 years and 78% by age of 51 years and was lower in comparison to *HNF1A/HNF4A* MODY. *RFX6*- MODY patients do not have good sensitivity to sulfonylureas, although they have detectable endogenous insulin 3 to 5 years post diabetes diagnosis (122). In individuals with *RFX6* -MODY*,* hyperglycemia results from impaired insulin secretion of the beta-cells but have a normal development of pancreatic islets (123). Hyperglycemia is also related with lower fasting and stimulated GIP levels. *RFX6*-MODY is also a subtype of diabetes with reported GIP deficiency (122). A case report from Japan

showed an improvement in glycemic control with GLP-1 receptor agonist liraglutide in *RFX6* related MODY (124).

1.5.2.4.3 WSF1

Wolfram gene (*WFS1*) is found on chromosome 4p16.1 and this gene has eight exons, which encodes an ER membrane-embedded protein. *WFS1* mRNA is found in several cell types and is highly expressed in pancreatic beta-cells. WFS1*(or* wolframin*)* protein has important role in ER membrane functions and regulation of ER calcium homeostasis. Dysfunctions induce ER stress responses, including apoptosis of cells (125).

Recessive mutations in *WFS1 gene* are related to commonly known Wolfram syndrome, a rare neurodegenerative disease also described as DIDMOAD (diabetes insipidus, insulin-deficient diabetes mellitus, optic atrophy and deafness) (126). Further description of Wolfram syndrome will follow in the chapter 1.5.3.1. However, reports indicate that heterozygous *WFS1* missense mutation can cause less severe phenotypes than Wolfram syndrome and are related with dominantly inherited young or adult-onset diabetes without other syndromic features. Exome sequencing has identified *WFS 1* in few families with non-autoimmune diabetes, like MODY (127,128). In addition, dominant *WFS1* mutations also have been published as cause for isolated hearing loss, optic atrophy and isolated congenital nuclear cataracts (129). Common variants in *WFS1* are also associated with T2D (130). De Franco et al published 5 patients with a unique congenital syndrome characterized with early diabetes, cataracts, and sensorineural deafness also related to a dominant heterozygous missense mutation in *WFS1*. Further functional studies suggest that major ER stress triggered by dominant *WFS1* mutations leads to premature death of beta-cell resulting in neonatal/infancy onset diabetes (129). These reports highlight the various genetic heterogeneity of *WFS1* and difficult genotype-phenotype correlations.

1.5.3 Diabetes-associated syndromes

1.5.3.1 Wolfram syndrome

Wolfram syndrome can be related to two different genes, most common caused by biallelic mutations in *WFS1* but also by mutations in *CISD2* gene. The diagnosis of Wolfram syndrome 1 (WS1) based on clinical features, requires the combination of: early insulin-dependent but non-autoimmune diabetes (typically within first 10 years of life) and progressive optic atrophy of both eyes before the age of 16. Additional findings include diabetes insipidus, sensorineural hearing loss, renal, renal tract and progressive neurologic abnormalities and psychiatric illness (126). Many patients with Wolfram syndrome are initially misdiagnosed as juvenile T1D. On average 4 years after the onset of diabetes, vision impairment occur and may be also misdiagnosed as diabetic retinopathy (131). More than 90% of patients with WS1 have recessively acting mutations in the *WFS1* gene. Over 200 different mutations are responsible for WS1(132,133).

A second rare and neurodegenerative type of Wolfram syndrome (WS2) is related to recessive mutations in *CISD2* (CDGSH iron-sulfur domain-containing protein 2), this gene is located on chromosome 4q22-q23. The 3 exons of these gene encode a “small intermembrane endoplasmic reticulum protein”. This zinc-finger protein is highly expressed in pancreas and brain cells. In addition to juvenile onset diabetes, optic atrophy, hearing impairment, neurological and psychiatric features, endocrine disorders like diabetes insipidus, and kidney dysfunction, unique features with upper gastrointestinal ulcers, mucocutaneous bleeding, and/or defective platelet aggregation are pathognomonic for WS2 (133,134).

1.5.3.2 Mitochondrial diabetes

Mitochondrial diabetes results from mutations in the mitochondrial DNA (mtDNA). Mitochondria are organelles within cells responsible for generating ATP through oxidative phosphorylation. Within our cells are hundreds to thousands of mitochondria and each mitochondrion contain 2 to 10 mtDNA copies. mtDNA is

maternally inherited and mutations can lead to dysfunction of the mitochondrial respiratory chain and impair ATP production, resulting in a range of clinical manifestations. The mtDNA contain only 37 genes which include 22 transfer RNAs and two ribosomal RNAs essential for mtDNA-specific translation of 13 mitochondrial encoded subunits of respiratory chain complexes. On the other hand our nuclear DNA encodes over 1,000 mitochondrially localized proteins, which are translated in the cytoplasm and then translocated to the mitochondria (135). Mitochondrial disease are genetically and phenotypically very diverse and the largest group of inborn errors of metabolism affecting 1:5000 individuals (136). The most common type of mitochondrial diabetes is related to m.3243A>G point mutation in *MT-TL1* (Mitochondrially encoded TRNA-Leu (UUA/G) 1) of mtDNA, which encodes a specifi tRNA for the amino acid leucine. In 1992 this point mutation was first described in a large family with several members, who had diabetes and/or hearing loss with maternal inheritance (137). Figure 1.6 shows the pedigree of one of these family. Of note the m.3243A>G can cause either classic MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) or maternally inherited diabetes and deafness (MIDD).

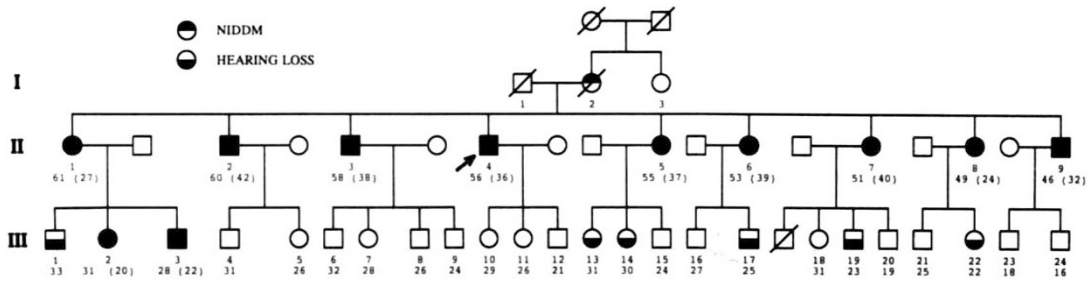

Figure 1.6: Pedigree of the first caucasian family with described MIDD. Diabetes and hearing loss were maternally inherited in all offsprings of generation I and maternal inheritance is demonstrated in generation III. The patient, whose mtDNA was analyzed, is indicated by an arrow. Adapted from van den Ouweland et al (137).

Mitochondrial diabetes should be considered in all individuals with diabetes and hearing impairment inherited from the mother, or in patients with diabetes and chronic progressive external ophthalmoplegia (CPEO). Additional clinical features

related with the m.3243A>G variant include macular retinal dystrophy, cardiomyopathy, myopathy, renal disease (focal segmental glomerulosclerosis), gastrointestinal disease (constipation or malabsorption), short stature and neurological phenotypes (138). The clinical phenotype is very heterogeneous, some will have diabetes alone, while others are oligosymptomatic or have multiple features, even in the same family. The mixture of wild-type and mutant mitochondrial DNA in each cell is named heteroplasmy and levels of heteroplasmy vary among the different type of tissues of an individuum. The variable phenotypes observed in mitochondrial disorders are influenced by the inherited quantity of mutant heteroplasmy load and the subsequent segregation of mutated mtDNA within diverse tissues and cell types (138–140). The penetrance for diabetes is over 85% at the age 70years in m.3243A>G mutation carriers but the age of onset varies with a mean age at diagnoso of 37 ± 11 years with a ranging from 11 to 68 years. About 20% of individuals will have an acute presentation with diabetic ketoacidosis, and therefore a misdiagnosis of T1D is likely. Most patients will require insulin therapy within months or few years, after they initially responded to diet or OHA. The use of Metformin is controverse, since it can interfere with mitochondrial function and might increase the risk for lactic acidosis (138).

It is estimated that 1% of all diabetes cases is caused by a mutation in the mtDNA, and the mutation m.3243A>G is the most common cause with over 85% of all mitochondrial diabetes. Other rarer mutations in the mitochondrial DNA associated with diabetes are following point mutations in the tRNA genes of *MT-TL1* (m.3254C>G, m.3256C>T, m.3264T>G, m.3271T>C), of *MT-TK* (m.8344A>G, m.8356T>C), of *MT-TS2* (m.12258T>C) and of *MT-TE* (m.14709T>C), and of *MT-ND6* (m.14577T>C). Diabetes and further endocrinopathies are also related with other mitochondriopathies for example due to large mtDNA deletations in Kearns–Sayre syndrome. In addition, diabetes can be associated in mitochondrial diseases due to autosomal recessive mutations in the nuclear genes *OPA1, MPV17, POLG* or *RRM2B*, which are important for maintenance of mtDNA (141).

1.5.3.3 Monogenic autoimmune diabetes

Early-onset diabetes can be autoimmune and caused by mutations in at least nine different genes related to immune regulation (*AIRE, CTLA4, FOXP3, IL2RA, ITCH, LBRA, STAT1, STAT3 and STAT5B).* These result in highly variable syndromes of autoimmunity, generally manifest in early infancy, have positive T1D antibodies and lifelong insulin therapy is required (3). Figure 1.7 gives an overview of major manifestations of monogenic causes of autoimmune diabetes.

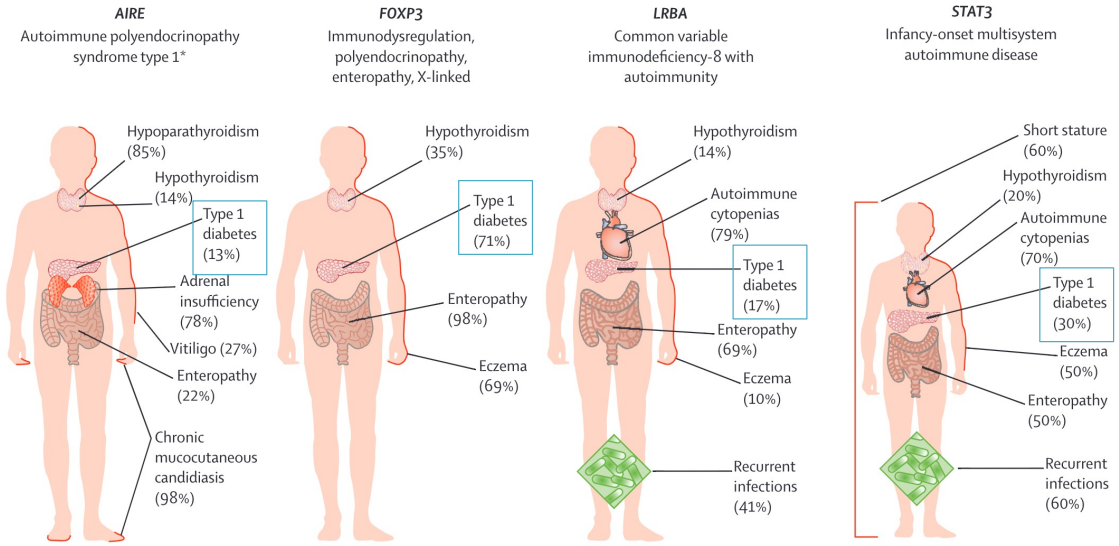

Figure 1.7: T1D as part of multiple manifestations of four monogenic causes of autoimmune diseases. Adapted from Johnson et al (22).

AIRE (autoimmune regulator) gene is important to establish central immune tolerance and to preclude autoimmunity. Loss of function mutations in this *AIRE* gene causes a rare autosomal-recessive disease named APS-1 (autoimmune polyendocrine syndrome type 1) (142).

T1D is also part of IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) that is related to the loss of function of the *FOXP3* (transcription factor forkhead box P3) gene, which is important for the function of the regulatory T cells. Genetic evaluation for this IPEX syndrome should be undertaken in any male offspring with infancy-onset diabetes and severe enteropathy (143). “IPEX-like”

phenotype is caused by heterozygous mutations in CTLA4 (cytotoxic T lymphocyte antigen-4), which is related to autoimmune lymphoproliferative syndrome. This syndrome can include also autoimmune diabetes and enteropathy as well as several cytopenia and thyroiditis. (144). Very early presentation of features like IPEX syndrome in girls can be caused by immunodeficiency 41 with lymphoproliferation and autoimmunity. This monogenic autoimmune syndrome results from recessively inherited loss-of-function mutations in *IL2RA* (IL-2 receptor α chain). IL-2 signaling is important for immune homeostasis and favours the transcription of FOXP3. Genetic testing for *IL2RA* mutations is recommended in individuals with early infancy onset of recurring infections (especially cytomegalovirus) and/or variable autoimmune disorders with enteropathy (22). *LRBA* (lipopolysaccharide responsive beige-like anchor protein) has an important role in the regulation and trafficking of CTLA-4 (cytotoxic T-lymphocyte antigen 4), an important molecule expressed on regulatory T cells with inhibitory effects. Recessive loss-of-function mutations in *LRBA* gene is related to common variable immunodeficiency 8 with autoimmunity (145).

Proteins encoded by the *STAT1, STAT3*, and *STAT5B* genes are transcription factors, which are part of the cellular immune response to cytokines and growth factors. Immunodeficiency 31C features are mucocutaneous candidiasis with variable autoimmune disorders resulting from autosomal dominant gain-of-function mutations in *STAT1* (signal transducer and activator of transcription 1), which result in increased interferon α signaling. Genetic testing for mutations in *STAT1* should be recommended in individuals with chronic mucocutaneous candidiasis (146). Another autosomal dominant gain-of-function mutation related to *STAT3* (signal transducer and activator of transcription 3), increase IL-17-producing T cells and cause infantile-onset multisystem autoimmune disease-1. *STAT3*-related disease should be suspected in children with early-onset of a spectrum of autoimmune findings and recurrent infections. Clinicians should test for *STAT3* mutations children with short stature and autoimmunity disorders. The *STAT5B* pathway is related to *IL-2RA, FOXP3* and growth hormone transcription and *STAT5B* deficiency leads to a lower cell count in regulatory T cells. Loss of function mutations in *STAT5B* results in immunodeficiency, allergic and/or autoimmune manifestations and decreased growth (3,22).

1.5.3.4 Other genetic syndromes associated with diabetes

A recent published novel syndrome of young-onset diabetes results from homozygous nonsense mutation in *TRMT10A*, a nuclear tRNA methyltransferase. Children have additional clinical features like microcephaly, epilepsy and intellectual disability (147).

Homozygous *DNAJC3* mutations can present with young-onset diabetes, pancreatic fibrosis/atrophy, hearing impairment, decreased growth, hypothyroidism and progressive neurodegeneration. *DNAJC3* gene encodes a protein that promote normal protein folding in ER. *DNAJC3* mutation should be considered in the differential diagnosis of suspected mitochondrial disease (148).

1.5.3.5 Diabetes secondary to genetic disorders of exocrine pancreas

CEL-MODY is an autosomal dominant disease of the exocrine pancreas and diabetes with pancreatic exocrine insufficiency already in childhood, usually 10-30 years before development of diabetes. For further description chapter 1.5.2.2.3.

Cystic fibrosis (CF) is a multisystemic disorder related to mutations in *CFTR* (cystic fibrosis transmembrane conductance regulator) gene, found on chromosome 7 with autosomal recessive inheritance. The CFTR gene codes for a specific cAMP-responsive chloride channel, which is located on the apical side of secreting epithelial cells. CF affects therefore several organs with secretory cells like the lung and pancreas as well as biliary, gastrointestinal and reproductive tract. To date over 2000 *CFTR* mutations were reported. In more than 65% of CF patients, a 3-base-pair deletion (F508del) is the most common mutation. *CFTR* mutations are summarized in 6 classes based on their major effect regarding CFTR function (149). CF has an estimated prevalence between 1/3,000 and 1/6,000 in populations with newborn screening (150). “Cystic fibrosis related diabetes mellitus” (CFRD) is a specific type of diabetes. With increasing life expectancy CFRD is common in patients with CF, around 19% of adolescents and 40-50% of adults with CF will require diabetes specific treatment with insulin (151).

Hereditary pancreatitis is an autosomal dominant inherited disease. Patients suffer from recurrent attacks of acute pancreatitis already in childhood, which will lead to chronic pancreatitis at early ages. One of the first reported mutations were identified in *PRSS1* (cationic trypsinogen) gene on chromosome 7, *SPINK1* (serine protease inhibitor Kazal type 1) on chromosome 5, and *CTCR* (chymotrypsinogen C) gene on chromosome 1 (152,153).

Pancreas agenesis can be related to biallelic mutations in *PDX1* gene, an important transcription factor for the development of the pancreas. Heterozygous mutation carrier can develop *PDX1*- MODY (154). Further reports showed that hypoplasia or agenesis of the pancreas can be also a result of inactivating *GATA6* mutations. GATA6 is a specific transcription factor in the regulation cell differentiation and organogenesis. This phenotype is associated with pancreas agenesis, congenital cardiac malformations (atrial or ventricular septal defects; singular pulmonary stenosis or tetralogy of Fallot) and can cause delays in development of children (155).

1.5.3.6 Monogenic forms of severe insulin resistance syndromes

Major characteristics of insulin resistance (IR) syndromes are the combination of specific skin feature with acanthosis nigricans and either very high fasting insulin concentrations (>150 pmoL/L) or increased insulin requirements in patients with already existing diabetes in usually lean patients.

1.5.3.7 Insulin signaling defects due to mutations in INSR gene

Genetic IR syndromes due to mutations of the insulin receptor (INRS) gene are type A insulin resistance, Donohue and Rabson-Mendenhall syndromes. Type A IR Syndrome is the mildest form with autosomal dominant inheritance. Key features are acanthosis nigricans and hyperandrogenism during puberty followed by diabetes onset during adolescence. Homozygous or compound heterozygous INRS mutations are responsible for Donohoe and Rabson-Mendenhall syndromes, the most severe forms. Babies with Donohue syndrome are small at birth and have early

onset diabetes with very high insulin levels (>1,000 pmol/L) and is associated with cardiomyopathy and hypertrichosis (156,157).

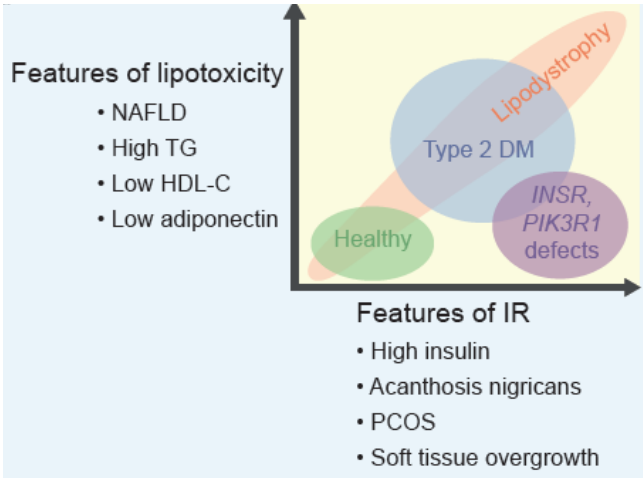

Figure 1.8: Monogenic forms of insulin resistance: clusters corresponding to degree of insulin resistance and features of lipotoxicity. Adapted from (43).

1.5.3.8 Monogenic lipodystrophies

Rare syndromes with lipodystrophy are a heterogeneous group that are characterized with a selective deficiency of adipose tissue with severe IR. Mutations in several genes associated with inherited lipodystrophies, are related to differentiation (*BSCL2, AKT2, PPARG*), maintenance (*LMNA, ZMPSTE24, CIDEC, PSMB8*), or function (*AGPAT2, CAV1, PTRF, LIPE, PLIN1*) of adipocytes. Recessively inherited mutations in *AGPAT2* or *BSCL2* are the main genetic cause for up to 80% of all patients with congenital generalized lipodystrophy (Berardinelli–Seip syndrome). These affected patients have loss of subcutaneous and visceral fat, but lipids are stored in the liver, which results in hepatic steatosis, hepatic insulin resistance and early onset of diabetes. Familial partial lipodystrophy (FPLD) is usually diagnosed after puberty. Heterozygous mutations in genes like *LMNA* or *PPARG* account for up to half of patients with FPLD (3,158,159).

MDP (Mandibular hypoplasia, Deafness and Progeroid features) syndrome is a rare disorder, which also causes premature aging. Mutations in *POLD1* gene, which encodes a universal DNA polymerase, can lead to subcutaneous lipodystrophy with diabetes and deafness, hypoplasia of the lower chaw, and in men to hypogonadism (160).

Another disease with progeroid features, partial lipodystrophy and low BMI is the autosomal-dominant inherited SHORT (Short stature, Hypermobility of joints, Ocular depression, Rieger's anomaly, Teething delay) syndrome. This syndrome with partial lipodystrophy is related to a mutational hotspot in *PIK3R1* gene. This gene encodes a phosphatidylinositol 3-kinase, which has key functions in pathways for insulin-signaling and growth factor regulation (161).

1.5.3.9 Ciliopathy-related insulin resistance and diabetes

Primary cilia are microtubule in the cell membranes, sensing environmental signals, and transducing extracellular signals within the cells. Mutations in genes regulating the structure and function of these cilia cause ciliopathy with metabolic disorder, obesity and diabetes (162).

Alström syndrome (ALMS) is a ciliopathy with features like obesity, insulin resistance/ T2D, hypertriglyceridemia, acanthosis nigricans, retinal degeneration, hearing impairment, dilated cardiomyopathy, worsening hepatic and/or renal function, but normal cognitive development. ALMS is related to biallelic mutations in the *ALMS1* gene, which encodes a major protein from the base of primary cilia with multiple functions (163).

Bardet-Biedl syndrome (BBS) is also a rare ciliopathy with multisystem involvement and autosomal recessive inheritance like ALMS, but the clinical features are very heterogenous and includes cognitive impairment. This syndrome is related to mutations in more than 26 different genes encoding for proteins related to the BBSome, chaperonins and the intraflagellar transport complex structure of cilia (164).

1.6 Diagnosis of monogenic diabetes

Genetic testing for monogenic diabetes has been possible since identification of the first MODY genes in the 1990s. MODY, although the most prevalent form of monogenic diabetes (approximately 0.5 to 5% of all patients with non-autoimmune diabetes) is still underdiagnosed (165). Mainly clinical examination and genetic evaluation with single gene testing or multigene panel were used to distinguish monogenic diabetes from other forms of diabetes. Awareness of monogenic diabetes among healthcare professionals varies and limited access to genetic testing laboratories are possible explanations that many cases with monogenic diabetes go unrecognized (42,165). Table 1.3 outlines the genetic tests available for monogenic diabetes.

At the beginning genetic testing for the most frequent mutations of NDM and MODY was based on Sanger sequencing of single or multiple genes. This technique is/was time consuming and potentially costly in case of multiple genes and was limited to few selected genes. The correct selection of genes to be tested depends on the existence of comprehensive clinical information regarding the phenotype as well as a indepth family history. Incomplete or still absent clinical features and/or family history details can be a limitation of the candidate gene approach. However, the targeted single gene approach with Sanger sequencing remains a cheaper method in cases with gene specific clinical characteristics or known mutation in the family. In the last decade, the next-generation sequencing (NGS) technique has been introduced into the diagnostic laboratories, which allows the simultaneous analysis of multiple genes. A single analysis can detect gene mutations previously identified by the use of serial Sanger sequencing or multiplex ligation-dependent probe amplification analysis (MLPA). NGS led to a higher mutation detection rate, because of the increased number of tested genes (166–168).

Genetic testing for monogenic diabetes with NGS has become increasingly cost-effective and less laborious. Two sequencing technologies are mostly used today: targeted sequencing of genes known to be involved in monogenic diabetes with the use of gene panels and whole exome sequencing (WES). WES analysis all protein-coding regions (< 2% of the genome). Large amounts of data generated with WES present bioinformatics challenges but enables the identification of pathogenic

variants in genes that were not initially considered. Further studies and analysis with sequencing additional family members and conducting segregation analysis are necessary to eliminate many of non-causative variants. As new genes are discovered, gene panels must be redesigned and revalidated. Gene panels and WES are both limited in their capacity to detect copy-number variations (for example, common whole gene deletion in *HNF1B*-MODY) or mitochondrial variants with low heterosplasmy (such as m.3243A>G in MIDD). Therefore, MLPA and Sanger sequencing of mitochondrial DNA are often used to complement the genetic analyses (169).

Table 1.3: Genetic tests for monogenic diabetes

Genetic Test	**Possible advantages**	**Possible disadvantages**
Sanger sequencing	Cheap – if single gene or few genes interpretation challenges: low	Limited to target gene/genes Difficulties with large heterozygous deletions
Deletion/duplication analysis (MLPA)	Cheap interpretation challenges: low	Limited to the target gene/genes
PCR (methylation-dedicated) for 6q24-TNDM	Cheap	Limited to few monogenic forms samples from parents necessary for the exact mechanism
NGS panels: Monogenic diabetes	Very good coverage of known monogenic DM genes Allows for expansion as new genes are discovered	Higher cost interpretation challenges: high Some panels may not detect deletions
Whole exom sequencing (WES) and whole genome sequencing (WGS)	Potential for novel gene discovery Increasing coverage of exons (with WES) and introns (with WGS)	Higher cost informed consent: complexer interpretation challenges: high incidental findings: high risk coverage of some known monogenic diabetes genes: can be incomplete. areas of high G/C content, repetitive elements regions: difficult to analyze

2 METHODS

2.1 Data collection

The aim of this single-center retrospective study was the evaluation of current diagnostic approach used in the diagnosis of monogenic diabetes in an adult diabetes population.

After approval of the Ethics Committee Medical University Graz (EC Nr: 35-075 ex 22/23) data were collected from all patients with molecular genetic testing for monogenetic diabetes. All individuals with a diagnostic work-up for monogenic diabetes had signed an informed consent for genetic analysis according to Austrian Genetic Engineering Act. Only patients, who did not object the pseudonymized use of data for scientific purposes, training, and further education, were included in this retrospective study. Data were collected from onsite and electronic medical records from patients with molecular genetic testing for monogenetic diabetes between 2010-2022 at the diabetes outpatient clinic of the Division for Endocrinology and Diabetology at the Department for Internal Medicine of the Medical University of Graz.

2.2 Primary and secondary endpoints

Primary endpoint was the positive rate of genetic tests performed.

Secondary endpoints measures were:

- Number of patients tested for monogenetic diabetes at the Division of Endocrinology and Diabetology between 2010-2022.
- Distribution of subtypes of monogenetic diabetes
- Clinical characteristics at time of testing (age of patient, BMI, diabetes duration, diabetes therapy, sex)
- Clinical characteristics at time of diabetes onset (age of patient, diabetes therapy
- Previous measurements (most recent HbA1c, C-peptide level, islet autoantibodies at time of genetic testing)

- Family history of diabetes (positive family history indicates diabetes in a first generation relative)
- Additional clinical features (hearing impairment, renal malformation, cardiomyopathy, other comorbidities suspected multisystemic disease, small for gestational age (SGA) in offspring)
- MODY probability at age of diabetes diagnosis, retrospectively by using the online Exeter Diabetes App: https://www.diabetesgenes.org/exeter-diabetes-app/ModyCalculator
- Case based description of diagnostic dilemmas or delayed diagnosis of monogenic diabetes.

2.3 Statistical analyses and literature search

The Microsoft® Excel for Mac version 16.70 was used for data collection and descriptive statistical analyses were carried out using IBM SPSS Statistics (version 27). The data are presented either as medians and IQRs (interquartile ranges) or as absolute numbers and percentages.

A thorough literature search was conducted using PubMed database with following relevant search terms: “monogenic diabetes”, “MODY”, “mitochondrial diabetes”, “diabetes mellitus” AND “genetic”, “GCK MODY”, “HNF1A MODY”, “HNF1B MODY” and other diabetes associated gene AND “diabetes”. Further search was conducted in “All databases” of NCBI like ClinVar, Genes, MedGen and OMIM.

3 RESULTS

3.1 Demographic data

This retrospective study included 105 patients (59 females (56%), 46 males) with molecular genetic testing for monogenic diabetes. During the time of the genetic testing, the median age of the patients was 37 years (29-48) and the median diabetes duration was 4 years (1-10). Age at diabetes diagnosis was at least 8 years and older (median 29 years, 22-38). 83 patients (79%) had a positive family history for diabetes in at least one generation. 7 patients had a positive family history for MODY. At time of diabetes diagnosis 70% (n=73) and at time of genetic testing 55% (n=58), patients were not treated with insulin therapy. Median BMI was 23.2 kg/m^2 (21-26) and median HbA1c was 52 mmol/mol (43-67) at time of genetic testing. 31 patients (30%) had at least one additional extra-pancreatic feature. 95 patients (91%) had a detectable C-peptide >0.5 ng/ml. In 9 (9%) patients at least one T1D autoantibody was positive, none of these patients received a positive genetic result. In 50% (n=52) the MODY probability calculator showed a score higher than 30%, but 32 patients (31%) were too old (>35 years) at time of diabetes diagnosis for the use of the calculator.

3.2 Diagnosis rate and identified variants

In 35 patients (33%) genetic testing revealed a variant in one of the known diabetes genes. *GCK* accounted for 49% (n=17) of all cases, 17% (n=6) had *HNF1A*, 14% (n=5) had *HNF1B*, 9% (n=3) each for *HNF4A* and MIDD. The main clinical fetures of patients with variants according to genetic subtypes and patients with non-monogenic diabetes are shown in Table 1.4. One person had a variant with unknown significance in *KLF11*. In patients with confirmed monogenetic diabetes time to correct diagnosis was median 10 years, in 9 patients more than 20 years elapsed until the correct diagnosis. 4 patients with a family history for MODY inheritance of the tested variant was verified.

In the 5 patients with confirmed *HNF1B*-MODY, kidney function and renal morphology were normal in 4 of 5. One patient with *HNF1B*-MODY due to whole-gene deletion had multiple extra-renal features with early-onset diabetes, pancreatic

hypoplasia with pancreatic exocrine dysfunction, vaginal aplasia, bicornuate uterus and hypomagnesaemia. In 5 patients with *GCK*-MODY preexisting diabetes therapy was metformin.
In all patients with MIDD the typical MELAS-mutation m.3243A>G was found.

Table 1.4: Main clinical characteristics and laboratory results at time of genetic testing in patients with monogenic diabetes according to genetic subtype and patients with diabetes and negative genetic testing results. *family history of diabetes in at least one generation; **insulin therapy; ***T1D autoantibodies; (ys=years; f/m=female/male); median+IQR or min-max in HNF4A and MIDD group.

	GCK	HNF1A	HNF1B	HNF4A	MIDD	Negativ
Patients (n)	**17**	**6**	**5**	**3**	**3**	**71**
Sex f/m	12/5	12/6	12/7	12/8	12/9	12/10
Age ys	33 (25-50)	43 (32-53)	36 (31-57)	42 (29-55)	44 (42-63)	37 (28-47)
DM family*	88 %	100 %	80 %	100 %	33 %	76 %
DM duration ys	5 (20-90)	18 (3-33)	13 (10-17)	5 (1-16)	13 (10-35)	2 (0.6-7)
Insulin** <6months	0 %	50 %	60 %	33 %	33 %	34 %
Insulin at testing	0 %	50 %	80 %	33 %	100 %	51 %
BMI kg/m^2	23 (20-24)	25 (22-29)	24 (22-27)	22 (20-22)	20 (19-26)	23 (21-26)
HbA1c mmol/mol	48 (44-51)	62 (54-81)	70 (52-88)	65 (54-72)	69 (63-105)	59 (47-71)
C-peptide ng/ml	1.03 (0.8-1.2)	1.15 (0.7-1.4)	0.63 (0.5-0.8)	1.26 (0.7-1.3)	0.93 (0.2-1.13)	1.34 (0.9-1.8)
T1D AAB***	0 %	0 %	0 %	0 %	0 %	9 %
Other features	24 %	0 %	80 %	0 %	100 %	29 %

3.3 Variants of unknown significance (VUS)

A 38-year-old female with BMI 19.5 kg/m^2 had a history of GDM and was on a low carbohydrate diet to archive normal blood glucose control (HbA1c 38 mmol/mol). C-peptide was in normal range with 1.01 ng/ml and T1D autoantibodies were tested negative. Her father had an known impaired glucose tolerance and his BMI was normal. WES of MODY associated genes revealed a missense-mutation, c.1448C>T, p.(Pro483Leu) in *KLF 11*-gene with unknown significance. Reported allele frequency data from public databases was too high to be the cause of disease

related to this variant. In the ClinVar database this variant is even considered benign (ClinVar; https://www.ncbi.nlm.nih.gov/clinvar/ variation/VCV000330640.3 (accessed March 5, 2023) Therefore, monogenetic cause of diabetes could not be confirmed.

3.4 Genetic testing

From 2010 until 2017 single gene testing (mostly *GCK, HNF1A, HNF4A* and *HNF5B*) by Sanger sequencing and MLPA analysis for the search of deletions was performed. In 2018 MODY- panel diagnostic by NGS was implemented with two targeted MODY gene panels: highly relevant genes: "*GCK, HNF1A, HNF1B, HNF4A, PDX1"* and in step two additional monogenic diabetes associated genes: "*ABCC8, AKT2, BLK, EIF2AK3, FOXP3, GATA6, GCGR, GCKR, GLIS3, GLUD1, HADH, IER3IP1, INSR, IRS1, IRS2, KCNJ11, KLF11, LIPC, MAPK8IP1, NEUROD1, NEUROG3, PAX4, PTF1A, RFX6, SLC19A2, SLC2A2, TCF7L2, WFS1, ZFP57".* In 2022 NGS with TrueSight One sequencing panels was changed to WES. 61 (58%) genetic analysis were performed with Sanger sequencing, which revealed in 20 patients (33%) a variant. 44 (42%) genetic tests were done with NGS, which revealed in 15 patients (34%) a positive result.

3.5 Case presentations

The objective was to derive more insight into the diagnosis of monogenetic diabetes, inclusive of issues related to diagnosis, genetic testing, and treatment changes. In-detail research of medical records was conducted in 3 patients, who were initially misdiagnosed as having type 1 diabetes.

3.5.1 Case presentation 1

A 51-year-old male with a pre-existing diagnosis of T1D since the age of 18, was referred to our diabetes outpatient clinic for a structured education course. Subsequently his prior diabetes treatment with multiple daily insulin injections (MDI)

was transferred to insulin pump therapy. History and screening for acute and chronic diabetic complications revealed two severe hypoglycemia prior to insulin pump therapy and diabetic peripheral polyneuropathy. His family diabetes history was remarkable for a sister and a great uncle with T1D and his mother with T2D. His HbA1c improved from 66 to 58 mmol/mol. Patients BMI increased to 28.5 kg/m^2 and an SGLT-2 inhibitor (10mg dapaglifloxin) was added off-label. 3 years after his initial presentation, his son presented with typical hyperglycemic symptoms at the age of 14 years. Pedigree is shown in Figure 1.9. His autoantibodies screen for T1D was negative and molecular genetic tests indicated a splice site mutation c.326+1G>A in the intron 1 of *HNF1A* gene. The same mutation was found in our patient and his sister. Following the molecular genetic diagnosis low dose sulfonylurea was started in addition to SGLT-2 inhibitor and his daily insulin dose decreased. Measurement of his current C-peptide was 0.66 ng/ml. Of note, our patient was on prandial insulin therapy for 12 years after his diabetes diagnosis. His sister was switched to sulfonylurea and responded to low dose gliclazide (10 mg) after 25 years of prandial insulin therapy. Her current C-peptide was 1.42 ng/ml.

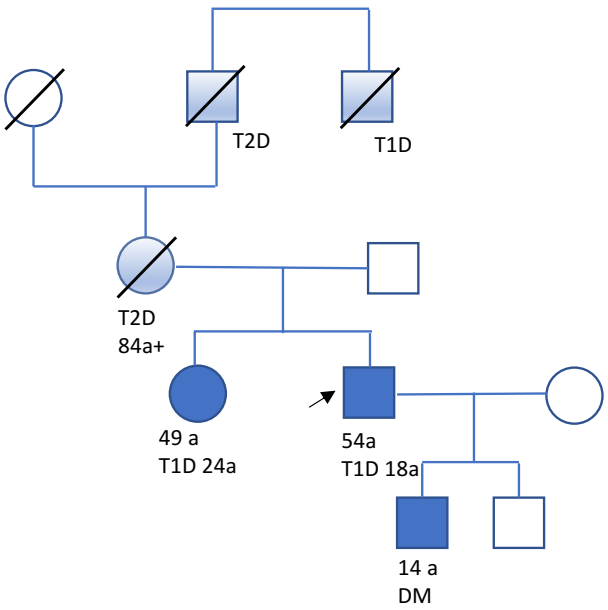

Figure 1.9: Pedigree Case 1
Autosomal dominant inheritance of monogenic diabetes related to *HNF1A* MODY. Circles denote females, squares denote males. Diagonal line stand for deceases family members. Filled circles/squares represent patients with diabetes and confirmed heterozygous *HNF1A* mutation. Arrow indicates described patient at time of genetic testing. Age at diabetes diagnosis is listed.

3.5.2 Discussion Case 1

Clinical features of *HNF1A* phenotype can overlap with T1D orT2D. *HNF1A*-MODY is highly prevalent in people with European ancestry and up to 95% of individuals

with *HNF1A*-MODY are misdiagnosed (64). Some MODY patients may be misdiagnosed as having T1D because they present with symptoms of hyperglycemia in their early 20s and treated immediately with insulin. Colclough et al (170) already reported that *HNF1A* gene mutations are found in 5-10% of patients diagnosed with T1D who have a positive diabetes family history. Additional selection criteria are negative autoantibody status and preserved endogenous insulin production by detection of C-peptide outside of the honeymoon period. In our patient and his sister, the initial diagnosis was based on clinical criteria, without measurement of T1D autoantibodies and C-peptide. Both patients had only prandial insulin therapy for several years, which showed prolonged preserved beta cell function. In T1D age at diagnosis impacts the decline of beta cell function over time, however most of adults T1D patient have a decreased C-peptide after 4 years of diagnosis (171). C-peptide and beta-cell/islet autoantibodies are highly sensitive and specific markers to distinguish T1D from non-T1D to aid diagnosis of monogenic diabetes (172). Early diagnosis is important since *HNF1A*-MODY affects 50% of children born to mutation carrier, with a high penetrance (above 90%) and treatment response to sulfonylurea depends on diabetes duration (65). *HNF1A* and *HNF4A*-MODY with longer diabetes duration (>11 years), who are overweight and have higher HbA1c, it is advised to add a sulfonylurea as an adjunct to the existing therapy, rather than replacing it entirely (173). In *HNF1A*-MODY patients, treatment with a SGLT2i and/or a GLP-1RA has been tested in small studies and they may be feasible (64).

3.5.3 Case presentation 2

A 39-year-old female with preexisting T1D presented with extreme fatigue and omitting her insulin therapy since the birth of her baby 8 week before. The pregnancy was complicated by preeclampsia, induction of labor and delivery was in 38th week of gestation with vacuum extraction. A manual extraction of the placenta was necessary, and the patient received blood transfusions due to significant blood loss. Her current BMI was 18 kg/m^2, HbA1c was 43 mmol/mol, renal function was impaired with serum creatinine of 1.85 mg/dl and thyroid levels indicated central hypothyroidism. Further endocrine follow-up diagnosed a Sheehan syndrome with partial hypopituitarism. Diabetes was diagnosed at the age of 31 and prandial insulin

therapy was started after 1 year of diagnosis. Until the pregnancy she was on prandial insulin therapy with excellent glucose control. C-peptide was 1.12ng/dl and T1D autoantibodies were negative. A molecular genetic test for MODY panel was negative. It was noted that the patient had bilateral hearing impairment with onset 1 year prior to diabetes diagnosis. A detailed medical history revealed further consultations for chronic obstipation and neurologic evaluation regarding numbness of feet and she had several miscarriages. The family history revealed a sister deceased in her early 30s with cardiac problems, a mother deceased in her early 50s and a maternal aunt had diabetes and deafness. Pedigree is shown in Figure 2.0. A genetic test for MIDD was performed and the specific m.3243A>G mutation was found in the *MT-TL1* Gen with a heteroplasmy level of 18% in the blood. Further family genetic testing showed that her brother, her aunt, and her niece are carriers of m.3243A>G.

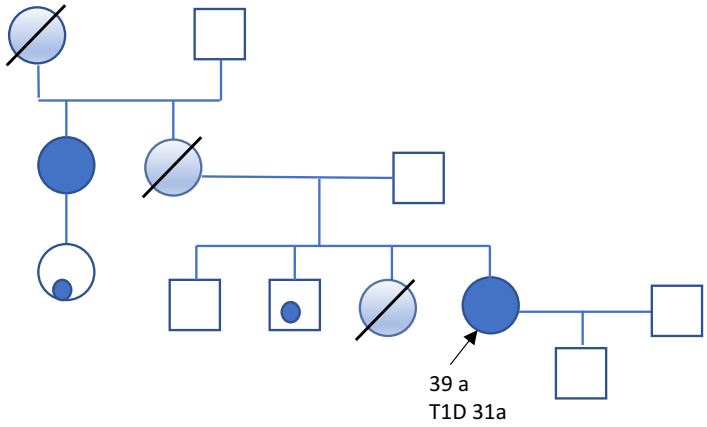

Figure 2.0: Pedigree Case 2
MIDD. Circles denote females, squares denote males. Diagonal line stand for deceases family members. Filled circles/squares represent patients with diabetes and hearing impairment and confirmed m.3243A>G mutation. Small, filled circle indicate carriers of m.3243A>G. Arrow indicates described patient. Age at diabetes diagnosis is listed.

3.5.4 Discussion Case 2

A diagnostic journey begins when a patient first seeks medical care for her symptoms and can take years before they reach their final correct diagnosis. The diagnostic odyssey of patients with mitochondrial diseases is complex and often reflect clinician unfamiliarity. Mitochondriopathies have a wide clinical heterogeneity and require a detailed medical and family history. A survey published by Grier et al, reported that patients saw on average 8 clinicians before the correct diagnosis was made (174). In our patient several features indicate an alternative diagnosis of monogenetic diabetes with negative T1D autoantibodies, detectable C-peptide and

low insulin requirements indicative for preserved beta-cell function. MODY probability calculator gave a score of 62.4% having MODY at time of first genetic testing. Additional extra-pancreatic features like early development of kidney disease are also part of the spectrum of *HNF1B* mutation. *HNF1B*-MODY and mitochondrial diabetes are the most common causes of syndromic forms of monogenetic diabetes. These patients often lack typical features or are oligosymptomatic. A recent report showed, that the mitochondrial DNA 3243A>G mutation is the 4th most common confirmed mutation for monogenic diabetes after mutations in GCK, *HNF1A* and *HNF4A* in patients with clinical suspicion for MODY and represented 8% of all monogenic cases. (175). An early diagnosis and most important correct diagnosis of mitochondrial diabetes has several implications for patients care with interdisciplinary follow-ups. Mothers with MIDD have a higher miscarriage rate than women with other types of diabetes and higher risk for preeclampsia (176). A mother with MIDD or asymptomatic carrier is likely to transmit the mitochondrial 3243A>G mutation to all her offspring. The penetrance of diabetes in an individual with m.3243A>G is age related but more than 85% will have diabetes by the age of 70 years (138).

3.5.5 Case presentation 3

A 60-year-old female with preexisting diagnosis of T1D since the age of 28 without any family history of diabetes was recently diagnosed with hypertrophic cardiomyopathy and atrial fibrillation. She reports an exercise-induced muscle weakness with elevated creatine kinase levels since the age of 40 and bilateral hearing loss since the age of 50. Her mother died at age 59 of a hematologic disorder and had T2D. Currently she was on intensified insulin therapy with MDI, had a HbA1c of 63 mmol/mol and a BMI of 26.2 kg/m^2. C-peptide level was 0.93 ng/ml. Screening for chronic diabetic complications showed normal kidney function and normal eye examination. A genetic test with WES including mtDNA was performed and the mutation m.3243A>G was found in the *MT-TL1* Gen with variant allele frequency (VAF) of 9,5%. Further family genetic testing showed that her brother and both daughters carried the same mutation. (Figure 2.1) Her brother has clinical myopathy, but her daughters are healthy.

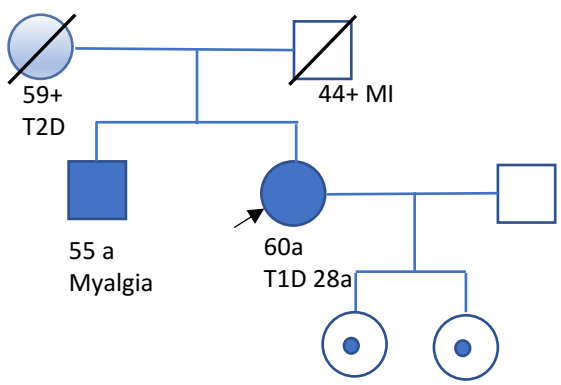

Figure 2.1: Pedigree Case 3
MIDD with maternal inheritance in all offsprings. Circles denote females, squares denote males. Diagonal line stand for deceases family members. Filled circles/squares indicate patients with features of MIDD and confirmed m.3243A>G mutation. Small, filled circle indicate carriers of m.3243A>G. Arrow indicates described patient. Age at diabetes diagnosis is listed. MI (myocardial infarction)

3.5.6 Discussion Case 3

Mitochondrial diabetes accounts up to 1% of individuals with diabetes but is oftentimes unrecognized by attending clinicians. Recognizing mitochondrial diabetes is not a simple task. The constellation of diabetes and pre-senile hearing loss, and/or a family history of these clinical features in maternal relatives are key features of a patient with MIDD but may be associated with broad range of other symptoms as summarized in chapter 1.5.3.2. Clinical characteristic features related with the m.3243A>G mutation can be heterogenous, also within the same family, as shown in our patients' family. Prevalence of diabetes in carriers with m.3243A>G is reported with 38% (136) and deafness usually precedes diabetes onset by 6 years (range 0-16ys). The combination of deafness and diabetes has a high positive predictive value for mitochondrial disease even in the absence of a maternal family history (136,138). Pattern recognition is of upmost importance to the clinician. Long-term follow-up is advised because of high risk for cardiomyopathy as seen in our patient and for sudden death due to arrythmias (177).

4 DISCUSSION

Monogenic diabetes is a heterogeneous category of disorders with hyperglycemia and compromise a range of clinical conditions, including NDM, MODY and diabetes-associated syndromes. Research on monogenic diabetes has improved our understanding of the pathophysiology of diabetes and the genetics of common diseases (178). Furthermore, monogenic diabetes has provided one of the best examples to date of the potential of precision medicine (43). The phenotypes of monogenic diabetes, T1D and T2D overlap. Monogenic diabetes represents an under-diagnosed condition with substantial implications for patients and their families. Reported data from England estimated that 80% of approximately 26,000 cases with diabetes are undiagnosed (165).

Improving diagnosis rates of monogenic diabetes

In this retrospective study, the diagnosis of monogenic diabetes was made in 32% of adult individuals with diabetes selected based mainly on clinical and laboratory features. Positive rate of referrals to the Exeter genetic diagnostic laboratory is around 27%, based on pre-selection of patients by healthcare professionals (165). The NHS England National Genomic Test Directory Testing Criteria for Rare and Inherited Disease related to monogenic diabetes has been set to keep the positive rate of tests performed at 25% overall, to ensure that resources are used effectively to diagnose monogenic diabetes (https://www.england.nhs.uk/wp-content/uploads/2018/08/rare-and-inherited-disease-eligibility-criteria-v4.pdf). So fare the described patient selection criteria are able to detect patients with monogenic forms of diabetes, however the count of missed individuals using these selection criteria is not known.

The MODY probability calculator helps to select individuals for genetic testing. This online tool is based only on clinical features like age at diagnosis, BMI, HbA1c, family history of diabetes and therapy with insulin or oral antidiabetic agents. In a European cohort of individuals diagnosed with diabetes less than 35 years of age the MODY calculator has shown good differentiation between monogenic and T1D or T2D. But dominant (heterozygous) variants of monogenic diabetes associated genes have also been described up to 6% in patients with apparent T2D with age of onset >40 years (179). No single clinical criterion was able to identify all patients

with monogenic diabetes (180). The current MODY calculator is soley validated for the 3 common subtypes (*HNF1A, GCK, HNF4A*) (181). In future it is planned to include additional biomarkers, such as C-peptide and islet autoantibodies. Although these criteria perform well in distinguishing monogenic diabetes from T1D, they are unlikely to reliably distinguish monogenic diabetes from T2D.

As expected *GCK* and *HNF1A* accounted for the large majority (66%) of monogenic diabetes diagnosis in our cohort, followed by *HNF1B* mutations. Diagnosing *HNF1B*-related disorders is clinically difficult due to high phenotypic heterogeneity among and between families, and incomplete penetrance and because of 50–60% rate of de novo mutations demonstrated in index cases. A *HNF1B* score was developed as a guide to use for rational genetic testing. The *HNF1B* score uses a weighted combined selection of specific characteristics. Glomerular cysts, hypomagnesemia, pancreas hypoplasia and genital tract abnormalities in females show high specificity. *HNF1B* gene analysis is recommended with a HNF1B score $\geq$ 8 (182). 20% of pathogenic mutations found in patients suspected to have MODY are in genes typically associated with syndromic presentations, including heterozygous, pathogenic mutations in *HNF1B,* the maternally inherited mitochondrial m.3243A>G variant, and biallelic, pathogenic mutations in WFS (183,184). As well described in a recent commentary (185), a focus should be to increase clinical knowledge and an update of current genetic testing strategy for monogenic diabetes.

Important implications of identifying and diagnosing monogenic diabetes

Distinct therapeutic responses have been demonstrated for several reported subtypes of monogenic diabetes. Despite the progress made, there remains an imperative to ensure prompt diagnosis for all individuals affected by monogenic diabetes. Since recognition of monogenic causes may have a major impact on treatment, their identification by genetic testing is critical for appropriate management. As demonstrated in case presentation 1 insulin therapy could have been avoided maybe for years with an earlier correct diagnosis of *HNF1A*.

Individuals with pathogenic activating mutations in channel genes *ABCC8* or *KCNJ11* respond well to in these cases high dose oral sulfonylureas (186,187). Also insulin therapy can be safely transferred to low dose oral sulfonylureas in patients deficient for *HNF1A* or *HNF4A* (65,188). On the other hand, diabetes treatment is unnecessary in individuals with mild to moderate lifelong non-progressive

hyperglycemia solely due to *GCK* MODY. Hypoglycemic treatment mostly do not improve blood glucose levels and *GCK* MODY do not develop diabetes complications (173,189). However, management of *GCK* MODY should be taken seriously during pregnancies. Under this circumstance insulin treatment might be necessary in a pregnant woman with *GCK* MODY, in the case of hyperglycemia occurs and/or when fetal ultrasonography shows accelerated intrauterine growth. When a mother with diabetes has a pathogenic *GCK* mutation and the fetus does not, maternal hyperglycemia will lead to fetal hyperinsulinemia and increased risk of macrosomia. On the other hand, when the mother and her baby share the same *GCK* mutation, the glucose set point to enhance secretion of insulin is similar in the mother and her baby, leading to normal fetal insulin levels and average birthweight. In case of paternal inheritance of a pathogenic *GCK* mutation and the mother has normal blood glucose levels, the maternal blood glucose levels are not enough to enhance adequate fetal insulin secretion to maintain optimal intrauterine growth, leading to higher risk of low birthweight (70,190).

Further clinical benefits of a correct diagnosis of monogenic diabetes are the impact on predicting the clinical course. As already mentioned, the risk for diabetes related vascular complications is very low in *GCK* but increased for retinopathy and cardiovascular events in *HNF1A* MODY (63,68). Also, the possibility to elucidate futher clinical features that are correlated with an underlying genetic etiology. These features may be already present, like renal/urogenital abnormalities in *HNF1B* or hearing impairment in MIDD or they may develop later like exocrine pancreas deficiency in *PDX1* and *HNF1B* or renal dysfunction and cardiac problems in MIDD.

Precision diagnosis in clinical practice in monogenic diabetes

In clinical practice, precision diagnosis involves the use of a combination of clinical assessments, laboratory tests, imaging studies, and genetic analysis to make an accurate diagnosis and develop a targeted treatment plan.

Key strategies that in combination can help clinicians achieve precision diagnosis now and in the future:

1. Collect detailed patient information: Comprehensive and accurate information about a patient's medical history, family history, symptoms, and physical examination findings can help clinicians narrow down potential diagnoses.

2. Utilize laboratory tests and imaging studies: Laboratory tests like C-peptide, T1D autoantibodies, further biomarkers and genetic testing, as well as imaging of brain, heart, pancreas and kidneys can help clinicians identify specific abnormalities that may be contributing to correct diagnosis.
3. Work collaboratively with specialists: Precision diagnosis often involves a multidisciplinary approach, to evaluate complex cases and develop targeted treatment plans, for instance in rare syndromic diabetes forms.
4. Use machine learning and artificial intelligence in future: Machine learning algorithms and artificial intelligence tools can help clinicians analyze large amounts of patient data to identify patterns and potential diagnoses that may not be immediately apparent through traditional diagnostic methods (191).
5. Consider personalized treatments: Precision diagnosis can lead to the development of personalized treatment plans to optimize treatment outcomes and improve quality of life of our patients.

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