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THE FINNMODY STUDY

CLINICAL CHARACTERIZATION OF MATURITY-ONSET DIABETES OF THE YOUNG (MODY) IN FINLAND

Jarno Kettunen

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Medicine of the University of Helsinki, in Niilo Hallman lecture hall on the 22nd of October 2021 at 12 o'clock noon.

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i. ABSTRACT

The most common form of monogenic diabetes is called Maturity-onset Diabetes of the Young (MODY), which accounts for 1–3% of all cases of diabetes. Initially, MODY was a diagnosis for a familial form of diabetes that occurred in the lean and the young, demonstrated no dependence on exogenous insulin, and followed a dominant pattern of inheritance. Today, the clinical manifestation is more heterogeneous, and MODY has increasingly become a genetic diagnosis. More than 90% of the pathogenic gene variants responsible for MODY reside in *GCK, HNF1A, HNF4A*, or *HNF1B*.

Although classical Mendelian diseases involve fully penetrant and distinctive phenotypes, heterogeneity in patients with MODY is pronounced. Even those with the same gene variant manifest with diverse clinical presentations. The characterization of gene–disease associations and heterogeneity in patients with MODY have inspired the three studies included in this thesis.

In Study I, our aim was to systematically assess hepatobiliary and pancreatic manifestations in 14 Finnish patients affected by pathogenic gene variants of *HNF1B*. The patients underwent magnetic resonance imaging and magnetic resonance cholangiopancreatography. In conclusion, half of the patients (7 of 14) had an anomalous finding of the biliary system, and 6 of them had bile duct cysts (BDCs). Although untreated BDCs have generally been associated with a substantial risk of malignant transformation, it is not known whether the BDCs of genetic origin are similarly premalignant.

In Study II, the aim of the international effort was to establish whether heterozygous protein-truncating variants (PTVs) in *RFX6* are a novel genetic aetiology for MODY. Comparing between independent patient and control cohorts, we found that the *RFX6* PTVs were enriched among the patients, whose clinical presentation was strongly suggestive of MODY, and among those routinely referred to genetic testing for MODY. In addition, the individuals heterozygous for the *RFX6* PTVs demonstrated dysglycaemia and lower levels of serum glucose–dependent insulinotropic polypeptide (a.k.a. gastric inhibitory polypeptide, GIP).

Study III was a multigenerational, longitudinal and cross-sectional family-based characterization study with a specific focus on the clinical and metabolic presentation of *HNF1A* p.(Gly292fs), the most common pathogenic variant responsible for HNF1A-MODY. The 12 families studied included 145 heterozygous carriers of the variant and their 139 first-degree relatives without the variant. Three of the 12 families were large multigenerational families who have continued their extensive follow-up ever

since they were first identified and reported by our group in the 1990s. In conclusion, the carriers were leaner than the non-carriers, and they demonstrated enhanced lipolytic activity. Plasma glucose levels were higher in carriers than in non-carriers throughout the OGTT, and suggestive of insulin deficiency, serum insulin levels were lower in carriers than in noncarriers during the OGTT response. Although most carriers developed diabetes at a young age, one-third remained free of diabetes at 33 years. The polygenic risk score for type 2 diabetes also modified the age at onset of diabetes in patients with HNF1A-MODY.

Studies I–III and numerous previous studies have indicated that patients with MODY are vastly heterogeneous. National efforts, including the studies conducted in Finland, might play a major role due to possible population differences. Personalized tailoring of medical therapy (*e.g.* a switch from insulin treatment to oral agents) is often possible regardless of the clinical presentation and origin of a patient, but further research is essential to explore individual predictors of the treatment response. Although the response has only rarely been assessed in engineered human cell line models, *in vitro* studies could provide novel mechanistic insights concerning MODY and other monogenic forms of diabetes.

To summarize, systematic studies on individuals with a pathogenic gene variant can uncover profound heterogeneity associated with monogenic diabetes. These studies provide a valuable source for genetic laboratories to produce high-quality gene reports. Precision medicine in monogenic diabetes is progressively becoming a reality.

ii. TIIVISTELMÄ (IN FINNISH)

Yleensä diabeteksen taustalla on yksittäisen syyn sijaan monen perintö- ja ympäristötekijän ryväs, mutta monogeeninen diabetes vaatii kehittyäkseen vain yksittäisen geenimuutoksen. Monogeenisen diabeteksen yleisin muoto on MODY (Maturity-Onset Diabetes of the Young), joka kattaa noin 1-3 % diabetestapauksista.

Alkujaan MODY viittasi harvinaiseen ja perheittäin esiintyvään diabetekseen, joka kehittyi hoikille nuorille ja periytyi vallitsevasti (dominantisti) puolille jälkeläisistä. Insuliinihoitoa ei tarvittu. Viime vuosikymmenten geenitutkimukset ovat kuitenkin paljastaneet, että yhä useamman MODY-potilaan oirekuva ja ilmiasu ovat huomattavasti alkuperäistä määritelmää monenkirjavampia. Kahdella perheenjäsenellä voi olla hyvin erilainen ilmiasu, vaikka molemmat kantavat samaa geenimuutosta.

Väitöskirjan ensimmäiseen osatyöhön osallistui 14 potilasta, jotka kantavat muutosta *HNF1B*-geenissä. MODY-diabeteksen lisäksi *HNF1B*-potilailla todetaan useamman elinjärjestelmän muutoksia. Kartoitimme potilaiden sappiteitä magneettikuvauksella, ja puolella tutkimukseen osallistuneista todettiin sappiteiden kystia. Sappitiekystojen on uskottu vaativan leikkaushoitoa.

Toisessa osatyössä etsimme uusien geenien yhteyttä MODY-diabetekseen. Osallistujien diabetes oli MODY-tyyppinen, mutta geeniseulonta ei paljastanut muutoksia tunnetuissa MODY-geeneissä. Poikkeuksellisen monella osallistujalla todettiin kuitenkin harvinainen *RFX6*-geenimuutos. Kansainvälisen yhteistyön tuloksena *RFX6*-muutokset voitiin yhdistää MODY-diabetekseen, joka kehittyi vain osalle kantajista. Kantajilla todettiin myös alhainen GIP-suolistohormonin taso.

Kolmannessa osatyössä jatkoimme 1990-luvulla käynnistynyttä monisukupolvista tutkimusta perheissä, joissa MODY-diabetesta aiheuttaa yleisin MODY-geenimuutos *HNF1A* p.(Gly292fs). Perheisiin kuului 145 geenimuutoksen kantajaa ja heidän 139 perheenjäsentään ilman muutosta. Tulokset paljastivat kantajien olevan perheenjäseniään hoikempia. Lipolyysi eli kehon rasvan hajoaminen oli kantajilla aktiivisempaa. Vaikka kantajien diabetes alkoi usein nuorena, heistä kolmanneksella ei ollut diabetesta vielä 33-vuotiaana. Tyypin 2 diabeteksen riski vaikutti MODY-diabeteksen alkamisikään.

Tämän väitöskirjan tutkimukset vahvistavat aiempia havaintoja MODY-diabeteksen monimuotoisuudesta. Vaikka MODY-diabeteksen tunnistaminen onkin haastavaa, yksittäisen potilaan diagnoosi saattaa

mullistaa diabeteksen hoidon. Joskus insuliinipistokset voidaan esimerkiksi korvata tablettilääkkein. MODY-tutkimus on tärkeää myös kansallisella tasolla, jotta Suomessakin yhä useampi pääsee oikeaan diagnoosiin ja siten yksilöllisen hoidon piiriin.

iii. CONTENTS

iv. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. **Kettunen JLT**, Parviainen H, Miettinen PJ, Färkkilä M, Tamminen M, Salonen P, Lantto E, Tuomi T. Biliary Anomalies in Patients with *HNF1B* diabetes. *Journal of Clinical Endocrinology and Metabolism* 2017: 102(6):2075-2082.

II. Patel KA*, **Kettunen J***, Laakso M, Stančáková A, Laver TW, Colclough K, Johnson MB, Abramowicz M, Groop L, Miettinen PJ, Shepherd MH, Flanagan SE, Ellard S, Inagaki N, Hattersley AT, Tuomi T, Cnop M, Weedon MN. Heterozygous *RFX6* protein truncating variants are associated with MODY with reduced penetrance. *Nature Communications* 2017: 8(1):888.

III. **Kettunen JLT**, Rantala E, Dwivedi OP, Isomaa B, Sarelin L, Kokko P, Hakaste L, Miettinen PJ, Groop LC, Tuomi T. A multigenerational study on phenotypic consequences of the most common causal variant of HNF1A-MODY. (Provisionally accepted, *Diabetologia*.)

* Equal contribution to the manuscript

v. ABBREVIATIONS

(hs)CRP	(High-sensitive) C-reactive protein
ACMG	American College of Medical Genetics and Genomics
AD	Autosomal dominant
ADA	American Diabetes Association
ALT	Alanine aminotransferase
AMP	Association for Molecular Pathology
APBJ	Anomalous pancreaticobiliary junction
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
Apo(M)	Apolipoprotein (M)
AR	Autosomal recessive
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BA	Bile acid
BDC	Bile duct cyst
BMI	Body mass index
Botnia PPP	The Prevalence, Prediction and Prevention of Diabetes – Botnia Study
CAKUT	Congenital anomalies of kidneys and urinary tract
CaSR	Calcium-sensing receptor
cffDNA	Cell-free foetal DNA
CGL	Congenital generalized lipodystrophy
CHI	Congenital hyperinsulinism
CKD	Chronic kidney disease
CndG	Candidate gene approach
CNS	Central nervous system
D+/-	Diabetic/non-diabetic
DEND	Neonatal diabetes, developmental delay and seizures (syndrome)
DM	Diabetes mellitus
DPP4	Dipeptidyl peptidase-4
DPP4i's	DDP4 inhibitors
EEC	Enteroendocrine cell
EHR	Electronic health record
EMSA	Electrophoretic mobility shift assay
Endo-ERN	The European Reference Network on rare endocrine conditions
ER	Endoplasmic reticulum
ESC	Embryonic stem cell
ESRD	End-stage renal disease
ExAC	The Exome Aggregation Consortium
FIMM	Institute for Molecular Medicine Finland
FISH	Fluorescence *in situ* hybridization
FPG	Fasting plasma glucose
FSIVGTT	Frequently sampled IVGTT
G-6-P	Glucose 6-phosphate
GCK	Glucokinase
GD	Gene discovery
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GH	Growth hormone

GIP	Glucose-dependent insulinotropic polypeptide (a.k.a. gastric inhibitory polypeptide)
GLP-1	Glucagon-like peptide 1
GLP-1RA	GLP-1 receptor agonist
gnomAD	The Genome Aggregation Database
GSIS	Glucose-stimulated insulin secretion
GT	γ-glutamyltransferase
HbA1c	Glycated haemoglobin
HCA	Hepatocellular adenoma
HDL	High-density lipoprotein
HEC	Euglycaemic hyperinsulinaemic clamp
hez	Heterozygous
HGC	Hyperglycaemic clamp
hiPSC	Human induced pluripotent stem cell
HK-II	Hexokinase II
HLA	Human leukocyte antigen
HNF(1α)	Hepatocyte nuclear factor (1α)
HNF1BD	*HNF1B*-related disease
HOMA	Homeostasis model assessment
hoz	Homozygous
IFG	Impaired fasting glucose
IGF1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
IPEX	*FOXP3*-associated polyendocrinopathy
IPF1	Insulin promoter factor-1
IUGR	Intrauterine growth restriction
IVGTT	Intravenous glucose tolerance test
K_{ATP} channel	ATP-sensitive potassium channel
Kir6.2	The potassium channel subunit of the K_{ATP} channel
KO	Knock-out
L	Linkage
LDL	Low-density lipoprotein
LMW	Low-molecular-weight
LRG	Locus Reference Genome (database)
MELAS	Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes
METSIM	The Metabolic Syndrome in Men study
MIDD	Maternally inherited diabetes and deafness (syndrome)
MODY	Maturity-onset Diabetes of the Young
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
ND	Neonatal diabetes
NFG	Normal fasting glucose
NGS	Next-generation sequencing
NGT	Normal glucose tolerance
NIPT	Non-invasive prenatal testing
NLS	Nuclear localization sequence (or nuclear localization signal)
OAT	Organic anion transporter
OGTT	Oral glucose tolerance test
OMIM	Online Mendelian Inheritance in Man (database)
P-	Plasma-
PEO	Progressive external ophthalmoplegia
PND	Permanent neonatal diabetes
POU	Pit-1 / Oct-1/2 / UNC-86 (homeodomain)
PTH	Parathyroid hormone
PTV	Protein-truncating variant
RCC	Renal cell carcinoma

RefSeq	Reference sequence in the NCBI database
RFX	Regulator factor X
SGLT2	Sodium-glucose cotransporter 2
SGLT2i's	SGLT2 inhibitors
SU	Sulfonylurea
SUR1	The SU receptor subunit of the K_{ATP} channel
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TND	Transient neonatal diabetes
tRNA	Transfer RNA
VCEP	Variant Curation Expert Panel
VDCC	Voltage-dependent calcium channel
WES	Whole-exome sequencing
WGS	Whole-genome sequencing
WHO	World Health Organization
VNTR	Variable number of tandem repeat
WS	Wolfram syndrome

1 INTRODUCTION

Outlining MODY, a familial form of atypical diabetes

Young-onset diabetes often manifests with an acute presentation suggestive of massive and progressive destruction of insulin-producing β cells, a hallmark of type 1 diabetes. Although the prognosis of type 1 diabetes was desperately fatal before the momentous discovery of insulin in 1921, diabetes in the young has not always signified a drastic natural history of type 1 diabetes. Unfortunately, early anecdotal observations of such young-onset but mild diabetes[1] fail to meet the current scientific standards.

Almost a century ago, in 1928, Dr Cammidge portrayed two families with glycosuria that followed a Mendelian dominant pattern of inheritance[2]. The "dominant variety" he presented was a hereditary and "almost invariably mild" form of diabetes. Regardless of whether the diabetes was of a long duration, complications hardly emerged, even in those with young onset. Unfortunately, blood sampling was rare back in the 1920s, and most, if not all, evidence in this study relied on urine samples. Therefore, the patients might have actually been affected by renal glycosuria, another Mendelian dominant condition[3] later associated with *SLC5A2,* and not by diabetes. Moreover, Dr Cammidge had not specified the diagnostic criteria used in this study (the first universal diagnostic criteria for diabetes would not be introduced for decades[4]).

In the 1960s and 1970s, screening for glucose tolerance among those without symptomatic diabetes gained growing interest, and reports on mild young-onset diabetes began to surface[5,6]. Although some patients ultimately progressed to type 1 diabetes[7,8], others did not present with any clinical deterioration during the follow-up[9–11]. Gradually, it became clear that young-onset diabetes is not a specific disease, but it covers a heterogeneous group of conditions[12].

In 1960, Dr Fajans and Dr Conn conducted an interventional study in 14 non-obese participants, most of whom had a family history of diabetes, and all of whom had been diagnosed with asymptomatic diabetes at a young age (16–33 years). Strikingly, all 14 participants improved their glucose tolerance with tolbutamide[5], an oral antihyperglycaemic agent that belongs to the class of sulfonylureas. To adhere with the then-standard diabetes classification, their diabetes was paradoxically called maturity-onset diabetes instead of young-onset diabetes[13]. The young, who presented with acute symptoms and dependence on insulin treatment, had juvenile-onset (young-onset) diabetes,

whereas those not presenting with acute symptoms and dependence on insulin treatment had maturity-onset diabetes by definition.

Finally, in 1974, Dr Tattersall formally described a novel type of diabetes with young onset and a distinctive, autosomal dominant pattern of Mendelian inheritance[14]. In addition to the inheritance pattern, the clinical presentation of this diabetes resembled neither maturity-onset nor juvenile-onset diabetes. Most patients in the three families were non-obese and had been diagnosed in their teens or early twenties but without a typical presentation of juvenile-onset diabetes. Symptoms (if any) were typically non-severe, complications were surprisingly few (excepted for severe retinopathy in some), and patients were clinically non-dependent on exogenous insulin. Although a subgroup of four patients demonstrated a delayed and subnormal insulin response to glucose, the response was nevertheless measurable. Two families had also a low renal threshold for glucose.

The following year, Dr Tattersall joined with Dr Fajans to further identify novel patients. As a result of their collaboration, a new term for this specific type of diabetes was coined: *m*aturity-*o*nset type of *d*iabetes of *y*oung people, MODY[15]. The acronym and the term (currently referred to as *m*aturity-*o*nset *d*iabetes of the *y*oung) have remained in medical jargon ever since. Of note, an alternative term, *Mason diabetes*[16] (after the first family presented by Dr Tattersall in his 1974 paper) disappeared by the 1990s.

Pioneering genetic studies in the 1970s identified a strong association between type 1 diabetes and the human leukocyte antigen (HLA) system[17–20]. Because the HLA system was not associated with MODY in successive studies[21,22], it became clear that the genetic architecture for all forms of young-onset diabetes was not common. Nor was MODY associated with the human proinsulin gene (*INS*)[23], and extremely rare variants in *INS* were later associated only with infrequent cases of MODY[24,25].

In conclusion, MODY was a rare form of diabetes whose clinical presentation and genetic background were clearly different from common forms of diabetes. Multiple sources have depicted various diagnostic criteria for MODY. Its clinical presentation has been associated with young onset (before 25–35 years of age at least in 1–2 family members), absence of obesity, a family history of diabetes extending to at least 2 or 3 generations, an absence of autoimmunity, and sensitivity to sulfonylureas and independence on exogenous insulin. However, these clinical criteria neglect some of the vast heterogeneity associated with MODY, which has been uncovered by accumulating evidence from genetic studies since the 1990s. A current overview on MODY and other Mendelian forms of diabetes will follow in the next chapter.

2 REVIEW OF THE LITERATURE

2.1 Monogenic forms of diabetes

2.1.1 Classification of monogenic forms of diabetes

There are no standardized practises to categorize monogenic forms of diabetes. This thesis has adopted a five-level classification with 1) MODY, 2) (monogenic) neonatal diabetes, 3) diabetes associated with monogenic autoimmune endocrinopathies, 4) diabetes associated with monogenic insulin resistance, and 5) other syndromic monogenic conditions accompanied by high-prevalent diabetes. Most classifications include MODY and neonatal diabetes. Monogenic mitochondrial diabetes is not strictly a Mendelian disease but is reviewed in section 2.5 *(Monogenic mitochondrial diabetes)*.

2.1.2 MODY is the main form of monogenic diabetes

Various subtypes of MODY *(Table 1)* account for 1–2% of all diabetes[26–28], and 1.2–3% of paediatric diabetes[27,29–35], whereas other types of monogenic diabetes are far less prevalent. For example, the prevalence of neonatal diabetes, the other main form of monogenic diabetes, is approximately 1:100 000 live births[28,36]. *GCK, HNF1A, HNF4A* and *HNF1B* harbour a clear majority (>90%) of the pathogenic variants identified in patients with MODY.

Table 1

Subtype, and an OMIM identifier	**Gene symbol, product**	**Proportion of MODY[37]**	**Method of the gene discovery**	**Inheritance model**	**Notes**	**Reference for gene discovery**
HNF4A-MODY (MODY1) # 125850	*HNF4A,* Hepatocyte nuclear factor 4α	5–10%	L (linkage) and FISH	AD	*see section 2.3 for further information*	[38]
GCK-MODY (MODY2) # 125851	*GCK,* Glucokinase	30–70%	CndG (candidate gene) and L	AD	*see section 2.2 for further information*	[39–42]
HNF1A-MODY (MODY3) # 600496	*HNF1A,* Hepatocyte nuclear factor 1α	30–70%	L and FISH	AD AR[43]	*see section 2.3 for further information*	[44]
IPF1-MODY (MODY4) # 606392	*PDX1,* Insulin promoter factor-1 (IPF1) [a]	<1%	L	AD AR?	† , *see text below*	[45]
HNF1B-MODY (MODY5 / RCAD) # 137920	*HNF1B,* Hepatocyte nuclear factor 1β	5–10%	CndG	AD	*See section 2.3 for further information*	[46]
NEUROD1-MODY # 606394	*NEUROD1,* Neurogenic differentiation 1 (NeuroD1)	<<1%	CndG	AD	†, varying presentation of diabetes, only a few patients reported[47–50]	[47]
KLF11-MODY # 610508	*KLF11,* Krüppel-like factor 11	<<1%	CndG	AD	†, *see text below,* mild diabetes with insulin resistance,	[51]

					currently not considered a subtype of MODY	
CEL-MODY # 609812	*CEL,* Carboxyl ester lipase	<<1%	L	AD	VNTRs in exon 11 associated with impaired β cell function, pancreatic lipomatosis, progressive exocrine dysfunction[52–54]	52
PAX4-MODY # 612225	*PAX4,* Paired box gene 4	<<1%	CndG	AD	†, *see text below,* few non-Europid patients reported[55–58]	55
INS-MODY # 613370	*INS,* Proinsulin (prohormone precursor to insulin)	<1%	CndG	AD	Impaired insulin expression or action[24,25,59], no definitive onset age to discriminate between the PND and MODY, *see also section 2.1.3*	24,25
BLK-MODY # 613375	*BLK,* B-lymphoid tyrosine kinase	<<1%	L	AD	† *see text below*[60,61], association with overweight and obesity?	60
ABCC8-MODY (* 600509)	*ABCC8,* Adenosine triphosphate (ATP)-binding cassette, sub-family C, member 8 [b]	<1%	CndG	AD	Typically, good response to SUs, no definitive onset age to discriminate between the PND/TND and MODY, can also present as CHI and later with diabetes[62,63],	62,64

					see also section 2.1.3	
KCNJ11-MODY # 616329	*KCNJ11*, Potassium channel, inwardly rectifying subfamily J, member 11 [b]	<1%	L/WES	AD	Typically, good response to (high-dose) SUs[65,66], no definitive onset age to discriminate between PND and MODY	[67,68]
APPL1-MODY # 616511	*APPL1*, Adaptor protein, phosphotyrosine interaction, PH domain, and leucine zipper containing 1	<<1%	WES	AD	Identified only in two families (inconclusive level of evidence)	[69]
WFS1-MODY	*WFS1*, Wolframin	<<1%	WES (and L)	AD	Hoz and compound hez *WFS1* variants are associated with WS[70], whereas p.(Trp314Arg) is responsible for dominantly inherited diabetes without syndromic features	[71]
RFX6-MODY	*RFX6*, Regulatory factor X6	<<1%?	WES	AD	*See section 2.4 and Study II for further information and references*	[72]

Table 1. Monogenic forms of diabetes associated with MODY[28,37,73,74]. Proportion of MODY (%), the proportion of all MODY attributable to the subtype (depending on the population screened and the criteria for diagnostic genetic testing, most estimates derive from data on Western countries); L, linkage study; FISH, fluorescence in situ *hybridization; CndG, candidate gene approach; AD, autosomal dominant; AR, autosomal recessive; RCAD, renal cysts and diabetes syndrome; VNTRs, variable number of tandem repeats; PND, permanent neonatal diabetes; TND, transient neonatal diabetes; SUs, sulfonylureas; CHI, congenital hyperinsulinism; WES, whole-exome sequencing; hoz, homozygous; hez, heterozygous; WS, Wolfram syndrome*

[a] *Alternatively, pancreatic and duodenal homeobox 1 (PDX1)*

[b] *ABCC8 encodes the sulfonylurea receptor subunit (SUR1) of the β-cell K_{ATP} channel, whereas KCNJ11 encodes the potassium channel subunit (Kir6.2) of the same channel*

[†] *Some variants reportedly associated with the subtype are present in population databases with rather high frequencies, which disputes the proposed gene–disease association. The gene could modulate the risk of polygenic diabetes yet not be responsible for MODY.*

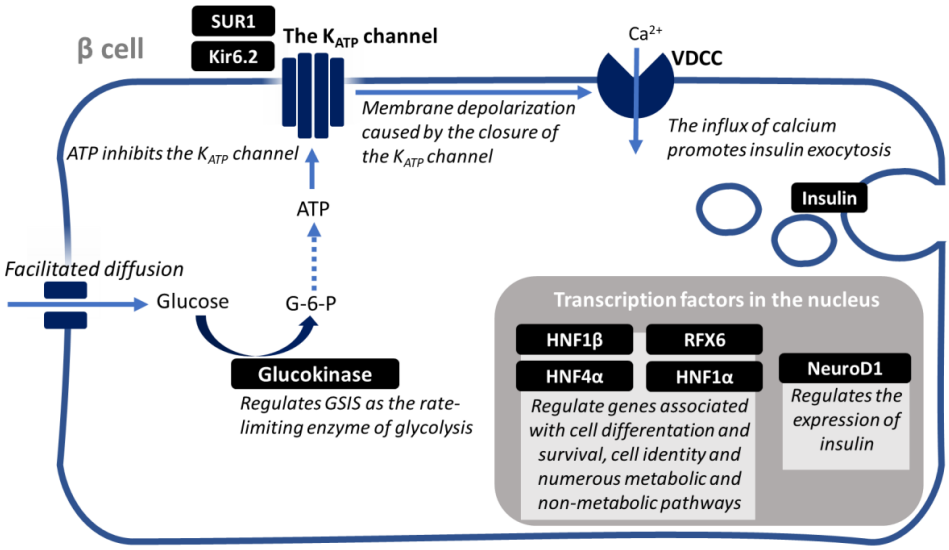

Figure 1. A schematic diagram of the β cell illustrates pathways and relative cellular locations of subtypes of MODY. The KATP channel consists of four subunits of Kir6.2 (encoded by KCNJ11), and four subunits of SUR1 (encoded by ABCC8). VDCC, Voltage-dependent Ca^{2+} (calcium) channel; G-6-P, Glucose 6-phosphate; ATP, Adenosine triphosphate.

Some genes, *e.g. KCNJ11* and *GCK,* are associated both with MODY and neonatal diabetes, which are two clinically distinct forms of diabetes (*Table 1, Table 2*). However, previously reported associations between a gene and MODY are not necessarily definitive, regardless of a definitive association between the same gene and neonatal diabetes. For example, insulin promoter

factor-1 (IPF1), which is a transcription factor encoded by *PDX1*, is one of the genes with limited evidence on the association with MODY, although the evidence for neonatal diabetes is definitive. IPF1 regulates pancreatogenesis and controls the expression of key β cell products such as insulin[45]. Mice with a heterozygous KO demonstrate impaired insulin secretion and glucose tolerance[75,76], but evidence for a phenotype associated with heterozygosity in humans has been conflicting and scarce (reported patients have either demonstrated obesity and hyperinsulinism or diabetes and hypoinsulinism without obesity[77,78]). In contrast, a biallelic inactivation of *PDX1* is strongly associated with a severe phenotype in humans, who present with pancreatic agenesis and, consequently, permanent neonatal diabetes and exocrine pancreatic insufficiency[45,79–81].

2.1.3 Neonatal diabetes is either transient or permanent

The second most common form of monogenic diabetes, neonatal diabetes (ND or NDM), is further divided into transient neonatal diabetes (TND, also TNDM) and permanent neonatal diabetes (PND, also PNDM). ND usually manifests before 6(–9) months of age. *De novo* variants are common. Numerous subtypes of ND include additional features such as low birthweight, developmental delay, epileptic seizures, *etc.* A coexistence of neonatal diabetes, developmental delay and seizures are referred to as DEND (*D*evelopmental delay, *E*pilepsy and *N*eonatal *D*iabetes) syndrome. Most subtypes of neonatal diabetes are recessive, hence arising from homozygosity or compound heterozygosity, with the notable exception of ND associated with *KCNJ11* and *ABCC8*. For further information, refer to a review[82]. *Table 2* includes the genes associated with ND in order of decreasing prevalence, as adopted from the review by Lemelman *et al.*[82].

Table 2

Gene	Inheritance model	Transient or permanent	An alternative medical therapy to insulin treatment, if available
KCNJ11	AD	PND or TND	SU
ABCC8	AD	PND or TND	SU
6q24	AD (paternal duplications)	TND	
INS	AD (AR rare)	PND or TND	
GATA6	AD	PND	

EIF2AK3	AR	PND	
GCK	AR	PND	One case reported with a SU response[83]
PTF1A	AR	PND	
FOXP3	X-linked	PND	
ZFP57	Maternal imprinting defect	TND	
GLIS3	AR	PND	
PDX1	AR	PND	
SLC2A2	AR	PND or TND	
SLC19A2	AR	PND	Thiamine (infrequently)
GATA4	AR	PND	
NEUROD1	AR	PND	
NEUROG3	AR	PND	
NKX2-2	AR	PND	
RFX6	AR	PND	
IER3IP1	AR	PND	
MNX1	AR	PND	
HNF1B	AD	TND	

Table 2. Genes associated with neonatal diabetes, adopted from [82]. If no alternative treatment is given, patients need insulin treatment for diabetes. AD, autosomal dominant; AR, autosomal recessive; TND/PND, transient/permanent neonatal diabetes; SU, sulfonylurea.

2.1.3.1 Diabetes associated with monogenic autoimmune endocrinopathies

Deficits in immune tolerance are responsible for a broad spectrum of clinical conditions, which include polygenic conditions such as type 1 diabetes and monogenic conditions such as *AIRE*-linked[84] APECED (autoimmune

polyendocrinopathy-candidiasis-ectodermal dystrophy)[30,85,86]. The prevalence of autoimmune diabetes is highly variable in each monogenic autoimmune endocrinopathy[30,85,86]. For example, in APECED, the prevalence of diabetes is 2% among 5-year-old children and 13% at the age of 30 years, whereas in *FOXP3*-associated polyendocrinopathy (IPEX), the prevalence of diabetes is as high as 71%[85]. Other genes involved in monogenic autoimmune endocrinopathies and occasionally or frequently co-presenting with diabetes include *STAT3* (30% of patients having diabetes)[85] and *CTLA4* (8% of patients having diabetes)[87,88].

The heterogeneity between individuals with the same genetic finding is considerable, and the individual phenotype could also reflect the polygenic risk for autoimmunity. For example, several (20%) of the 60 Finnish patients with APECED eventually developed autoimmune diabetes[89], which could reflect the high background risk of type 1 diabetes in Finland.

2.1.3.2 Diabetes associated with monogenic insulin resistance

Polygenic insulin resistance contributes to or interacts with obesity, fatty liver disease, type 2 diabetes, certain medications, and chronic inflammation as well as infection. Polygenic insulin resistance, therefore, affects a substantial proportion of the population. Monogenic forms of insulin resistance, in contrast, are extremely rare and include monogenic lipodystrophies, primary insulin signalling defects and other syndromes with severe insulin resistance[90–93].

Lipodystrophies, either congenital or acquired, disrupt adipocyte energy storage. Secondary to the adipocyte function, glucose metabolism in liver and muscle becomes impaired. Congenital generalized lipodystrophies (CGL), manifesting with a global absence of subcutaneous adipose tissue soon after birth, are autosomal recessive disorders associated with genes such as *AGPAT2, BSCL2, CAV1, PTRF* and *PPARG*. Also, *LMNA* has been associated with CGL but with an autosomal dominant inheritance pattern. Autosomal dominant inheritance is more typical of familial partial lipodystrophies characterized by a decrease in adipose tissue primarily in the lower extremities. Associated genes include *LMNA, PPARG, PLIN1, CIDEC, LIPE, AKT2, ADRA2A, CAV* and *PCYT1A*.[90–93]

Primary insulin signalling disorders hamper the action of insulin on its receptor (generalized insulin signalling defects, primary "receptoropathies") or downstream of it ("postreceptor", partial insulin signalling defects)[94]. A few features are common in both generalized and partial defects, as well as in lipodystrophies. For example, high levels of circulating insulin, whatever the cause, can activate IGF1 receptors, which predisposes to *acanthosis nigricans*. In addition, partial insulin signalling defects (for example, those associated with *AKT2*) and lipodystrophies generally demonstrate secondary consequences of increased insulin signalling (such as metabolic dyslipidaemia and fatty liver disease). In primary receptoropathies, however, compensatory hypersecretion of insulin might fail to activate insulin

receptors.[94] Primary receptoropathies are either congenital or acquired. Congenital forms are attributable to pathogenic variants in *INSR,* encoding the insulin receptor (also referred to as type A insulin resistance syndrome), whereas acquired receptoropathies is an intermittent, permanent or resolving autoimmune condition characterized by inactivating autoantibodies to the insulin receptor (type B insulin resistance syndrome)[93–95]. *INSR*-related receptoropathies may involve a severe clinical presentation called Donohue syndrome (leprechaunism) or a milder presentation called Rabson–Mendenhall syndrome[96].

Other syndromic conditions with diabetes are far beyond the scope of this thesis. In the following sections, we shall dissect some intriguing features of the most common subtypes of MODY.

2.2 GCK-MODY (formerly MODY2)

2.2.1 Glucokinase couples plasma glucose to insulin secretion

Glucokinase (GCK, or hexokinase IV, HK-IV) is a 52 kDa two-domain hexokinase isozyme expressed in the central nervous system (CNS, reviewed in [97,98]) and, in the scope of this thesis, in liver and pancreas. GCK and other classic hexokinases catalyse the phosphorylation of glucose into glucose-6-phosphate (G-6-P), which is the first step of glycogen synthesis and the first step and the rate-limiting step of glycolysis. After glucose has undergone glycolysis in pancreatic β cells, ATP produced in the pathway triggers glucose-stimulated insulin secretion (GSIS).

GCK, located in human chromosome 7p, presents as three isoforms (A–C), each comprising 12 exons. The length of the first exons is distinctive to each isoform. The 465-residue isoform (A) is expressed in the pancreas, whereas isoforms (B,C) are found in the liver[99]. A conventional pancreatic reference for reporting *GCK* variants is NM_000162.X, which is an mRNA reference sequence (RefSeq) maintained by the National Center for Biotechnology Information (NCBI) database.

2.2.2 The prevalence of GCK-MODY

GCK-MODY, arising from inactivating variants in *GCK,* has covered a major proportion of the patients with MODY after it was discovered in 1992[39–42]. An early estimate from France concluded that 56% (18 of 32) of the families with MODY had an inactivating *GCK* defect[100], and the current estimation ranges between 30 and 70%[101–104] – largely depending on the age and ethnic distribution of the population, as well as clinical practices to identify patients with diabetes and/or MODY. Systematic screening to assess the population prevalence of GCK-MODY are few, but an investigation among pregnant

women in Ireland estimated that the population prevalence could be up to roughly 1 in 1000[101].

Contrary to the inactivating *GCK* variants that present as hyp***er***glycaemia (GCK-MODY), a few variants in *GCK* are activating and promote the enzymatic activity of the enzyme, hence increasing insulin secretion, and are responsible for hyp***o***glycaemia and congenital *hyper*insulinism (CHI)[105,106] (recently reviewed in [107]). The activating variants map to the allosteric site of GCK. The allosteric site can harbour both inactivating and activating variants, some of which are further pathogenic and others benign. Unfortunately, CHI falls out of the scope of this thesis.

2.2.3 GCK-MODY presents with persistent and mild fasting hyperglycaemia

Ever since birth, the glucose-sensing defect[100] associated with GCK-MODY produces persistent hyperglycaemia with fasting plasma glucose (FPG) ranging from 5.4–5.5 to 8–8.4 mmol/l. In a multicentre study, patients demonstrated a mean FPG of 6.8 mmol/l[108], and only 2% of patients had FPG <5.5 mmol/l[108]. In 71% of the patients, the 2-hr glucose increment during the OGTT was <3 mmol/l[108]. Also, a guideline report found 90% of patients having an increment <4.6 mmol/l[109].

However, in some patients with GCK-MODY, the 2-hr glucose increment during the OGTT can be more pronounced, as evidenced by a mean 2-hr increment of ~6 mmol/l in those heterozygous for *GCK* p.(Gly261Arg)[110]. In addition, patients with GCK-MODY can further display features of another form of diabetes, which modifies the OGTT response (see Figure 4 in [111]).

Fasting hyperglycaemia (FPG ≥5.5 mmol/l) in a patient with an inactivating variant in *GCK* is considered diagnostic of GCK-MODY. Of these patients, 42–68% formally meet the diagnostic criteria for diabetes[112,113].

2.2.4 *Insulin secretion in patients with GCK-MODY*

Whereas β cells usually elicit the maximal response with glucose levels around the upper normal range (5.5–6.0 mmol/l), the maximal response in patients with GCK-MODY occurs at 6.5–7.5 mmol/l[114]. These patients have also showed a right shift in the dose-dependent glucose response curve[115]. Postprandial hepatic glycogen synthesis is impaired[116]. The counterregulatory mechanisms to hypoglycaemia occur at higher glycaemic levels in patients with GCK-MODY than in healthy controls or in patients with T2D[117]. In a murine model, glucagon responses originated directly from α cells, whereas epinephrine responses appeared to be CNS-mediated[118].

2.2.5 Complications and treatment of GCK-MODY

Antihyperglycaemic treatment proved little efficacy in patients with GCK-MODY (*N*=799), whose HbA1c was similar regardless of whether they were being treated at referral to genetic testing (p=0.11)[119]. A small subgroup discontinued treatment for three months without deterioration in a glycaemic control[119]. Another small study replicated the findings[120].

GCK-MODY is not associated with micro- or macrovascular complications nor comorbidities associated with T2D[112,121], and the patients have an increased risk for background retinopathy but no other complications[122]. Owing to a low rate of complication *and* inefficacy of medication, guidelines (*e.g.* [29]) recommend discontinuing antihyperglycaemic medication.

However, evidence for GCK-MODY with HbA1c over 7.6% (~60 mmol/mol) or with high-dose medication is incomprehensive. Follow-up data on the elderly patients are scarce.

2.2.6 Gestational diabetes and GCK-MODY during pregnancy

During pregnancy, maternal glycaemia is a major determinant of foetal glycaemia. By the second trimester, a foetus begins to secrete insulin, a major regulator of foetal growth. Maternal hyperglycaemia, leading to foetal hyperglycaemia, can therefore elicit foetal insulin hypersecretion and consequent overgrowth of the foetus (macrosomia), a phenomenon called the Pedersen hypothesis[123–125]. Besides increasing the risk of macrosomia, uncontrolled gestational hyperglycaemia predisposes to maternal and neonatal adverse outcomes.

To recognize hyperglycaemia during pregnancy, mothers might be screened for gestational diabetes (mellitus) (GDM). GDM is a condition characterized by hyperglycaemia (FPG 5.1–6.9 mmol/l[126,127(section 3.B.1.4.)] or FPG ≥5.3 mmol/l in the Finnish guidelines[128]) with onset or first recognition during pregnancy[126,127(section 3.B.1.4.)].

An appropriate genetic diagnosis of GCK-MODY provides clinical implications for pregnant women[129–131]. The need for antihyperglycaemic treatment during pregnancy is determined by the foetal genotype of *GCK*[129], which has been indirectly determined based on foetal growth[132] by biweekly ultrasounds (after 26 gestational weeks). Chorionic villus sampling and amniocentesis are not recommended because of the risk of miscarriage[129,130].

If a foetus has inherited a maternal inactivating variant in *GCK*, the altered glucose sensing affects mother and foetus similarly. Foetal growth is normal, and the mother can abstain from antihyperglycaemic treatment. On the contrary, should a foetus not have inherited the maternal variant, maternal hyperglycaemia associated with GCK-MODY predisposes to foetal

macrosomia (usually defined by *e.g.* a foetal abdominal circumference above the 70–75th percentile[129–131]).

Complying with studies on gestational diabetes[133,134], the guidelines for gestational GCK-MODY in the UK recommend initiating insulin with FPG ≥6.7 mmol/l. Those with FPG ≤6.6 mmol/l[130] can opt for foetal growth screening. There is no rationale to infuse insulin during labour[130].

Of the pregnant women with GCK-MODY on insulin, 56% had experienced self-reported hypoglycaemia (23% had experienced severe hypoglycaemia)[131]. Hypoglycaemic events might challenge adherence to the guidelines[135]. The miscarriage rate (19%) in pregnancies with GCK-MODY has been similar to that of the background population (15–20%)[131].

Newly available non-invasive prenatal testing (NIPT) of cell-free foetal DNA (cffDNA) could become a gold standard of determining a foetal genotype[136]. NIPT techniques eliminate the need for indirect reasoning of foetal genotype in serial ultrasounds, besides providing a novel method to conduct future studies[136].

2.3 Hepatocyte nuclear factors (HNFs) associated with three subtypes of MODY, namely HNF1A-MODY, HNF4A-MODY and HNF1B-MODY

2.3.1 An introductory glance at the HNF family

Hepatocyte nuclear factors (HNFs, also stylized as *hepatic nuclear factors*) are tissue-specific transcription factors expressed in the liver, where they were first found[137], and also in several other tissues such as the kidneys and pancreas[138–141]. Considering the high degree of homology between HNF family members and a plethora of independent and interdependent roles across numerous transcriptional networks, HNFs participate in diverse and distinctive functions in various tissues. Furthermore, during embryonic development, HNFs interact with pathways that differ from those in later life. Additional complexity arises from autoregulation and crossregulation as well as from alternative splicing isoforms. Biological functions related to HNFs are still not uniform across species of mammals, although HNFs demonstrate a high level of conservation in vertebrates[138–142]. Taken together, outlining the molecular consequences of defective HNF function across diverse biological pathways during development and adult life has been exceptionally difficult.

Nonetheless, inactivating variants in genes encoding HNF can produce a pathophysiological clinical presentation in humans. To date, among the HNF subfamilies, HNF1 and HNF4 have been associated with MODY (see *Table 3*

for the RefSeq transcripts used to report variants in these genes), whereas FOXA (formerly HNF3) and HNF6 (ONECUT[140,143]) have not. Until recently, MODY associated with HNFs has been attributable to the presence of inactivating gene variants in heterozygosity. However, MODY might infrequently arise from hypomorphic homozygosity, as exemplified by individuals with homozygous p.(Ala251Thr) variants in *HNF1A*[43]. Each HNF subfamily includes molecular domains that are specific to the subfamily, but the subfamilies also have common features. For example, all HNFs can transactivate and bind to DNA.

Table 3.

Gene	RefSeq transcript (LRG transcript)	Length
HNF1A	NM_000545.8 (LRG_522)	631 amino acids
HNF1B	NM_000458.4	557 amino acids
HNF4A	NM_175914.4 (with the polyA tail removed in LRG_483)	452 amino acids

Table 3. The NCBI database maintains mRNA reference sequences (RefSeq) to report human gene variants in HNF1A, HNF1B or HNF4A. "X" denotes a version number. The corresponding LRG transcripts (in the Locus Reference Genome database) for HNF1A and HNF4A are in parentheses.

RefSeq, reference sequence; LRG, locus reference genome

2.3.1.1 HNF1 subfamily (HNF1α and HNF1β)

The HNF1 subfamily comprises two members, namely HNF1α[137] encoded by *HNF1A* (alias *TCF1,* chromosome 12q) and HNF1β[144] (formerly HNF2 and vHNF1 for "variant HNF1") encoded by *HNF1B* (alias *TCF2,* chromosome 17q). Inactivating pathogenic variants in *HNF1A* and *HNF1B* are responsible for two subtypes of MODY: HNF1A-MODY (formerly MODY3) and HNF1B-MODY (formerly MODY5).

HNF1α and HNF1β include a Pit-1 / Oct-1/2 / UNC-86 (POU) homeodomain and regulate the expression of hundreds genes as homodimers or heterodimers[138–142] by binding numerous binding targets on DNA[145]. HNF1α and HNF1β target the palindromic consensus sequence (GTTAATNATTANC)

and share a high degree (>80%) of sequence homology in the DNA-binding domain[140]. Besides displaying a high conservation across mammals, both HNF1α and HNF1β have homologues in non-mammals including fish and frog[146–152].

HNF1A harbours an N-terminal dimerization domain at residues 1–32, a DNA-binding domain with a bipartite POU domain at residues 91–279, and a C-terminal transactivation domain at residues 281–631[153,154]. The two subunits of POU interact with DNA and are named the POU_S domain (for POU-specific domain, residues 91–181) and POU_H domain (for POU-homeodomain, residues 203–279), with a structurally disordered region intervening in these subdomains[153,154]. POU_S stabilizes the protein, whereas POU_H orchestrates interaction between HNF1α and DNA[153,155], and the POU subdomains can target DNA from opposing directions *(Figure 2)*. Residues 33–97 form a flexible link between the dimerization and DNA-binding domains, and residues 197–205 act as a nuclear localization sequence (NLS, alias nuclear localization signal).[138–142,156–158]

HNF1α, expressed in the adult human (and rodent) gastrointestinal tract, liver, kidney (especially the proximal tubule[159]) and pancreatic β cells as well as immune system[140,145,160,161], has three isomers arising from alternative splicing and polyadenylation patterns[141,162]. The HNF1α(A) isomer displays ~5-fold less activity than the two truncated isomers, (B) and (C). HNF1α(A) is the main human and rodent isomer in the kidney and liver. HNF1α(A) also predominates in rodent pancreas throughout life and in foetal human pancreas, whereas HNF1α(B) is the leading isomer in adult human pancreas[141,162].

HNF1β also contains three main domains: an N-terminal dimerization domain (at residues 1–32), a DNA-binding domain with a bipartite PUO element (POU_S at residues 90–187, POU_H at residues 235–311), and a transactivation domain (at residues 311–557)[142]. In adult humans, besides in the tissues that express HNF1α, HNF1β is expressed in lung and reproductive tissues, although the hepatic expression is low[140,141]. Of the three isomers (A–C), HNF1β(A) predominates in pancreatic islets and HNF1β(B) in the liver and overall pancreas[141].

Haploinsufficiency (loss of function) has been the most widely accepted molecular mechanism associated with HNF1A-MODY, although some studies have proposed dominant-negative effects (especially regarding variants in the transactivation domain at residues 281–631 and the variant p.(Arg272Cys)[163])[154,163–171]. However, the evidence on negative-dominant effects has typically relied on luciferase reporters with supraphysiological levels of mutated protein expression or other conditions uncharacteristic to *in vivo* cells. Therefore, negative-dominant mechanisms are probably not relevant in living human cells.

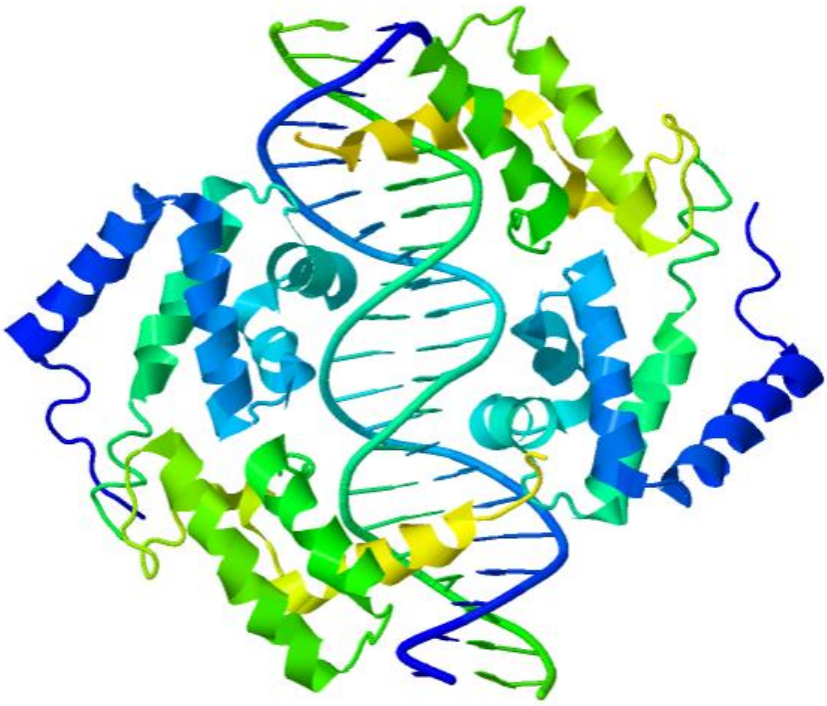

Figure 2. HNF1α binds to the DNA helix in this cartoon illustration. This protein structure of PDB ID 1IC8[153] *by* rcsb.org[172,173] *is created with JSMol, an open-source Java viewer for chemical structures in 3D,* http://www.jmol.org/.

2.3.1.2 HNF4 subfamily

The two members of the HNF4 subfamily, comprising HNF4α (alias NR2A1 for nuclear receptor subfamily 2, group A, member 1, encoded by *HNF4A* on chromosome 20q, formerly referred to as *TCF14*) and HNF4γ (encoded by *HNF4G* on chromosome 8q), also belong to a group of nuclear receptors[140]. Pathogenic, inactivating variants in *HNF4A* are associated with MODY, whereas those in *HNF4G* are not.

Unique to nuclear receptors, HNF4α can transactivate without a ligand. HNF4α is either an orphan or an adopted orphan nuclear receptor since evidence on fatty acids serving as ligands of HNF4α is not wholly conclusive[174,175].

Widely present in numerous adult tissues such as the liver and pancreas, HNF4α binds to DNA as a homodimer and consists of six structural domains (A–F): the transactivation domain AF-1 (activation function 1, alias A/B domain); DNA-binding domain including two zinc fingers (C domain) and followed by a hinge region; ligand-binding region (E domain); homodimerization region; second transactivation domain AF-2 (AF-1/2); and an F domain[140,176,177]. Unlike other subfamilies of HNFs, HNF4 members include an F domain, which is a negative regulatory element whose activation represses co-activators of the transactivation domain (AF-2). The F domain might additionally be able to suppress other regions besides AF-2[176,178]. HNF4α has 12 potential isomers, partly explained by the two alternative promoters, P1 and P2. Alternative splicing contributes to distinct spatial and tissue-dependent activities in human physiology and development[178–183]. The highest variation between the isomers occurs in the F and A/B regions (P1 is located downstream of P2 and selects the exon 1B, whereas P2 selects exon 1A, located upstream of both the exon 1B and the promoter P1)[184].

2.3.2 The gene discoveries of the HNFs responsible for MODY

In 1996, an international research collaboration used a linkage analysis and fluorescence *in situ* hybridization (FISH) to discover that *HNF4A* (20q13) is associated with MODY1[38] (now, HNF1A-MODY) and *HNF4A* (12q24) with MODY3[44] (now, HNF1A-MODY). The next year, a Japanese group using a candidate gene approach introduced *HNF1B* as the last HNF gene so far to be associated with MODY (MODY5, now HNF1B-MODY)[46]. After the original paper had discreetly implied an association between *HNF1B* and unspecified nephropathy, the clinical presentation associated with *HNF1B* disease gradually came to include major renal manifestation[185–188] (see 2.3.3.4).

2.3.3 Clinical characteristics in humans with HNF defects

2.3.3.1 Age at onset of diabetes and progressive hyperglycaemia

Although young onset has always been one the most fundamental features of MODY, the variability of the onset age has been exceedingly large. Some patients with MODY have developed diabetes decades after adolescence. However, some patients with mild to absent symptoms might have remained unaware of their diabetes. Routine screening is crucial in identifying those patients.

Already in 1974, Dr Tattersall had recognized that a considerable proportion of patients with MODY developed diabetes after 30 years of age; 23 of 38 (61%) individuals with diabetes had been diagnosed before the age of 30 (at a mean age of 18.4 years), and 15 (39%) after. Of the 23 diagnosed before the

age of 30, a majority (13) were diagnosed through routine screening and only 7 patients because of symptoms. Among those with a late diagnosis (after 30 years), the mean age at onset was 53 years (6 had been diagnosed in routine screening and 5 because of symptoms)[14].

MODY was originally a purely clinical diagnosis. The first families with MODY presented with distinctive phenotypes that fulfilled at least most clinical criteria set for MODY. For example, the penetrance of MODY in the R.W. family (a large multigenerational family from Michigan characterized by Dr Fajans), later identified to represent HNF4A-MODY, was rather high (95% of the variant carriers had diabetes, most by 14 years of age)[189]. Nevertheless, although the initial setting might have been distinctive in the families, a later deterioration of glucose tolerance was extremely variable[190].

In the late 1990s and early 2000s, a gradually growing number of patients could be referred to genetic diagnostic testing. MODY started to transform from a purely clinical diagnosis into a hybrid of a genetic and clinical diagnosis of diabetes segregating with a gene variant. Genetic testing has also been diluting the importance of clinical diagnostic criteria. If a gene variant has previously been pathogenic for MODY without a doubt, the clinical impact of such a variant must be evident in all patients with the same variant. Therefore, a patient without a classic clinical presentation of MODY yet diabetic and heterozygous for a pathogenic gene variant, is practically diagnosed with MODY. The phenotypic spectrum has become ever more heterogenous.

Consequently, whereas a review article published in 2001 had concluded that the onset of diabetes of MODY was *"usually"* before age 25[191], a review in 2008 was somewhat more restrained. In this paper, the penetrance of HNF1A-MODY was 63% by 25 years, 79% by 35 years, and 96% before 55 years[192]. In other words, almost one-third did not develop diabetes by age 25.

Population-based approaches have further questioned whether MODY is quite as penetrant as once thought. In the UK biobank, for example, less than 10% of the individuals heterozygous for an *HNF4A* (p.Arg114Trp) had diabetes by the age of 40 years, although the variant has previously been associated with strongly penetrant MODY[193]. A recent French study has also questioned the previous estimates on penetrance[194], as well as a recent preprint[195]. Yet, population-based studies cannot fully substitute family-based and cohort-based studies, which allow systematic, labour-intensive and occasionally more invasive approaches to characterize patients[111].

Many reviewers imply that glycaemic control progressively deteriorates in HNF1A-MODY (and occasionally in HNF4A-MODY)[196]. The evidence is scarce, however. In one study, FPG increased among the individuals with pathogenic *HNF1A* variants annually by 0.0446 mmol/l[108], whereas glucose-stimulated insulin secretion displayed an annual decline of 1–4% among those with HNF4A-MODY (the R.W. pedigree)[197]. Whether weight gain could partially be responsible for the worsening glycaemic control and whether the deterioration differs from that associated with other types of diabetes are unanswered questions to my knowledge.

Sulfonylureas might correct defective insulin signalling in a cell model of HNF1A-MODY[198] (preprint data), but human data *in vivo* are few. Perhaps a specific class of medication or glycaemic control could restore or maintain β cell function to some extent, as Dr Fajans proposed in 2011: *"Our experience suggests that good [glycaemic] control slows this decline [in estimated β cell function], but there are no controlled trials to prove this and such studies are needed"*[199].

Of the *HNF1B*-related phenotypes, structural kidney anomalies and impaired renal function are apparently far more common than diabetes. A systematic review on the phenotype of 211 patients with pathogenic *HNF1B* variants[200] concluded that only 95 carriers (45%) had diabetes, with a mean age at diagnosis of 24 years (SD ±13, range 0–61). The *HNF1B*-related disease (HNF1BD) expands far beyond diabetes, as later discussed.

The affected individuals with HNF-associated MODY manifest a highly variable age at onset of diabetes, which is also evident in the family members carrying the same causal variant. This variability has few known modifiers, and most studies have focused on HNF1A-MODY only. On average, women might develop HNF1A-MODY 3.0 years earlier than men[201], but not all studies have confirmed this result, which could obviously be subject to ascertainment bias. For example, a diagnosis of asymptomatic diabetes is far more likely among young women who undergo routine screening for gestational diabetes mellitus than among young men without such screening. BMI has no effect on the onset age[201].

In line with previous studies[162,202], Lango Allen *et al.* reported that those with pathogenic *HNF1A* variants in exons 1–7 (affecting most *HNF1A* isoforms) develop diabetes 5.2 years earlier than those with variants in exons 8–10 (affecting only the A isoform)[201]. A polygenic risk score (PRS) for T2D with 17 genetic variants modified the age at onset only moderately, by 0.35 years per each risk variant[201]. An earlier study from 2003 also suggested some modification by the loci 5p15, 14q24 and 9q22 (one of Dr Lango Allen's 17 variants, rs10811661, is located at 9q21 near *CDKNA2A/2B*)[203]. A murine locus named *Moda1* modulated the age at onset in a mouse model[204]. Of note, none of the murine homologs of the 17 genes included in Lango Allen's T2D-PRS belongs to *Moda1* (the closest being *NOTCH2,* as *Notch2* in murine chromosome 3 is tens of millions of nucleotides distant from *Moda1*).

A study in 2008 concluded that PTVs (protein-truncating variants) are associated with an earlier age at onset than missense variants (18 vs 22 years). Also, the localization of missense variants modulates the onset (dimerization/DNA-binding domain vs transactivation domain, 20 vs 30 years)[202]. However, an update is crucial, as these studies precede the introduction of the current genetic databases and other bioinformatic tools.

Recent studies on the genetic modifiers of the age at onset are even fewer. Among those with a PTV responsible for HNF1A-MODY, each allele of a common *HNF1A* variant (p.Ile27Leu) significantly decreased the age at onset. However, the effect was rather modest (only by 1.6 years per allele, 95% CI -2.6; -0.7)[205]. Exposure to hyperglycaemia during foetal development

(a.k.a. intrauterine or maternal hyperglycaemia) affects the age at onset[206,207] by a mean decrease of -5.1 years[201]. To distinguish whether the parental inheritance model (maternal inheritance) modulates the age at onset independent of intrauterine hyperglycaemia is far from clear.

In conclusion, modifiers of the age of onset of HNF1A-MODY remain largely unidentified. The data for HNF4A-MODY and HNF1B-MODY are even scarcer.

Finally, *HNF4A* and possibly *HNF1A* can present with "a dual phenotype". The young and adults with inactivating variants in *HNF4A* are to develop HNF4A-MODY, whereas neonates can demonstrate an opposite phenotype with transient or persistent[208] hyperinsulinaemic hypoglycaemia and, even more often, macrosomia (believed to be secondary to intrauterine hyperinsulinism)[209,210]. In the first report on the neonatal phenotype, the carriers' median birthweight was 790 g higher than that of non-carrier relatives, and among the carriers, the prevalence of macrosomia (defined as a birthweight >4,000 g) was at 56%, whereas the prevalence was only at 13% among the non-carrier relatives. Of the 54 neonates with an *HNF4A* variant, 8 (15%) had experienced transient neonatal hypoglycaemia (low insulin levels were documented in three babies, but there was no systematic evaluation of serum insulin)[209]. Macrosomia was equally common in all neonates heterozygous for the familial variant regardless of inheriting it maternally or paternally[209]. Hence, the observation cannot merely arise from maternal hyperglycaemia during the pregnancy. As for *HNF1A,* a more limited series of studies have analogously proposed a dual phenotype[211–213], but if such a phenotype does exist, the risk of macrosomia and/or hyperinsulinaemic hypoglycaemia is likely far more subtle than that associated with *HNF4A*[209].

2.3.3.2 β cell function and insulin sensitivity

As initially suggested in 1974 and later made evident by several researchers listed in detail in *Table 4*, the pathogenic variants in *HNF1A* and *HNF4A* lead to impaired β cell function, characterized by a clear deterioration of the first-phase and, to a lesser extent, second-phase (late-phase) insulin secretion. The studies on β cell function are relatively small, and direct comparisons between the heterozygous carriers of variants with diabetes and the carriers without diabetes are astonishingly few. Vaxillaire *et al.* found that β cell function was similar in the non-diabetic carriers (*N*=3) and in the control group (*N*=11). Indeed, evidence is limited, but progressive hyperglycaemia[108] is believed to parallel worsening β cell function. Insulin secretion in *HNF1B* disease has been evaluated in few individuals, with no clear indication of defective insulin secretion to my knowledge. However, as heterogeneity in

the *HNF1B*-related clinical spectrum is vastly broad and often involves pancreatic hypoplasia (see 2.3.3.3), future studies with a larger number of individuals are essential in uncovering possible defects in insulin secretion in some proportion of these patients.

The question on insulin sensitivity is far more complex *(Table 4)*. There is no evidence for a strong effect on insulin sensitivity associated with inactivating variants in *HNF1A* and *HNF4A*. However, the variants might modulate insulin sensitivity indirectly. An increase in insulin sensitivity could, for example, originate from the body composition (in one study, those affected by an inactivating variant in *HNF1A* were leaner than the control group[214], although the difference was not statistically significant) or from a control group's genetic susceptibility to insulin resistance (in another study, the control group comprised of non-diabetic individuals with a family history of type 2 diabetes[215], which might predispose to insulin resistance[216–220]). *Vice versa,* as reported by Vaxillaire *et al.*[214], a decrease in insulin sensitivity could be secondary to body composition (those affected by an *HNF1A* variant had a higher BMI than the control group, although this difference was not statistically significant) or, as suggested by the authors, to pancreatic deficiency and/or chronic hyperglycaemia. Considering HNF4A-MODY, one study[189] has speculated on the presence of decreased insulin sensitivity, but the assumptions for the model used to estimate insulin sensitivity were possibly violated[221] *(Table 4)*. To conclude, evidence on insulin sensitivity is inconclusive.

In contrast, limited evidence on *HNF1B* implies that hepatic insulin sensitivity is decreased, and peripheral insulin sensitivity is normal in HNF1B-MODY[222].

Of note, following a glucose challenge or a stimulus containing protein/fat besides carbohydrates, an inappropriate suppression of glucagon secretion from α cells has been reported in HNF1A-MODY[223–225].

Table 4.

Study and associated genes	Herman *et al.* 1994[189], variant in R.W. family later identified as *HNF4A,* p.(Gln255Ter)[38]	Tattersall 1974[14] (In two of the three clinically diagnosed families with MODY, including family M., MODY was later learnt to arise from *HNF1A* variants)
Method	FSVIGTT (with tolbutamide), and a separate glucose infusion protocol	50 g OGTT
Study population	4 D+ carriers and 5 D- carriers	4 patients from the three families, of whom at least two were from family M
Control population	6 D- non-carrier relatives, and an unrelated control group of healthy individuals (11 for the FSIVGTT study and 7 for the glucose infusion)	None
Results	*D- carriers vs D- controls:* FSIVGTT: Glucose and insulin concentrations ↔ Insulin sensitivity (S_i) ↔ Rate of glucose disappearance (K_g) ↔ (but ↑ as compared with an additional control group) Glucose effectiveness (S_g) ↔; Estimated insulin secretion rate ↓ *D+ carriers vs D- controls:* Insulin sensitivity (S_i) ↔ (or ↓, because the mean S_i was clearly but insignificantly lower in D+ carriers; however, a small sample size precludes definite reasoning and/or statistically significant results, with a note that some clinical features in MODY probably violate model assumptions[221]); Glucose effectiveness (S_g) ↓ Rate of glucose disappearance (K_g) ↓ (also ↓ *vs* D-carriers) Acute insulin response (AIR) ↓ Estimated insulin secretion rate ↓	Insulin response during the OGTT ↓ (delayed and subnormal (No numeric data available)

Study and associated genes	Lehto *et al.* 1997[215], *HNF1A*, p.(Gly292fs) in families B–D, and p.(Thr160Met) in family A	Hansen *et al.* 1996[227], poster likely *HNF1A*, as the diabetes was linked to the chromosome 12q	Byrne *et al.* 1995[226], variant in the R.W. family later identified as *HNF4A* p.(Gln255Ter)[38]
Method	75 g OGTT and Botnia clamp (comprising sequentially performed IVGTT and HEC[111])	FSIVGTT with 33 time points[111], tolbutamide at 20 mins	Graded glucose infusions
Study population	57 carriers with diabetes; 18 carriers without diabetes (14 D+/D- carriers vs 264 D+/D- familial/population controls in the Botnia clamp study; 30 carriers vs 2677 related/unrelated non-carriers to assess incremental insulin during an OGTT)	7 participants with MODY in linkage with 12q, probably representing HNF1A-MODY	10 carriers (with NGT or mild hyperglycaemia)
Control population	For carriers with diabetes: 1068 population controls with diabetes and 12 non-carrier relatives with diabetes; for 18 non-diabetic carriers, 92 population controls and 138 non-diabetic non-carrier relatives	8 non-related matched subjects (matched for age and sex?); mean age 40 vs 39, *N.S.,* mean BMI 21.3 vs 22.8, *N.S.*	6 noncarrier relatives
Results	*D+/- carriers vs D+/- non-carriers*: IVGTT: Insulin secretion (first- and late-phase) ↓ *D- carriers vs population controls*: IVGTT: Insulin secretion (first phase) ↓ HEC: insulin sensitivity ↔ (6.3 vs 6.4 in) *D+ carriers vs NIDDM population controls and D- carriers vs family/population controls:* OGTT: Fasting and incremental insulin ↓	*Affected vs controls:* Insulin secretion after an intravenous glucose load ↓ but response to tolbutamide ↔ Insulin sensitivity (S_i) ↔ Glucose effectiveness (S_g) ↔ (1.5 vs 2.2) by Bergman's minimal model[111]	Insulin secretory response to glucose levels >7 mmol/l ↓

(continued)

Study and associated genes	Vaxillaire *et al.* 1999[214], *HNF1A,* p.(Gly31Asp) (later revised unlikely pathogenic for MODY[229]), p.(Arg55fs) [which is, by a modern annotation, p.(Arg54fs)], p.(Tyr122Cys), p.(Arg171Ter,) and p.(Gly292fs)	Surmely *et al.* 1998[228], *HNF1A,* p.(Gly292fs)
Method	IVGTT, HGC and arginine test, HEC	Two-step HGC; HEC
Study population	OGTT: 18 carriers (10 with diabetes, 4 with IGT and 4 with NGT) IVGTT+HGC+HEC: Of these 18, 5 with diabetes, 2 with IGT (mean BMI at 22.6) as well as 3/4 without diabetes (mean BMI at 23.8)	5 patients with the variant from one family (4 had overt diabetes)
Control population	OGTT: 28 FDRs, IVGTT/HGC:/HEC: 11 unrelated healthy controls, and 6 patients with GCK-MODY (both groups had a mean BMI at 21.5)	12 healthy controls
Results	*Carriers vs FDRs*: OGTT: relative insulin response ↓ *Carriers with NGT vs controls*: Arginine test: Delayed insulin response IVGTT+HGC: first- and second-phase insulin secretion ↔/↓; HEC: insulin sensitivity ↔/(↓) (among carriers with NGT, only the one also obese demonstrated decreased insulin sensitivity) *D+/IGT+ carriers vs healthy controls / a control group with GCK-MODY:* IVGTT+HGC: first- and second-phase insulin secretion ↓ Arginine test: insulin secretion ↓ HEC: insulin sensitivity ↓	*Carriers vs control group:* At fasting before glucose infusion: carbohydrate oxidation rate ↔ endogenous glucose production ↔ HGC: early- and late-phase insulin secretion ↓ glucose utilization ↓ nonoxidative glucose disposal (glycogen synthesis) ↓ carbohydrate oxidation ↓ HEC: peripheral insulin sensitivity ↔ (mild dysregulation of glucose production?)

(continued)

Study and associated genes	Pearson *et al.* 2004[232], *HNF1B* (see paper for the variants), *HNF1A* (?)	Tripathy *et al.* 2000[230], as a combined analysis on *HNF1A,* p.(Gly292fs), p.(Arg131Gln), p.(Arg272Cys), p.(Leu107Ile), p.(Ser315fs), p.(Gly375fs); and *HNF4A* (by a modern annotation) p.(Lys86fs), p.(Leu315_Leu316insVal), not specific to either form of MODY but still in line with overall evidence
Method	Fasting samples for HOMA model, IVGTT study with tolbutamide	OGTT-derived composite index of insulin sensitivity (a.k.a Matsuda index)[111,231]; for a subgroup IVGTT/HEC
Study population	8 *HNF1B* variant carriers, 30 *HNF1A* variant carriers (of whom 5 and 10 carriers, respectively, participated in the IVGTT study)	118 with a pathogenic variant in *HNF1A* and 13 with a pathogenic variant in *HNF4A*
Control population	30 healthy controls (fasting samples), 10 patients with T2D (the IVGTT study)	Two control groups: GADA+ and relatives of T2D patients
Results	*HNF1A variant carriers vs healthy controls:* HOMA: insulin sensitivity ↔, β cell function ↓ *HNF1B variant carriers vs healthy controls:* HOMA: insulin sensitivity ↓, β cell function ↔ *HNF1A variant carriers vs HNF1B variant carriers/patients with T2D:* Response to tolbutamide (relative to the glucose-stimulated response) ↑	*NGT carriers vs NGT controls:* IVGTT: first-phase insulin secretion ↓ OGTT: insulin sensitivity (Matsuda) ↑, insulin secretion (incremental insulin, insulin/glucose ratio) ↓ *IFG/IGT carriers vs IFG/IGT relatives of T2D patients:* same as above, with HEC: insulin sensitivity ↑ (as defined by higher insulin-mediated uptake of glucose, M-value) *Diabetic carriers vs diabetic control groups:* same as above, but those with GADA+ and diabetes could elicit the poorest insulin secretion

(continued)

(continued)

Study and associated genes	Brackenridge *et al.* 2006[222] *HNF1A* p.(Gly292fs), p.(Glu132Lys), p.(Arg229Pro), p.(Arg272His), c.1623+1G>A, *HNF1B* p.(Ser151Pro), p.(Arg181Ter), p.(Pro159fs), p.(Arg295Pro), p.(Pro328fs), c.544+1G>T
Method	Two-step HEC (some patients with high FPG required an initial insulin infusion) with [6,6-$^2H_2$]glucose as a tracer
Study population	6 patients with *HNF1A* variants (of whom 1 had diabetes), 6 patients with *HNF1B* variants (of whom 3 had diabetes)
Control population	Age-, sex-, and BMI-matched healthy individuals who had no FDR with diabetes
Results	*HNF1A group and HNF1B vs controls at basal or high-dose steady state:* Endogenous glucose production (=appearance) rate (R_a) ↔ Glucose disposal rate (R_d) ↔ *HNF1A group vs controls at low-dose steady state:* Endogenous glucose production rate (R_a) ↔ *HNF1B group vs controls at low-dose steady state:* Endogenous glucose appearance rate (R_a) ↑ (~hepatic insulin sensitivity ↓ - note an unknown role of renal cortical gluconeogenesis, contributing to ~20% of endogenous glucose production in non-diabetic conditions, but 25–31% in type 1/2 diabetes[233])

Table 4. An extensive list of studies on β cell function and insulin sensitivity in individuals with pathogenic variants in HNF1A, HNF1B or HNF4A. HEC, euglycemic hyperinsulinaemic clamp; HGC, hyperglycaemic clamp; IVGTT, intravenous glucose tolerance test; FSIVGTT, Frequently sampled IVGTT; NIDDM, non-insulin dependent diabetes; D+/-, diabetic/non-diabetic; FDR, first-degree relative; NGT, normal glucose tolerance (in the OGTT); IFG, impaired fasting glucose (in the OGTT); IGT, impaired glucose tolerance (in the OGTT); FPG, fasting plasma glucose; HOMA, Homeostasis model assessment. Our review[111] includes a short introduction to methods.

2.3.3.3 Exocrine pancreatic insufficiency and other pancreatic phenotypes

To assess human β cell mass *in vivo* is tremendously difficult, but evidence from animal models[234] and human cell lines[235] has indirectly indicated that *HNF1A* inactivation could damage mitochondrial function and reduce β cell survival. However, evidence is contradicting, as exemplified by pancreatic donor islets from a 33-year-old woman heterozygous for a pathogenic variant *HNF1A* p.(Thr260Met)[236]. Her β cell mass was normal, but she had more α cells than average and a consequent increase in α to β cell ratio[236]. Donor islets demonstrated basal insulin secretion above normal and a normal intracellular insulin content, but glucose failed to elicit a normal biphasic insulin secretion, and interestingly, so did membrane depolarization by KCl. In contrast, the cAMP-provoked insulin secretion by the phosphodiesterase inhibitor isobutylmethylxanthine was normal. Moreover, her α cells exhibited a poor glucagon response to low glucose or noradrenaline, and the KCl displayed a paradoxical inhibitory effect on α cells[236]. However, with data from one patient only, further research is crucial.

Pancreatic volume, when assessed by computed tomography and adjusted for body surface area, was reduced in 15 patients with HNF1A-MODY (mean 34.5 ml/m^2) compared with non-diabetic controls (45.7 ml/m^2; $p < 0.02$)[237]. The volume was still clearly larger in those with HNF1A-MODY than in those with T1D (21.4 ml/m^2; $p < 0.001$)[237]. The authors postulated that the primary pathophysiology could involve reduced insulinotropic (and possible paracrine) regulation on acinar cells. Reduced pancreatic volume was significantly associated with exocrine dysfunction. Interestingly, 5 (33%) of those with HNF1A-MODY and about half of those with T1D and T2D had exocrine deficiency (as defined by faecal elastase tested twice at <200 μg/g). However, the authors also speculated that the findings in patients with HNF1A-MODY might also represent a primary (functional or developmental) defect associated with *HNF1A*, and not consequences of the reduced insulinotropic regulation. Future research on the individuals with inactivating *HNF1A* variants who have not progressed to insulinopenia and associated hyperglycaemia could partly elaborate the mechanisms behind the observations.

HNF1B-related disease (HNF1BD) typically presents with pancreatic hypoplasia[188,238] (hypoplasia in most patients involves the tail, *cauda,* and body, *corpus,* which are both derivatives of a dorsal pancreatic bud during foetal development[200,239,240]) and exocrine dysfunction involving both acinar and ductal cells (in up to 28–86%[188,241] of the patients, although the figures could be overestimated[200])[242]. Exocrine dysfunction can occasionally be symptomatic (10% or 3 of 29 patients had symptoms in a study, which also identified an association between exocrine dysfunction and diabetes[241]. Attributing to a central role of *HNF1B* in organogenesis (see 2.3.4), pancreatic hypoplasia is commonly considered to arise directly and not from insulinopenia[237].

2.3.3.4 Renal phenotypes

2.3.3.4.1 HNF1B

HNF1B-related disease (HNF1BD) involves both functional and structural presentation in the kidneys[185,186,200,243–248]. Whenever polycystic kidneys, the most common clinical manifestation of HNF1BD[185,186,200,243], coexist with diabetes, HNF1BD is occasionally referred to as renal cysts and diabetes syndrome (RCAD). In addition, a variety of other renal and extrarenal features and symptoms are associated with HNF1BD[200,243]. At a macroscopic or microscopic level, possible structural and functional findings related to kidneys are extremely diverse *(Table 5)*. However, absence of a renal phenotype does not exclude HNF1BD because the macroanatomy of the kidneys (in imaging studies) and renal function can remain unaffected in some patients.

The relative prevalence of the phenotypes in HNF1BD is obviously different in cohorts of patients with diabetes and in other patient cohorts, such as those with renal manifestations[249]. In addition, the type of gene variant influences the phenotype (see *2.3.3.7 Contrasting intragenic variants with whole-gene deletions*). HNF1BD is the most frequent monogenic aetiology for congenital anomalies of the kidneys and urinary tract (CAKUT), with *HNF1B* accounting for 10–30% of the cases manifesting with CAKUT[250–252]. Foetal and paediatric cohorts also imply that *HNF1B* is responsible for isolated renal dysplasia as the most common aetiology[253].

About half of the patients develop some level of chronic kidney disease (CKD), and around one-third progress to moderate-to-severe CKD[200,252]. Few available kidney biopsies have demonstrated glomerulocystic disease and renal dysplasia (two patients) or mild mesangial glomerulonephritis (one patient)[252]. Of the characteristic renal features, hyperuricaemia is the only manifestation originating in the proximal tubule[253]. Although hyperuricaemia might be secondary to renal insufficiency, specific proposed mechanisms involve reduced expression of *HNF1B*-regulated proteins such as organic anion transporter (OAT)1, OAT3/OAT4 and/or uromodulin and a consequent increase in the reabsorption of uric acid in the proximal tubule[253]. Hyperuricaemia occasionally manifests as young-onset gout or trophi[254,255].

The renal features of HNF1BD further include hypermagnesiuric hypomagnesaemia and hypocalciuria[256,257], a combination also present in Gitelman syndrome[258]. Hypermagnesiuria is possibly secondary to a loss of *HNF1B*-regulated *FXYD2* expression in the distal convoluted tubule[256]. Up to about half of adult patients with HNF1BD allegedly demonstrate potassium loss in urine and consequent hypokalaemia, which, interestingly, could also be unrelated to hypomagnesaemia[247,253,256,258]. Hypocalciuria, on the other hand, might be associated with a downregulation of calcium-sensing receptor (CaSR)[259].

During a follow-up, a majority of children (72%) with HNF1BD and renal cysts demonstrate no increase in the cyst number and present with a mean annual GFR decline of -0.33 ml/min/1.73m², whereas the children with an increase in cyst number also decline clearly in terms of renal function (-2.8 ml/min/1.73m²)[260].

Case reports have further suggested an association between *HNF1B* and chromophobe renal cell carcinoma (RCC)[261,262], but malignant associations of the HNF1BD still warrant further studies[263].

Table 5

Polycystic kidneys (with a prevalence reported from >70% up to >90%)
Solitary renal cysts or oligocystic kidneys
Hyperechogenic kidneys (20-30% of foeti with hyperechogenicity have HNF1BD[264])
Collecting system anomalies
Congenital anomalies of kidneys and urinary tract (CAKUT)
Familial juvenile hyperuricaemic nephropathy
Other dysplasias or structural nephropathies with or without cysts (including hypoplastic glomerulocystic kidney and renal interstitial fibrosis)
Hypoplastic kidneys, renal agenesis
Horseshoe kidney
Oligomeganephronia
Duplex system kidneys
Progression to ESRD and kidney transplantation

Table 5. HNF1BD can demonstrate numerous manifestations in the kidneys[185,186,200,243–248].

2.3.3.4.2 HNF1A

Although the main phenotype of HNF1A-MODY is diabetes, the clinical spectrum of HNF1A-MODY expands beyond the pancreas. Of the three families characterized by Dr Tattersall in 1974[14], the two families (M and R, later diagnosed with HNF1A-MODY[265]) had demonstrated glucosuria. In a study from the late 1990s, patients with HNF1A-MODY expressed a lower renal threshold for glucose than the patients with T1D as determined by

gradual increases in plasma glucose following carbohydrate consumption and/or interruptions of insulin administration. Those with HNF1A-MODY indeed had a lower renal threshold (6.5 ± 0.9 mmol/l) than those with T1D (10.7 ± 0.5 mmol/l)[266], as defined by peak plasma glucose. However, glucose sampling every 30 mins could have been too diffuse to measure the maximal peak in plasma glucose (especially after carbohydrate ingestion), should it occur between measurements. Furthermore, plasma glucose was not adjusted for serum insulin. In another study, 7 diabetic carriers of pathogenic *HNF1A* variants and 13 non-diabetic carriers underwent an OGTT, and all those with plasma peak glucose >8.4 mmol/l during the OGTT displayed glucosuria[265].

The individuals with pathogenic *HNF1A* variants also presented with low-molecular-weight (LMW) proteinuria, although microalbuminuria was not a frequent finding among the patients[159].

Interestingly, an observational study from Poland identified kidney malformations in 5/56 (8.9%) patients and unilateral kidney agenesis in 2/56 (3.6%) patients with HNF1A-MODY[267]. The figures are far higher than in the general population (0.6% and 0.08%, respectively[267]), which obviously inspires systematic research in patients with HNF1A-MODY worldwide. Also, a rarely identified microdeletion encompassing *HNF1A* has co-presented with renal cysts (see also section 2.3.3.7 *Contrasting intragenic variants with whole-gene deletions*)[268].

One study has proposed that germline variants in *HNF1A* could predispose to renal tumours such as clear RCC[261], but the evidence is extremely limited.

2.3.3.4.3 HNF4A

To date, 18 cases (including 8 unrelated probands) with a distinctive phenotype of MODY and atypical Fanconi renotubular syndrome have been associated with a specific variant in *HNF4A,* p.(Arg85Trp)[269–271]. Typically, Fanconi syndrome presents with impaired tubular reabsorption of multiple compounds in the kidneys (involving glucose, amino acids, phosphate, LWM proteins, bicarbonate and urate, urine loss of glucose, amino acids, phosphate), which predisposes the affected (children) to growth failure and rickets[269]. Those with *HNF4A* p.(Arg76Trp) can demonstrate typical Fanconi features but also features not observed in the typical Fanconi syndrome, namely nephrocalcinosis, renal insufficiency, hypercalciuria with hypocalcaemia and hypermagnesaemia[269].

2.3.3.5 Hepatic phenotypes

Patients with HNF1BD often express fluctuating levels of liver enzymes (including alanine aminotransferase, ALT; γ-glutamyltransferase, GT; and

aspartate aminotransferase, AST), which does not commonly co-present with liver insufficiency or jaundice[200,243,272,273]. The cause of enzyme fluctuations is unclear, but as the adult expression of HNF1β only persists in cholangiocytes and not in hepatocytes[140], the release of liver enzymes is speculatively secondary to disturbances of the intrahepatic or extrahepatic bile system.

Liver and biliary phenotype of the HNF1BD further include dyslipidaemia (*i.e.* hypertriglyceridaemia and low levels of HDL cholesterol)[232] and, more rarely, neonatal cholestasis or adult-onset cholestasis[264,274–277]. A limited series of patients have also implied neonatal ductal plate malformations with duct dysplasia as well as a paucity of bile ducts[274,276–278], primary ciliopathy affecting cholangiocytes[279] and extrahepatic biliary atresia, as well as minor dilatations of bile ducts[277,280]. Interestingly, conditional KO of pancreatic *Hnf1b* in mice involves a lack of cilia (see section 2.3.4).

Through spontaneous inactivation of the initially unaffected allele, patients with an inactivating germline variant in *HNF1A* could be at risk of developing multiple hepatocellular adenomas (HCAs), namely, liver adenomatosis[281–283]. Biallelic inactivation of *HNF1A* constitutes a major aetiology (30–50%) of HCAs. The other three subtypes are β-catenin-activated HCAs (10–15%), inflammatory HCAs (35–50%[284]) and unclassified HCAs (5–10%)[284–287]. *HNF1A*-associated HCAs express characteristic features such as marked steatosis[284]. Unlike β-catenin-activated HCAs, solitary *HNF1A*-associated and inflammatory HCAs have low malignant potential[288]. Despite some propositions of a higher risk of malignant transformation in *HNF1A*-associated adenomatosis[288], a presence of multiple *HNF1A*-associated HCAs was not associated with malignant transformation in a recent study, although the HCA diameter showed an increase during the follow-up among the affected patients[289]. Sanger sequencing may miss some of the patients, as exemplified by a family with diabetes and liver tumours (including HCAs, adenomatosis and hepatocellular carcinoma) cosegregating with an in-frame deletion of exons 2–3 of *HNF1A*[290] and a child with HCA and diabetes associated with an *HNF1A* translocation[291].

C-reactive protein (CRP), mainly secreted from hepatocytes, is an acute-phase protein. Because the expression of CRP is under the direct control of *HNF1A,* circulating CRP levels are significantly lower in patients with HNF1A-MODY than in diabetic or non-diabetic control groups[292–294]. Early hopes of using high-sensitive CRP (hsCRP) as a discriminative biomarker for HNF1A-MODY have broadly vanished, as the overlap between patients with HNF1A-MODY and patients with other types of diabetes clearly hampers the discriminative value of hsCRP[295,296]. Moreover, as *HNF4A* does not regulate the expression of CRP, hsCRP cannot distinguish between patients with HNF4A-MODY and those with other types of diabetes[295].

Apolipoprotein M (apoM), mainly produced by the liver and kidneys, is present in various lipoprotein particles, predominantly HDL particles. Although animal and early human data found an association between *HNF1A* and circulating apoM levels[297], *HNF1A* was not later associated with circulating apoM levels in humans[298]. Moreover, *HNF1A* has not been associated with high-density lipoprotein (HDL) levels[299], which is the main

target of apoM. To know whether *HNF1A* and *HNF4A* inactivation further modulates the hepatic secretion of other proteins[300], more research is needed.

To some extent, HNF1α also controls the expression of organic cation transporter 1 (OCT1, encoded by *SLC22A1*). OCT1 mediates hepatic uptake of numerous drugs *e.g.* morphine, tropisetron, ondansetron, tramadol and metformin[301]. Although the clinical significance of the *HNF1A*-mediated OCT1 expression remains poorly understood, an association between *HNF1A* polymorphism and mortality among opioid addicts[302] signals that possible interactions must not be overlooked.

HNF4α regulates the hepatic lipid metabolism in a complex network. As a result, *HNF4A* inactivation leads to reduced levels of serum triglycerides, Apo-AII, Apo-CIII and ApoB[303–305]. Low triglyceride levels could be secondary to a decrease in ApoB and Apo-CIII, which are normally enriched in triglyceride-rich lipoproteins[303,304,306].

2.3.3.6 Other phenotypes

The features associated with the HNF1BD further include genital tract malformations (*e.g.* bicornuate unicollis uterus is a common finding in women[185,243]) and, in a small patient series, elevated levels of parathyroid hormone (PTH)[307]. In addition, the HNF1BD might present with intrauterine growth restriction (IUGR)[238,275–277,308] by an unknown mechanism. However, as HNF1BD is only infrequently associated with neonatal diabetes, IUGR hardly arises from hypoinsulinaemia[238].

A whole-gene deletion of *HNF1B,* which is associated with the 17q12 microdeletion (see 2.3.3.7 *Contrasting intragenic variants with whole-gene deletions*), displays neuropsychiatric features such as autism spectrum disorders, developmental delay, schizophrenia and cognitive impairment (seizures and mild facial dysmorphias are more rarely reported)[309–312], whereas those with intragenic variants in *HNF1B* lack this phenotype. In a recent French report, 12.7% of children with *HNF1B* deletion experienced special educational needs[313]. Hearing loss might also develop in HNF1BD, but a mechanism – if any – remains unclear[200,243]. *Figure 3* summarizes most features associated with HNF1BD.

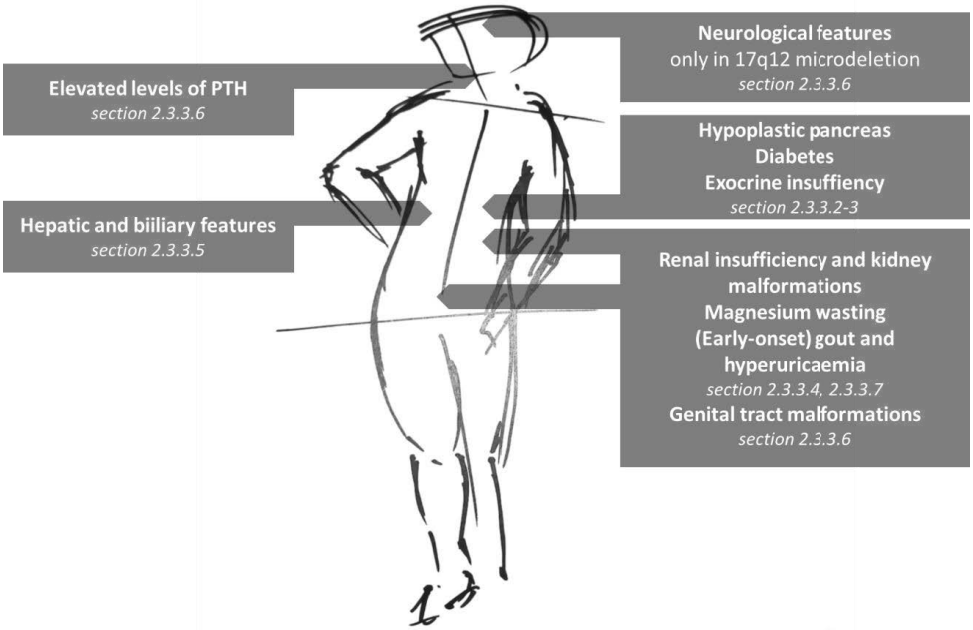

Figure 3. Features associated with HNF1B-related disease are diverse and further characterized by enormous variation at an individual level. PTH, parathyroid hormone.

For a short discussion on incretin responses associated with *HNF1A,* see 2.3.6.3.

The 12q24 microdeletions encompassing *HNF1A* are rare, especially those specifically limited to the band 12q24.31 (harbouring *HNF1A*). The associated symptoms have included diabetes, renal cysts, recurrent neonatal hypoglycaemias, macroglossia, overgrowth, developmental delays and seizures among other neuropsychiatric and neuropsychological symptoms[268,314–318], but whether *HNF1A* contributes to symptoms other than diabetes remains unclear.

Biomarkers distinguishing MODY from other types of diabetes have always been of profound interest[319] (as for hsCRP, see 2.3.3.5 above). However, most proposed biomarkers are robust surrogates, *e.g.* for insulin resistance, hence not demonstrating an actual consequence of the genetic defect.

CD36 is a central fatty acid transporter[320] with a role in insulin resistance, diabetes and beyond[321–323]. In type 2 diabetes, circulating levels of CD36 (presumably of erythrocyte origin) are increased, whereas non-diabetic control individuals predominantly express CD36 from the endothelium[321]. In

a small series of patients, the circulating levels of CD36 were, expectedly, lower in those with HNF1A-MODY than in those with type 2 diabetes. Compared with unaffected euglycaemic family members, those with HNF1A-MODY demonstrated lower levels of circulating CD36[324], but the results were not adjusted to BMI. Likewise, owing to the association between low ghrelin and insulin resistance[325], ghrelin has become one of the latest biomarkers suggested to identify patients with MODY[326]. However, prospective and larger series of patients are crucial to evaluate how CD36 and ghrelin perform as biomarkers of MODY when compared with robust but far more inexpensive markers associated with insulin resistance, such as hsCRP, BMI and HDL cholesterol.

Preliminary data have indicated that individuals with HNF1A-MODY display some alterations in urinary steroid metabolite profiles as compared with type 2 diabetes or healthy controls[327]. However, the study relied on a low sample size with less than 20 individuals in each group (patients with HNF1A-MODY, patients with type 2 diabetes, and control patients without diabetes) and lacked data on a few important confounding factors (such as insulin sensitivity[328]). By an unconventional false discovery rate of 10% chosen by the authors, an expected number of false positive results among different steroid metabolites would be five ($^{10}/_{100}$ · $49\ ratios\ of\ metabolites\ analyzed \approx 5\ false\ positive\ results$). As not even five but only one comparison between HNF1A-MODY and type 2 diabetes reached statistical significance (with p=0.004), the chances are that the difference might have represented type 1 error (false positive finding). Further studies are crucial to elucidate the results.

2.3.3.7 Contrasting intragenic variants with whole-gene deletions

Most inactivating variants in *HNF1A*, *HNF4A* and *GCK* are intragenic, which include microinsertions and microdeletions, nonsense and nonconservative missense variants, splice site acceptor/donor variants, as well as promoter variants. *De novo* variants account for 0–7% of the cases.[74,329–331] The prevalence of partial or whole-gene deletions in patients with inactivating *HNF1A, HNF4A* and *GCK* variants is only 1.2–1.9 %[305]. In contrast, approximately half the patients with HNF1BD have a whole-gene deletion of *HNF1B*. This deletion is commonly attributable to a wider microdeletion at 17q12, which typically encompass 1.2–1.5 Mb and up to 14 genes in addition to *HNF1B*[74,246,264,312,332,333]. Of all *HNF1B* variants, 40–60% are *de novo,* but of the *HNF1B* deletions, 50–60% (even up to 70%) are *de novo*[74,246,264,312,332,333].

As discussed in section 2.3.3.7, neuropsychiatric and neuropsychological symptoms are common in patients with whole-gene deletions of *HNF1B* (and possibly *HNF1A*), but these symptoms are rare to absent in patients with intragenic variants. When contrasting whole-gene deletions with intragenic variants in those with HNF1BD, the genotype-phenotype differences also

include kidney function (less affected by whole-gene deletions[249], supporting evidence also provided by [246]), body composition (those with whole-gene deletions are leaner[249]) and insulin treatment (those with whole-gene deletions are more often on insulin[249]). No clear evidence on the differences in plasma electrolyte levels exists[256,258], although those with a whole-gene deletion have occasionally demonstrated lower levels of plasma magnesium than those with intragenic variants[312].

2.3.4 HNF1A, HNF1B and HNF4A during embryonic development and beyond

To elucidate adult human phenotypes associated with the HNFs, one cannot neglect foetal development. However, as human foetal samples are rarely accessible, most knowledge on the HNFs during embryogenesis and foetal development derives from rodent models (see *Figure 4* for an overview).

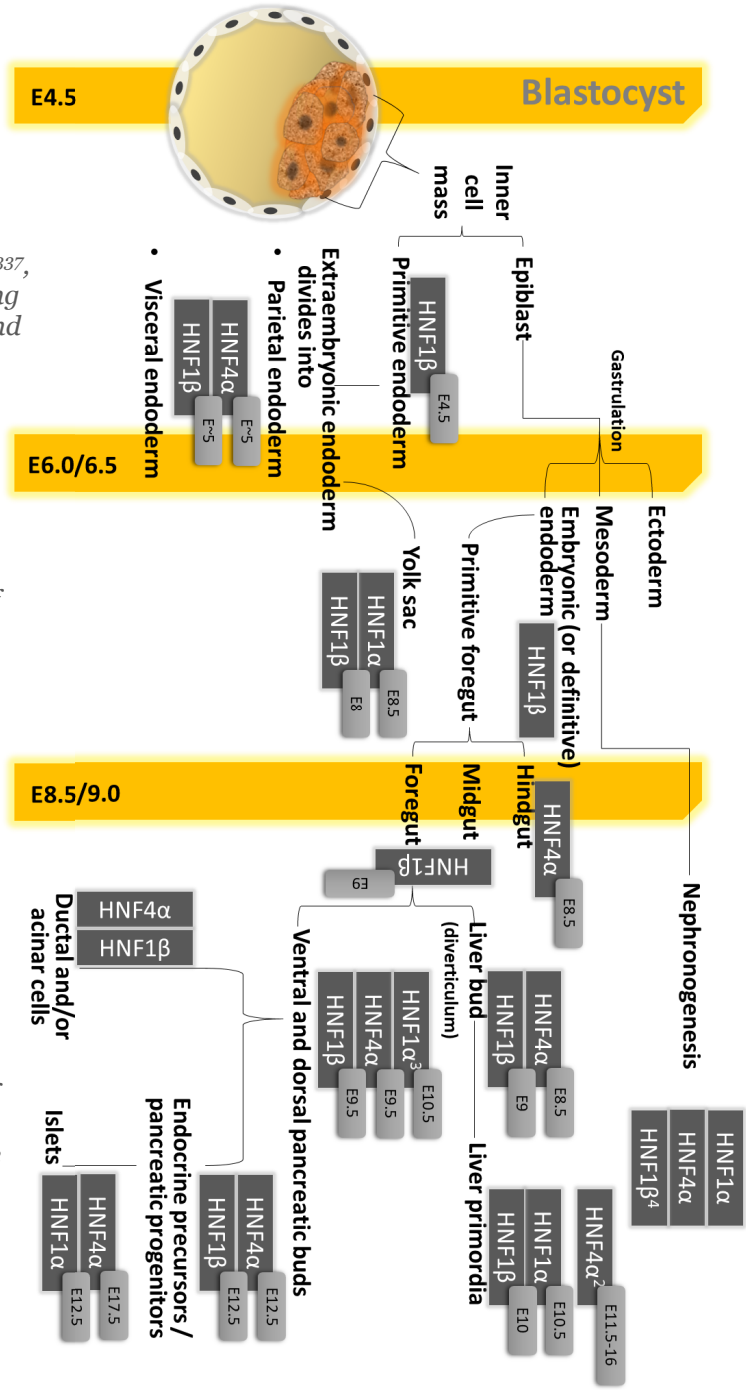

Figure 4. A schematic illustration on Hnf1α, Hnf1β and Hnf4α in murine development and organogenesis[334–337], specifically focusing on the pancreas and liver[338–350]. As highlighted by an early embryonic appearance, HNF1β plays a central role in pancreatic and urogenital embryogenesis. Of note, the primitive endoderm is referred to as a hypoblast in humans.

[2] *in peripherally located hepatocytes*

[3] *mainly in epithelial cells*

[4] *for further insights on the temporal and spatial patterns of the HNFs during nephrogenesis, see [140,243,351]*

E, embryonic day.

Murine HNF1β is essential for extraembryonic visceral endoderm differentiation, besides serving as a major regulator of organogenesis. It displays a wide presence in the endoderm-derived foetal foregut and midgut in the embryo proper at E8 as well as in the liver and both pancreatic buds at E9.5[338,352–354]. A sequence of *Hnf1b – Hnf6 – Pdx1* triggers a differentiation of pancreatic bud cells into pancreatic progenitors[355]. Emphasizing the importance of HNF1β in organogenesis, a failure of visceral endoderm differentiation occurs in *Hnf1b* null mice already before gastrulation[338,353]. Conditional and chimeric models have further evidenced the key role of *Hnf1b* in murine pancreatogenesis[347,354], nephrogenesis[140,243,350,351], bile duct morphogenesis[356], liver function and hepatic insulin resistance[357].

Akin to *Hnf1b* null mice, *Hnf4a* null mice are also early lethal (<E10.5) due to an absence of HNF4α in the visceral endoderm and the consequent failure of ectoderm survival and gastrulation[340]. HNF4α acts as a master regulator of lipid metabolism, bile acid metabolism, drug metabolism, and blood coagulation in hepatocytes[358,359], and it influences the early foetal fate of pancreatic and hepatic cells and their gene signatures[360]. A complete loss of hepatic HNF4α (at 4-6 weeks of life) triggered gradual weight loss, and mice manifested a high mortality rate (>70%) by 8 weeks of age[358]. Conditional hepatic models also suggest *Hnf4a* to control proliferation and tumorigenesis[359].

In contrast to *Hnf1b* and *Hnf4a* null mice, *Hnf1a* null mice are viable – although infertile – and demonstrate a complex phenotype, which includes impaired liver function as evidenced by perturbations in hepatic glucose metabolism[361] and a decrease of hepatic expression of phenylalanine hydroxylase (and consequent phenylketonuria), as well as growth hormone insensitivity, renal Fanconi syndrome (see also 2.3.3.4) and non-insulin dependent diabetes presenting with a reduced pancreatic expression of *Pklr* and *Glut2 (Slc2a2)*, but no alterations in insulin or glucokinase expression[361–365].

Although both *Hnf1a* and *Hnf1b* null mice demonstrate an impaired response to glucose, the insulin response to arginine is impaired only in *Hnf1a* but not in *Hnf1b* null mice[363]. The growth retardation observed in *Hnf1a* null mice probably derives from IGF-1 deficiency[362], but considering the complex phenotype of the murine KO, other factors might also affect the growth.

Obviously, nullizygous models hardly represent human disease. In humans, diabetes and other clinical features arise in heterozygosity, whereas mice with heterozygous variants in *Hnf1a*[362,366] or *Hnf1b*[338,353] lack any specific phenotype. Conversely, no human with a homozygous defect in *HNF1A, HNF1B* or *HNF4A* has been identified to date[141] (biallelic hypomorphism represents a different phenomenon[43]).

Rodents display higher expression levels of *Hnf1a* and *Hnf4a* than humans[141]. In mice, a highly expressed functional allele could compensate for a loss of an inactive allele of *Hnf1a* or *Hnf4a*, which could explain why heterozygosity is damaging to humans but not mice[305]. However, such compensation cannot explain the case for *Hnf1b*, whose expression levels are

similar in humans and mice[141]. Recently, non-coding RNA molecules have been proposed to establish some interspecies differences[367,368].

Akin to murine foeti, human foeti express *HNF1A, HNF1B* and *HNF4A* in the pancreas, liver and kidneys[140,184,369,370]. HNF1β and HNF1α are further present in the human foetal gastrointestinal tract[140]. In each human and rodent tissue, the pattern of HNF isoform expression is distinctive to each species and changes during the embryonic development and beyond; for example, in humans, up to 23% of HNF4α expressed in the foetal pancreas originates from the P1 promoter, whereas only P2 promoter-driven transcripts are present in the adult pancreas[141,179,184,371].

In a study with genetically modified human embryonic stem cells (ESC), *HNF1A* insufficiency diverted the endocrine cell fate from β cells into α cells (a heterozygous and complete ablation of *HNF1A* presented with an increase in α cell population and monoclonal glucagon cells as well as a decrease in monohormonal insulin cells)[368]. ESCs and human iPSC-derived (hiPSC) models have also highlighted that a loss of *HNF4A* affects foregut endoderm signatures and, therefore, transitions to the hepatic fate[360,372,373] and probably differentiation to fully mature β cells[360,374]. A hiPSC model of *HNF1B* unexpectedly found that, during pancreas development, *HNF1B* inactivation displayed only an indirect decrease in *PAX6* expression but a compensatory increase in a set of other genes[375].

2.3.5 An uncomprehensive list to exemplify HNFs at a cellular or molecular level

Beyond antenatal development, the regulative roles of HNFs persist in numerous adult tissues. The molecular evidence on HNFs in transcriptional networks has particularly accumulated through studies focused on pancreatic and hepatobiliary cell models and animal tissues. Pancreatic and hepatic tissues from humans are rarely available for research purposes.

Although HNF1α is not an essential transcription factor for most target genes, the knock-out (KO) of *Hnf1a* alters the transcription of hundreds of genes in murine pancreas and liver[235]. Whereas the pathways associated with downregulated genes following the KO are similar in the pancreas and liver (and involve membrane transport and steroid and lipid metabolism among other metabolic functions), the upregulated genes are more cell-specific[235]. In pancreatic β cells, the HNF1α regulates glucose metabolism[166,167,235,363,376,377] (especially glycolysis and GSIS[235,368,378]); metabolic oscillators *e.g.* fructose bisphosphatases 1/2 (Fbp1/2), and phosphofructokinase (Pfkl/Pfkp)[379,380]; electron transport; endoplasmic reticulum (ER) stress response[381]; lipid metabolism[382]; cellular growth, communication and adhesion[235,383]; intracellular structural integrity; amino acid and ion transport and metabolism; proteolytic activity; oxidative phosphorylation and mitochondrial metabolism[167,235] (further modulated by

the human-specific lncRNA, LINKA or LINC01139, a downstream target of *HNF1A*, which is absent in rodents and possibly explains some interspecies differences between humans and rodents)[368]; as well as a number of other signalling, metabolic and regulative functions[235,236,365,365,377,384] and target genes, such as *Slc2a2* (encoding glucose transporter 2, Glut2), *G6pc2*[385] (encoding glucose-6-phostatase catalytic subunit), and *Mafa*[386] (encoding musculoaponeurotic fibrosarcoma homolog A).

Interestingly, whereas the loss of HNF1α in the pancreas compromises β cell survival and proliferation, the loss of HNF1α in the liver promotes proliferation and oncogenesis and deteriorates epithelial cell identity[235,285,387–390]. Of note, *HNF1A* could also promote cell survival under some conditions in the pancreas, as evidenced by human pancreatic cancer stem cells[391].

In addition, HNF1α regulates the expression of CRP and ApoM (see 2.3.3.5 *Hepatic phenotypes*), bile acid (BA) metabolism[392], and protein expression in the gastrointestinal tract[393–395] and kidneys (such as sodium-glucose cotransporter 2 (SGLT2), which is responsible for 90% of the glucose reabsorption in the proximal tubule epithelial cells of the kidney[396,397]). HNF1α also plays a role in other tissues of endoderm origin, such as the stomach, spleen, thymus, skin (keratinocytes, melanocytes) and testis[140,398–400].

HNF1B regulates protein expression in the kidneys[401] and could be associated with cell divisions[402] or ciliary disruptions[403]. Whether HNF1BD is a primary ciliopathy[279,347,404–406] has remained unclear.

HNF4α modulates fatty acid metabolism in the liver[407] and intestine[408], as well as bile acid and drug metabolism in the liver[409]. This nuclear receptor also regulates renewal of intestinal stem cells[408]; controls intestinal absorption, transport and metabolism of vitamin A[410]; and impacts intestinal Wnt/β-catenin signalling[411]. Further functions include upregulation of proteins such as sex-hormone binding globulin (SHBG)[412,413], ApoC-III (see also section 2.3.3.5) and transthyretin[414]. HNF4α modulates intestinal inflammation and mucosal permeability[415], tissue-specific circadian oscillations[416], and adult hepatocyte differentiation[417], besides facilitating recruitment of RNA polymerase II[372].

In human adult pancreas, P2-driven isoforms of HNF4α cannot transactivate HNF1α as efficiently as P1-driven isoforms expressed in hepatocytes. Another transcription factor, β cell-specific NKX6-1 (homeobox protein Nkx-6.1, encoded by *NKX6-1*), is a master regulator of human pancreatic HNF1α[378]. In addition, *HNF1A* and *HNF4A* also target some common genes in pancreatic islets synergistically[366].

2.3.6 Medical treatment of MODY arising from HNF variants

Randomized controlled trials, cross-over trials and case-control studies on the treatment of HNF1A-MODY, HNF1B-MODY and HNF4A-MODY are

astonishingly few. In addition, a low-carbohydrate diet (especially low in fast carbohydrates) can be beneficial in achieving appropriate glycaemic control, especially for those with early and mild stages of diabetes[418].

2.3.6.1 Sulfonylureas and meglitinides

Sulfonylureas (alternatively spelled sulphonylureas) depolarize the β cell by binding and closing the ATP-sensitive K^+ (K_{ATP}) channel located on the cell membrane. The depolarization leads to an increase in intracellular calcium concentration, eventually triggering insulin secretion[419].

Sulfonylureas (with a typical dose well below the daily maximum) are potent drugs to treat both HNF1A-MODY[420–428] and HNF4A-MODY[197,331] with an efficacy that can persist for decades[197,420,425]. Indeed, the first reports on sulfonylurea sensitivity preceded the first gene discoveries of MODY[5,14,197,429]. To quantify the sensitivity to sulfonylureas, a small cross-over trial in 36 patients, published in 2003, evaluated patients' response to metformin and gliclazide, which belongs to the second-generation sulfonylureas. Compared to metformin, the response to gliclazide was ~5-fold among those with HNF1A-MODY. Comparing between HNF1A-MODY and type 2 diabetes, the response to gliclazide was ~4-fold in those with HNF1A-MODY[420]. Those with HNF1A-MODY demonstrated a decrease in fasting glucose of 0.9 mmol/l when on metformin, whereas those with type 2 diabetes experienced a decrease of 1.3 mmol/l[420]. Despite early suggestions of hyperexcitability to sulfonylureas in those with HNF1A-MODY[423,424], supported by prolonged sulfonylurea half-life in *Hnf1a* null mice, human studies have not found any evidence on differences in sulfonylurea metabolism in patients with either HNF1A-MODY or HNF4A-MODY[420,427,430].

Patients with diabetes and the HNF1BD often need insulin treatment, as suggested by several review articles. However, patients may initially manage with oral sulfonylureas or sulfonylurea-like meglitinides. In one series, 21% of patients demonstrated a persisting response to sulfonylureas during a follow-up[249,431]. Oral antidiabetics might therefore also be a good alternative to exogenous insulin in HNF1BD.

Meglitinides (namely nateglinide, mitiglinide, and repaglinide, the last of which is also available in Finland) resemble sulfonylureas in their mechanism of action, but their binding affinity to the K_{ATP} channel is weaker than that of sulfonylureas. Major differences between the two classes of antidiabetics reside in pharmacokinetics; meglitinides are quickly metabolized. After the rapid absorption, meglitinides display a short onset of action (<15 minutes) with a maximal peak plasma concentration within one hour and a half-life within one hour[432,433].

Comparing nateglinide (30 mg) and sulfonylurea (glibenclamide, 1.25 mg) as well as placebo, nateglinide displayed the most potent postprandial glucose control after a test meal in 15 Finnish patients with HNF1A-MODY. After 140

minutes of light exercise, glibenclamide induced symptomatic hypoglycaemia in six patients, whereas nateglinide did not in any patient[223]. In a case report of three adolescents, meglitinides decreased the patients' HbA1c levels without predisposing to hypoglycaemic events[434].

To summarize, the sensitivity to sulfonylureas and sulfonylurea-like meglitinides is a characteristic feature of HNF1A-/HNF4A-MODY. However, in a study from the UK, only 36.1% of patients with HNF1A-/HNF4A-MODY could reach an HbA_{1c} target (≤58 mmo/l or 7.5%) at two years of follow-up with sulfonylurea monotherapy or diet[120]. Higher HbA_{1c} and higher BMI, as well as a longer duration of diabetes, predicted an inferior response to these therapies[120]. Although the data from one specialized centre in the UK is more encouraging[425], clinicians should recognize the patients who require adjunct therapies.

To evaluate the frequency of symptomatic and asymptomatic events of hypoglycaemia in patients with MODY and treated with sulfonylureas/ meglitinides, a clinical or observational trial with continuous glucose monitoring could be beneficial. To my knowledge, however, these studies have not been conducted.

2.3.6.2 Biguanides (metformin)

Metformin is an efficient and low-cost antihyperglycaemic agent with a negligible risk of hypoglycaemia and a conventional first-line medical therapy for type 2 diabetes. However, uncovering the main mechanism of this drug's action has been blatantly challenging. The suppression of hepatic glucose production was believed to be the main mechanism, but today, even the primary organ targeted by this drug is unclear (liver vs intestines); metformin targets several pathways in the human body.[435]

Although sulfonylureas and meglitinides are the primary therapy in HNF1A-MODY and HNF4A-MODY, metformin could provide a sensible second-line alternative[418] for patients without intermediate/severe renal insufficiency should sulfonylureas (or meglitinides) have contraindications. Metformin can also be considered if sulfonylureas fail as a monotherapy. Two case patients with HNF4A-MODY had achieved successful glycaemic control on metformin monotherapy[422]. As metformin could additionally provide some cardiovascular benefits[436,437] besides improving antiglycaemic control, metformin could potentially benefit some patients with MODY.

In contrast to a wide acceptance of sulfonylureas (or sulfonylurea-like acting meglitinides) as the first-line therapy for HNF1A-/HNF4A-MODY, guidelines do not completely agree on the primary antihyperglycaemic treatment of HNF1BD. As patients with HNF1BD are heterogeneous, simplified guidelines might undermine between-individual variation. Some reviewers suggest that metformin could be the first-line drug (whenever renal function allows)[418,438], which is a logical consequence of hepatic insulin resistance

associated with HNF1BD (see section 2.3.3.2). Many other guidelines, however, recommend using insulin (or sulfonylureas) as first-line drugs.

2.3.6.3 DPP4 inhibitors and GLP-1 receptor agonists

Enteroendocrine cells (EECs) align in the intestine and are further divided into K cells, L cells and, as recently suggested, intermediate K/L cells with features of both cell types. EECs respond to dietary nutrients (such as carbohydrates, triglycerides, proteins and some amino acids) by producing a group of hormones called incretins. Of the two classic incretins, glucagon-like peptide 1 (GLP-1) is secreted from L cells, whereas glucose-dependent insulinotropic polypeptide (GIP, originally gastric inhibitory polypeptide) is secreted from K cells.

An oral administration of nutrients leads to increased plasma levels of GIP and GLP-1, which potentiate insulin secretion from pancreatic β cells, whereas the intravenous route omits this augmented response. The difference between orally and intravenously stimulated insulin secretion is referred to as the incretin effect. Classically, the incretin effect has become apparent by contrasting insulin responses between the oral challenge and intravenous challenge of equivalent doses of glucose (an OGTT vs an IVGTT). Currently, there are two classes of antihyperglycaemic drugs that employ the incretin system: GLP-1 receptor agonists (GLP-1RAs) that directly activate GLP-1 receptors and DDP4 inhibitors (DPP4i's) that block the rapid enzymatic degradation of incretins by dipeptidyl peptidase 4[439,440].

Type 2 diabetes is associated with a compromised incretin effect, which involves decreased sensitivity to GIP[441], whereas patients with MODY demonstrate unchanged sensitivity to GIP. GIP preserved its capacity to upregulate insulin secretion during a test meal in patients with HNF1A-/HNF4A-MODY, demonstrating a strong correlation between insulin and GIP responses, whereas GLP-1 and insulin responses were not correlated[442]. In general, GIP levels or GLP-1 levels were surprisingly similar in patients with HNF1A-/HNF4A-MODY, and in control groups with or without diabetes, only modest differences existed[442]. Also, incretin levels during an OGTT challenge in another study were similar in patients with HNF1A-MODY and in a control group without diabetes[224]. Although patients with HNF1A-MODY presented with an impaired incretin effect after an OGTT[224], another study with exogenous GIP and GLP-1 infusions demonstrated normal insulinotropic efficacy in patients with HNF1A-MODY, possibly predicting a good response to pharmacological interventions by DPP4i's or GLP-1RAs[443].

The antihyperglycaemic potency of DPP4i's or GLP-1RAs in patients with HNF1A-MODY or HNF4A-MODY, either as a monotherapy or add-on therapy (typically on top of sulfonylureas), has mostly been subject to case reports[444–449]. In a randomized controlled cross-over trial in 16 patients with

HNF1A-MODY, sulfonylurea (glimepiride) monotherapy decreased FPG by -2.8 (± 0.7) mmol/l, whereas GLP1-RA (liraglutide) did so by -1.6 (± 0.5) mmol/l. The difference was not statistically significant, probably due to the small number of participants. However, those on liraglutide experienced fewer hypoglycaemic episodes than those on sulfonylureas[450].

In a recent randomized and double-blinded crossover trial in patients with HNF1A-MODY, adding DPP4i (linagliptin) to glimepiride did not meet the primary end point of improved mean amplitude of glycaemic excursions, but the combination of glimepiride and DPP4i was nonetheless more effective than glimepiride alone in reducing HbA_{1c} (by -0.5%, p=0.0048). The result could also be achieved by a lower daily dose of glimepiride (-0.7 mg, p=0.0099), yet with a similar rate of hypoglycaemia[451].

In conclusion, GLP-1RAs or DPP4i's might act as a valuable adjunct therapy on top of sulfonylureas (or meglitinides). Although GLP1-RAs/DPP4i's might be inferior or non-superior to sulfonylureas as a monotherapy, GLP-1RA/DPP4i-based monotherapy might be useful for patients with mild hyperglycaemia or for those who have contraindications for sulfonylureas. In addition, there are no systematic trials on heavily overweight patients with MODY, who might additionally benefit from the weight loss associated with the GLP1-RAs.

2.3.6.4 SGLT2 inhibitors

Under physiological conditions, the human renal glomeruli filter ~200 g of glucose each day, but virtually all glucose is reabsorbed by a secondary active reabsorption in the proximal convoluted tubule (PCT) through sodium–glucose cotransporters 1 and 2 (SGLT1, encoded by *SLC5A1*; and SGLT2, encoded by *SLC5A2*). SGLT2, located in the early PCT (segments 1–2), accounts for ~90% of the reabsorption, whereas SGLT1 reabsorbs the remaining glucose in the late segment (3). However, a therapeutic inhibition of SGLT2 increases the magnitude of glucose absorbed by SGLT1.[452–454]

SGLT2 inhibitors (SGLT2i's) are a class of oral antihyperglycaemic drugs to inhibit renal SGLT2. This inhibition induces glucosuria, essentially improving glycaemic control in patients with diabetes without the risk of hypoglycaemic episodes.[452–454]

HNF1α normally regulates the expression of SGLT2, which is responsible for ~90% of renal glucose reabsorption (see 2.3.5). A relative deficiency of SGLT2 in patients with HNF1A-MODY probably contributes to the low renal threshold for glucose observed in patients[265,396] (see also 2.3.3.4.2). Logically, patients with reduced expression of SGLT2 hardly benefit from further blocking of SGLT2. However, a preliminary study strikingly implied that a single dose of SGLT2i's induced more pronounced glucosuria in patients with HNF1A-MODY than in those with type 2 diabetes[455]. The rationale remains unclear. In future, researchers should evaluate the differences in the

compensatory glucose reuptake by SGLT1 after blocking SGLT2 in patients with MODY compared with other types of diabetes.

Although all patients with any form of diabetes, including HNF1A-MODY, can be at risk of developing euglycaemic ketoacidosis when treated with SGLT2i's, even patients with type 1 diabetes can now use SGLT2i's as an adjunct therapy if they take appropriate precautionary measures to address the risk of ketoacidosis[456]. Despite the concerns associated with the use of SGLT2i's[455], surprisingly few reports on ketoacidosis associated with SGLT2i's in patients with HNF1A-MODY have emerged[457].

2.3.6.5 Insulin treatment

An insulin regimen is essential for all patients without a sufficient capacity to produce endogenous insulin, such as those with type 1 diabetes. In addition, regardless of a specific subtype of diabetes, insulin treatment stands as the last frontier to maintain glycaemic control in any patient[458,459].

Although numerous patients with MODY often discontinue insulin injections and adopt an alternative antihyperglycaemic therapy at the genetic diagnosis, the re-initiation of insulin treatment might become inevitable again after a progressive deterioration of glycaemic control[196] (see also section 2.3.3.1). Another transient need for insulin could emerge during pregnancy (2.3.6.6), as metformin and insulin are the only therapies currently available to treat hyperglycaemia in pregnant women.

2.3.6.6 Treatment and monitoring during pregnancy

The diagnosis of HNF1A-/HNF4A-/HNF1B-MODY might precede pregnancy or emerge during a pregnancy, owing to increased insulin resistance during the second and third trimesters, as well as active routine screening efforts to identify GDM. However, whereas patients with GCK-MODY typically exceed the reference value of plasma glucose already at fasting (2.2.6), patients with MODY associated with HNFs might maintain normal fasting glycaemia yet demonstrate increased postprandial levels of glucose. The OGTT is therefore crucial in identifying early presentation of MODY (see also 2.3.3.2).

Because most antihyperglycaemic drugs are not safe or certified during pregnancy, a transient switch to insulins and metformin must take place until the childbirth. Glibenclamide, once considered safe during pregnancy[460], is no longer recommended as it crosses the placenta and increases the risk of macrosomia and neonatal hypoglycaemia[461].

Neonates whose parent has a variant associated with HNF4A-MODY might present with hypoglycaemia and macrosomia at birth (see 2.3.3.1). Whenever maternal or paternal inheritance of a pathogenic *HNF4A* variant could have occurred, biweekly monitoring of foetal growth is recommended after 28 weeks of gestational age, even in the absence of maternal hyperglycaemia, and an elective Caesarean section is suggested between weeks 35 and 38[209]. Of note, the obstetrics department in our hospital generally considers labour induction or an elective Caesarean section at weeks 38–40 in all patients on insulin therapy during pregnancy.

Foetuses heterozygous for a pathogenic variant causal of HNF1BD often demonstrate IUGR (2.3.3.6). Foetal structural anomalies in the kidneys might also present antenatally[462,463]. Some affected neonates develop cholestatic emergencies (2.3.3.5). Hence, numerous foetal implications imply a need for personalized antenatal and neonatal monitoring. In addition, mothers might need careful monitoring of kidney function and cholestatic laboratory tests during pregnancy, as we suggested in a Finnish review[128].

Of note, non-invasive prenatal testing (see 2.2.6) has also become available for mothers with a variant in *HNF4A*[136].

2.3.7 Microvascular and macrovascular complications

Type 1 and type 2 diabetes increase the risk for microvascular and macrovascular complications[464,465]. Microvascular complications have classically included retinopathy, neuropathy and nephropathy, but the list has recently been reinforced by various additions, *e.g.* cerebral microvascular complications[466]. The microvascular outcome in both type 1 and type 2 diabetes largely depends on glycaemic control. Intensive management of diabetes reduces the risk by approximately 70%, as mirrored by a decrease in HbA_{1c}. Still, a rather considerable proportion of the risk arises from factors unrelated to HbA_{1c}, albeit possibly involving dimensions of glycaemic control, such as glycaemic variability[464,465].

Although a decrease in HbA_{1c} is a direct proxy for a decrease in average plasma glucose levels, it also reflects insulinotropic effects associated with most antihyperglycaemic drugs. Therefore, the association between microvascular complications and hyperglycaemia could partially be indirect and secondary to insulin deficiency. For example, insulin deficiency is associated with endothelial dysfunction and impaired lipid metabolism (including elevated free fatty acid levels)[467,468]. To divide microvascular complications into primarily glycaemia-related or/and insulin-related outcomes might provide future insights for personalized medicine, especially among patients with insulin-deficient forms of diabetes, such as HNF1A-MODY.

Intensive glycaemic control does not alleviate the risk of macrovascular complications, such as atherosclerosis and other cardiovascular diseases, to

the extent seen in the context of microvascular complications. However, the macrovascular and microvascular interact, exemplified by diabetic nephropathy, serving as a risk factor for macrovascular diseases. Notably, the medical therapies for dyslipidaemia and hypertension have significantly improved macrovascular outcomes in recent decades[464,465].

In 1998, our group published a study that reported a high prevalence of microvascular and macrovascular complications in HNF1A-MODY. However, if the patients were matched for the duration of diabetes and glycaemic control, the complication profile was similar in all different forms of diabetes, and the glycaemic control served as a major determinant of the complications[469]. Studies from France[470] and Poland[267] reported similar figures.

In 2010, a UK study identified that HNF1A-MODY is associated with 2.6-fold cardiovascular mortality and 1.9-fold all-cause mortality[471] compared to unaffected family members. These findings translated into a recommendation to initiate statins at the age of 40[74], regardless of glycaemic control. The original publication, however, did not adjust the mortality for the glycaemic control.

Indeed, six years later, an appropriate glycaemic control in HNF1A-MODY was associated with a reduced risk of microvascular and macrovascular complications. Complications are more infrequent in patients with HNF1A-MODY than in patients with type 1 diabetes (matched for BMI, age, ethnicity, diabetes duration; retinopathy 13.6% vs 50%, $p < 0.0001$; microalbuminuria 5% vs 8.3%, n.s.; nephropathy 5% vs 8.3%, n.s.; coronary artery disease 6.7% vs 8.3%, n.s.)[425]. In addition, the complication rate was generally lower than in previous reports on MODY. About 20% of patients with HNF1A-MODY received statins, which might beneficially affect the outcome. Yet, although the patients with HNF1A-MODY with or without complications had similar HbA_{1c} levels, a paucity of long-term data on HbA_{1c} measurements could have underestimated the all-life glycaemic burden.

2.4 Monogenic diabetes and RFX6

2.4.1 RFX6 controls embryonic pancreatogenesis and maintains cell identity in adult β cells

Members of the regulator factor X (RFX) family, with a high level of evolutionary conservation across species, share a winged helix-turn-helix domain, which interacts with DNA by recognizing the major groove with an

adjacent X box motif. One of the eight RFXs identified to date, regulatory factor X6 (RFX6), plays a crucial role in pancreatic development in vertebrates, including humans[472–478].

In mice, RFX6 is expressed throughout the definitive endoderm at the embryonic day (E) 7.5. From the gut endoderm at E9.0, the protein expression of RFX6 eventually spreads to multiple foci of the gut and pancreas[475]. In *Rfx6* null embryos, the endocrine pancreas fails to initiate a sequential gene activation downstream of Neurogenin3, hence displaying an absence of cells producing insulin (β cells), glucagon (α cells), somatostatin (δ cells) and ghrelin (ε cells), but pancreatic polypeptide (PP cells) remains present[475]. Early lethality follows within a few days after birth[475]. *Rfx6* is also present in the murine gut endoderm, and eventually the small intestines and colon[475,479], promoting the enteroendocrine progenitors to differentiate into enteroendocrine cells (EECs) that secrete peptide hormones (GIP, GLP-1, cholecystokinin)[479].

Studies on the human Endo-βH2 cell line[480] and the postnatal conditional deletion of *Rfx6* (>95%) in mice[481] have indicated that the adult loss of RFX6 is far less drastic than the embryonic loss of RFX6. Without RFX6, adult β cells demonstrate reduced insulin content and secretion, as well as downregulation of L-type Ca^{2+} channels, which is crucial for insulin exocytosis evoked by membrane depolarisation. Therefore, it can be concluded that RFX6 maintains β cell identity[480,481].

2.4.2 Biallelic RFX6 inactivation causes Mitchell-Riley syndrome in humans

In humans, homozygosity and compound heterozygosity of inactivating *RFX6* variants manifest as Mitchell-Riley syndrome (OMIM # 615710), which is characterized by pancreatic hypoplasia and neonatal diabetes, intestinal atresia and/or malrotation, gallbladder aplasia and other extrahepatic biliary defects and is occasionally accompanied by additional features such as IUGR[482–486]. In some patients, the onset of diabetes occurs after the neonatal period, possibly implicating variability between different gene variants[486,487].

Case reports often state that patients die early in the neonatal period or early infancy, but obviously, therapeutic approaches might alter the outcome. Interestingly, among the reported cases listed in a recent review article[488], those who died within 6 months of age (N=5) had a lower mean weight (1333 g) than those reportedly alive (1813 g, N=9, p=0.016, Mann–Whitney U test; data are unadjusted for gestational age as gestational age was similar in both groups, p=0.31, means 34.4 vs 36 weeks). However, owing to the rare prevalence of this recessive disorder, data on possible heterogeneity are scarce. Despite an apparent phenotypic overlap between Mitchell-Riley syndrome and Martínez-Frías syndrome (OMIM % 601346, with pancreatic hypoplasia, intestinal atresia and biliary atresia occasionally with

tracheoesophageal fistula but without diabetes)[489,490], Martínez-Frías syndrome has not been associated with *RFX6*[475].

2.4.3 Pancreatic hypoplasia and similarities to HNF1BD

Pancreatic body and tail, both of which originate from the ventral pancreatic bud, are typically absent in patients with Mitchell-Riley syndrome. Indeed, human iPSCs with a biallelic *RFX6* inactivation display no ventral pancreatic bud[491]. Intriguingly, the structural pancreatic defect is similar to that observed in most patients with HNF1BD, who also lack the pancreatic body and tail (2.3.3.3). In addition, HNF1BD has some features of primary ciliopathy (2.3.3.5, 2.3.5), and some members of the RFX family have also been associated with defective primary cilia[492]. Primary cilia control cell identity/polarity and cell division in various tissues[493–496] and regulate glucose homeostasis through the paracrine interactions between islet cells[406]. Although primary cilia are not essential for endocrine cell development *per se*[497,498], and although the *Rfx6* null mice express unaffected ciliogenesis in islets[475], the loss of ciliary control can nevertheless somehow disrupt tissue organization in the pancreas[497,498]. Future research needs to evaluate whether *RFX6* affects the endocrine fate in the pancreas via ciliary regulation by the exocrine cells.

2.5 Monogenic mitochondrial diabetes

Mitochondria are present in high numbers (from hundreds to thousands) in most eukaryotic cells, where these intracellular organelles produce the main source of cellular energy in a process called cellular respiration. In addition to producing energy, mitochondria contribute to numerous cellular functions[499].

Mitochondrial DNA (mtDNA) is the 16,569-nucleotide circular chromosome inside the mitochondria that encodes a small but important fraction of mitochondrial genes not encoded by the nuclear DNA[500]. Because mitochondria are of maternal origin[501–503], a person can inherit a pathogenic gene variant in mtDNA from their mother but not from their father (with rare exceptions[501–503]). The offspring and the mother can demonstrate extremely heterogenous phenotypes as a result of an uneven and highly random distribution of unaffected and affected mitochondria in each cell and tissue from fertilization and embryonic development until adulthood. Having multiple forms of genetic material in each cell or individual is referred to as heteroplasmy. The level and distribution of heteroplasmy (in each organ)

define the clinical phenotype of a patient. People can perfectly tolerate a low level (1–2%) of clinically irrelevant heteroplasmy.[504]

2.5.1 The phenotypes of MIDD and MELAS, mitochondrial syndromes

The most common gene variant responsible for MIDD (maternally inherited diabetes and deafness, OMIM # 520000), or monogenic mitochondrial diabetes on the whole, is mt.3243A>G in *MT-TL1*. *MT-TL1* encodes a transfer RNA for leucine ($tRNA^{Leu}_{UAA}$) in the mitochondrial chromosome[505–510] and manifests with diabetes with a prevalence of 20–50%[511,512]. More rare gene variants associated with monogenic mitochondrial diabetes include genes for other tRNAs, namely *MT-TE* and *MT-TK*[513,514]. By some estimates, mitochondrial diabetes might account for up to 3% of all diabetes[512].

The mt.3243A>G transition is responsible for two clinically defined syndromes, MIDD and MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes, OMIM # 54000). Obviously, heteroplasmy modifies and determines the actual clinical phenotype at an individual level, and a phenotypic overlap between the two clinical syndromes is common. Diabetes associated with this variant develops at a highly variable age (from childhood to late adulthood) with a mean onset at approximately the late 30s[515].

A mitochondrial defect generally affects the organs with high energy requirements, including (but not limited to) pancreatic β cells, striated muscle tissue, the central nervous system and the auditory system[510,512]. In one patient series, hearing loss was the most prevalent finding, present in around one-third of patients at diagnosis and in more than half of the patients during follow-up. Only 15–20% of the patients had originally presented with diabetes or seizures, which were the two next common findings. During a follow-up, however, their prevalence increased to 40–49.9%[511].

A patient with a multiorgan presentation involving metabolic, neurological, muscular, ophthalmological and/or cardiac features (*Figure 5*) should be referred to genetic testing for mitochondrial disorder. After diagnosis, various medical specialities coordinate to initiate an appropriate strategy for management[510,512].

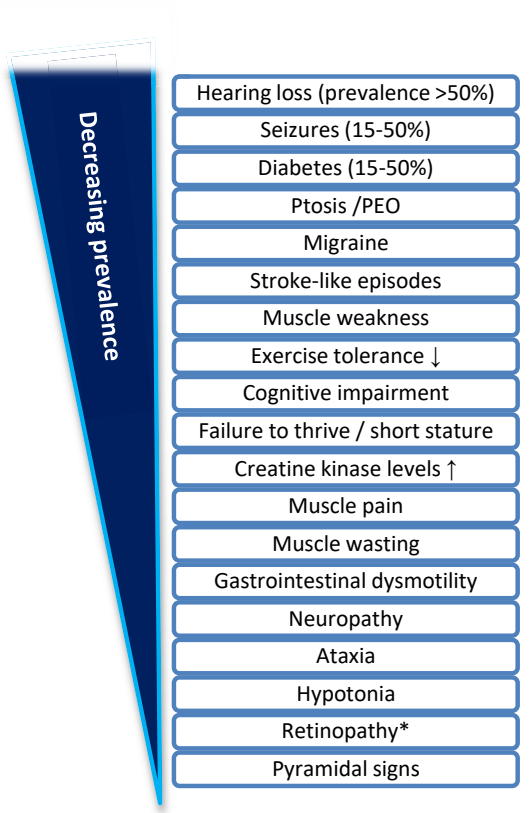

Figure 5. Phenotypes arising from the mitochondrial variants associated with diabetes in order of decreasing prevalence, as evaluated at the onset of the disease[512]*. Other rare endocrinopathies reported in patients include adrenal insufficiency, hypoparathyroidism, GH deficiency, hypogonadism and syndrome of inappropriate anti-diuretic hormone secretion (SIADH)*[515,516]*.*

PEO, progressive external ophthalmoplegia.

** A specific type of retinopathy, pigmentary retinal dystrophy, is more characteristic than a classic form of diabetic retinopathy.*

2.5.2 Insulin secretion and sensitivity

Mitochondrial disorders often present with an insidious onset of diabetes, and only one-fifth of the patients had experienced acute symptomatic hyperglycaemia. On the other hand, 45% progress to insulin treatment within 2–4 years after diabetes diagnosis[509,515]. However, the numbers of patients in observational and prospective studies have been limited, and the ascertainment bias might be substantial.

Nondiabetic and diabetic individuals with mt.3243A>G typically demonstrate impaired insulin secretion in at least one of the several tests performed (IVGTT, HGC, arginine test, graded/oscillatory glucose infusions)[505,517,518]. Owing to the virtually normal insulin secretion in the arginine test[517], mitochondrial defect probably deteriorates glucose sensing rather than insulin production. The results on insulin sensitivity (by HEC) have been contradictory. Some studies have implied insulin resistance at

least in those with overt diabetes[517,519,520], whereas others have not[521,522]. Roughly a decade ago, Finnish researchers used HEC (using labelled glucose) with PET (positron emission topography) to find that the mitochondrial variant mt.3243A>G disrupts β cell function[523] and the peripheral insulin sensitivity in muscles[523] and subcutaneous adipose tissue[524] but leaves the hepatic insulin sensitivity unaffected[524]. These changes preceded the onset of overt diabetes. Indeed, mitochondria interact with hexokinase II (HK-II)[525] and various other levels in human physiology.

2.5.3 Therapeutic implications

RCTs for mitochondrial disorders are few and have not produced any definitive evidence on the treatment of mitochondrial diabetes by any medical therapy or dietary supplement (such as coenzyme Q10)[526]. Physical exercise maintains general health and might also be beneficial for those with mitochondrial conditions[512].

Lactic acidosis belongs to the clinical spectrum of MIDD/MELAS, but it is also a possible adverse effect associated with the use of metformin, especially in those with pre-existing risk factors (such as renal or cardiac insufficiency). Therefore, guidelines for mitochondrial diabetes recommend avoiding metformin with pre-existing hyperlactataemia or monitoring patients carefully when on metformin[512,527]. A similar recommendation probably applies for thiazolidinediones (pioglitazone)[512]. The risk of hypoglycaemia could be lower when on short-acting sulfonylureas instead of long-acting ones[515]. Among the novel antidiabetic therapies, DDP4i's are probably a safe option[527]. GLP1-RAs and SGLT2i's could prove advantageous in the future, but the present data remain scarce[527].

Antidyslipidaemic therapy (especially statins and fibrates) might deteriorate mitochondrial function in muscles, hence predisposing to worsening (skeletal or cardiac) myopathy[512]. Balancing between benefits and adverse effects is essential when initiating statins/fibrates.

3 AIMS AND STUDY DESIGN

3.1 Aims and objectives

FINNMODY, a subproject of the Botnia Study[218] since 2014, has identified and characterized patients with a suspected or established diagnosis of monogenic diabetes. The patients' family members have also been invited to enrol. In addition, FINNMODY has been coordinating the follow-up of families previously diagnosed with monogenic diabetes in the Botnia Study.

The specific aims for the three studies included in this thesis were

I. to investigate the phenotype in patients with HNF1BD, with a focus on the biliary features
II. to assess *RFX6,* novel candidate genes associated with MODY, and introduce a preliminary phenotype of RFX6-MODY
III. to delineate clinical features associated with the *HNF1A* variant p.(Gly292fs), the most common variant responsible for HNF1A-MODY, using a family-based design

These aims cohere with the general objectives of the FINNMODY study, which are to assess the prevalence of monogenic forms of diabetes in Finland, with an emphasis on MODY and MIDD:

a. to characterize variant–disease associations and assess whether genetic and non-genetic factors modulate the clinical presentation;
b. to describe clinical features associated with monogenic diabetes and to provide recommendations for the diagnostic work-up, treatment and monitoring;
c. to evaluate how monogenic gene variants deteriorate glucose metabolism; and
d. to identify new gene variants and genes associated with monogenic diabetes.

3.2 Patients and methods

3.2.1 Patient recruitment

The FINNMODY study has a total of 1080 participants, including Botnia families with an identified genetic aetiology of monogenic diabetes and enrolled before the initiation of the FINNMODY study. Of the 1080 participants, 485 have been identified with a gene variant associated with monogenic diabetes, and most (>99%) of the genetic diagnoses are classified as MODY. *Figure 6* summarizes the number of participants. The patient selection for Study II, which was a collaborative effort between the Finnish, British and Belgian counterparts, is separately discussed.

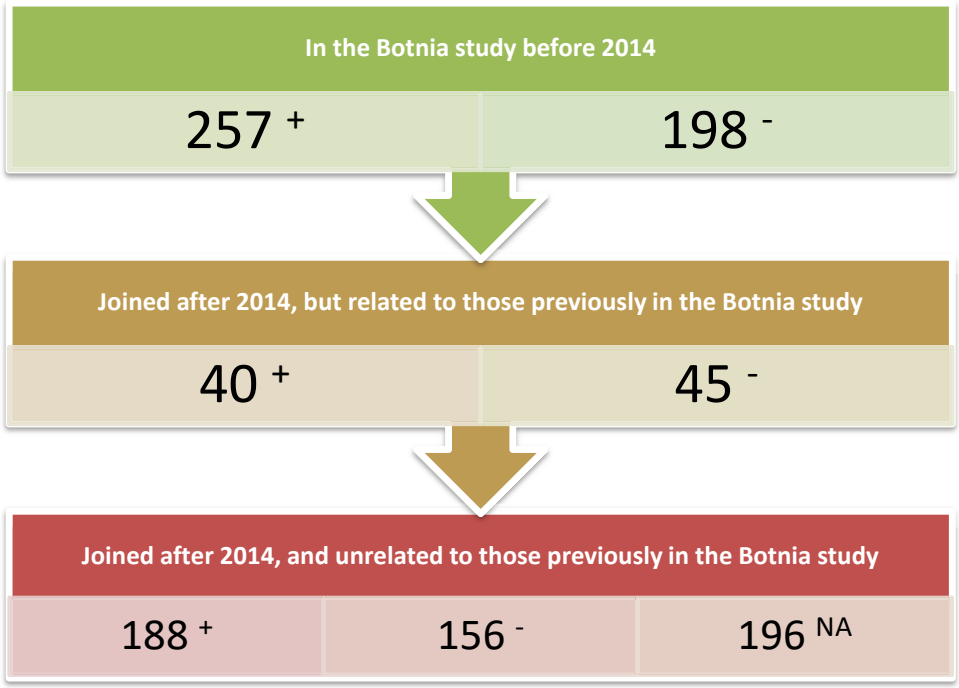

Figure 6. Participants of the FINNMODY study until the 15th of December 2020.

+, participants with pathogenic or likely pathogenic gene variant(s) responsible for a monogenic form of diabetes (including mitochondrial diabetes);

[-], participants, whose (first-degree) relative has a monogenic form of diabetes, but who themselves do not have the familial variant;

[NA], participants from families without an identified aetiology of monogenic diabetes (or not targeted for genetic testing)

3.2.2 Advertising and recruitment

Somewhat unique to the FINNMODY study, possible participants have directly contacted FINNMODY online, by phone or by regular mail. Of those initially enrolled, >90% eventually proceeded to blood sampling after fulfilling the minimal inclusion criteria (a relative or total absence of features commonly associated with type 1 and type 2 diabetes and/or a multigenerational family history of diabetes).

Multiple interviews and advertisements in Finnish medical and patient journals have increased awareness of monogenic diabetes among the diabetologists and patients *(Figure 7)*.

Figure 7. A few examples of increasing awareness of monogenic diabetes. The first picture (top left corner) is an advertisement published in Duodecim (a Finnish medical journal). The picture in the middle was shot during the Annual Meeting of the Finnish Diabetes Association (photograph by Paula Kokko, published with her kind permission). The other pictures represent interviews and articles published in various journals (the magazines in the bottom left-hand corner, Folkhälsan; in the top right-hand corner, Mediuutiset, and the bottom right-hand corner, Diabetes ja lääkäri).

3.2.3 Genetic testing

For the first few years of the FINNMODY project, research assistant Paula Kokko performed all the in-house genetic testing by the conventional Sanger method (introduced by Frederick Sanger and colleagues in 1977[528]). External laboratories, especially those located in Exeter (United Kingdom) and in Kuopio (the University of Eastern Finland) provided services for next-generation sequencing (NGS)[529].

In 2018, with assistance from FIMM (Finnish Institute of Molecular Medicine, University of Helsinki), the FINNMODY study pioneered its own NGS panel with 19,084 probes for screening for exons (± 50 nucleotides) and promoters of the target genes associated with monogenic diabetes. The mean exonic read depth of the target genes was above 85 in more than 85% of the samples, whereas the four most common MODY genes (*GCK, HNF1A, HNF1B, HNF4A*) displayed a mean read depth of about 150 in ≥95% of the samples. The variants initially called by the FIMM annotation system proceeded to a robust filtering process (utilizing the population frequency data and *in silico* predictors *etc.*) before being clinically interpreted[530]. To date, no clinically significant panel call (with adequate read depth) has been a false negative in confirmatory analysis by Sanger sequencing.

3.2.4 Other laboratory testing, imaging studies and ethics

Our laboratory personnel, especially medical laboratory technologist Paula Kokko, have performed most laboratory testing for analyses associated with glucose metabolism (such as insulin, C-peptide, glucagon, proinsulin), along with various other samples (such as autoantibodies, free fatty acids). Prof Marja-Riitta Taskinen's Lipid Metabolism Study Group has kindly provided their assistance to analyse selected samples of lipid metabolism (such as cholesterol and apolipoprotein measurements). In addition, routine laboratory testing (such as blood count) has been performed in local hospital laboratories. (Please refer to the method sections of each study and the electronic supplementary material, Table 2 of Study III in this thesis for further information.)

Authors H.P. and E.L. (of Study I) designed the protocol for the magnetic resonance imaging primarily targeted to the upper abdominal region (magnetic resonance cholangiopancreatography) and performed by the HUS Medical Imaging Center, a division of the Helsinki and Uusimaa Hospital District.

Ethical committees of Helsinki and Uusimaa Hospital District have reviewed and approved the protocols of the Botnia and FINNMODY studies. Over the years, several amendments have addressed the modifications to the study protocols. The protocols must also adapt, for example, to advancements in genetic techniques and renewing legislation, as exemplified by the

modifications motivated by the *General Data Protection Regulation* (GDPR, EU 2016/679) entering into force.

3.3 General concepts and pitfalls related to the study design

Owing to a rather low prevalence and lack of pathognomonic features, a general challenge is to identify sufficient numbers of patients with monogenic diabetes. The patients with characteristic features suggestive of monogenic diabetes are more likely to be identified and diagnosed, which might introduce ascertainment bias.

Ascertainment bias, however, does not always present as a major issue. For example, the aim of Study I, which proposed a new phenotype, was not to estimate a prevalence of this phenotype or how severe it is but to acknowledge the presence of a phenotype in general. In Study II, the replication outside the original discovery cohort confirmed the primary results at the population level. The population data probably serve as a potentially less biased estimate of a prevalence of the condition. Study III specifically emphasized the role of ascertainment bias.

4 RESULTS AND SPECIFIC DISCUSSION OF EACH STUDY

4.1 Study I

In Study I, 14 participants with HNF1BD underwent magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP), two procedures never systematically performed together in those with HNF1BD. MRI and MRCP results on the hepatobiliary and pancreatic structures indicated that biliary anomalies were surprisingly common; half (7) of the 14 patients manifested with biliary anomaly, and 6 of the 7 presented with bile duct cysts (BDCs, a.k.a. choledochal cysts). BDCs are associated with a high risk of malignancies. Malignancies were not present in our patients.

4.1.1 Secondary unpublished results

Patients with HNF1BD arising from intragenic variants display more severely impaired renal function than those with a hemizygous deletion of *HNF1B* (and typically associated with a ~1.4 Mb microdeletion on 17q12) (see 2.3.3.7). This pattern was evident *(Figure 8)* in our 16 patients with HNF1BD, 10 of whom had a hemizygous deletion and 6 an intragenic variant. A mixed linear model (with a random intercept for each patient and fixed effects from the age and variant type, deletions vs intragenic variants) in patients of Study I indicated that the 10 patients with a deletion displayed an approximately 77 ml/min/1.72m^2 higher estimated glomerular filtration rate (based on the CKD-EPI equation[531]) than the 6 patients with intragenic variants (Akaike information criterion, 3951.9 vs 3929.1).

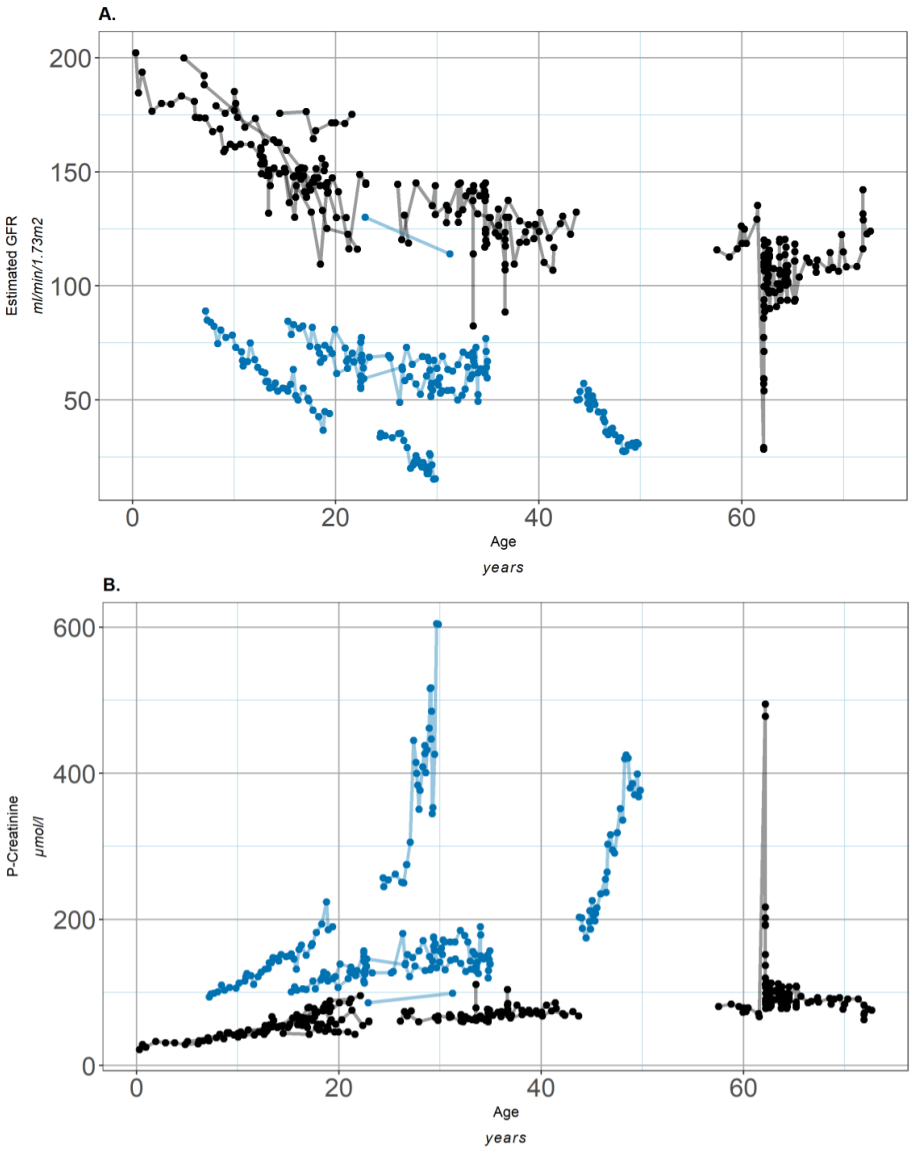

Figure 8. Panel A. Glomerular filtration rate (GFR), estimated by the CKD-EPI equation[531] and plotted against the age at measurement, is lower in those with HNF1BD attributable to intragenic variants (6 patients, represented by blue dots connected to visualize the follow-up) than in those with a hemizygous deletion (black dots; 10 individuals). As the CKD-EPI is applicable for those over 18 years of age, the underage estimates might be misleading or inexact. Panel B. The same data, but plasma creatinine has replaced GFR on the y-axis.

Contrasting a reported association between HNF1BD and primary hyperparathyroidism[307], elevated levels of plasma parathyroid hormone (PTH) mostly co-occurred with high levels of plasma creatinine, likely indicative of secondary (renal) hyperparathyroidism *(Figure 9)*.

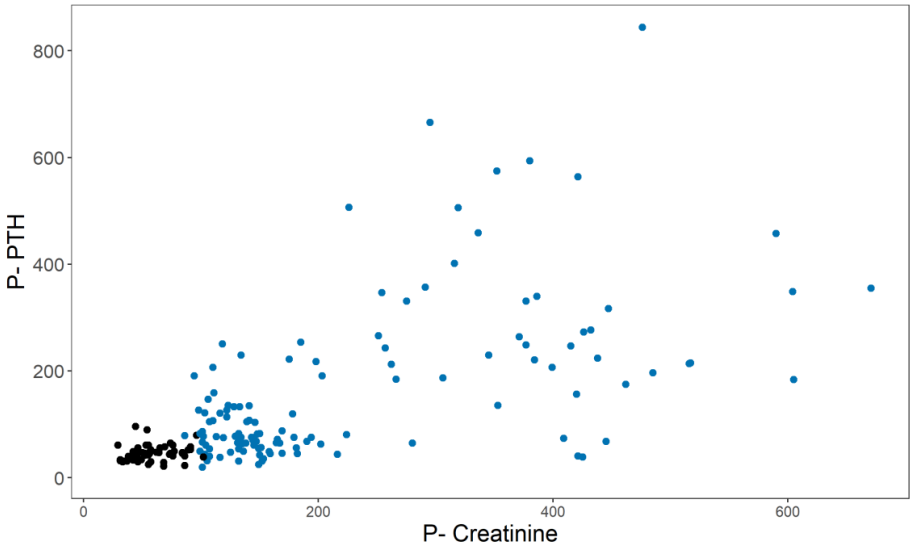

Figure 9. Plasma PTH (ng/l) plotted against concomitant plasma creatinine (µmol/l) during the follow-up in our patients with HNF1BD. The figure includes all measurements available for each patient (excluding measurements after a renal transplantation). Black dots represent those with a whole-gene deletion, and blue dots represent those with intragenic variants.

The reported clinical spectrum of HNF1BD includes hypermagnesiuric hypomagnesaemia. Low levels of plasma magnesium further predispose to urine potassium wasting (possibly via reduced inhibition of renal potassium channels in the kidneys) and consequent hypokalaemia[532,533]. Hypokalaemia has been reported in up to half of patients with HNF1BD (see 2.3.3.4). However, hypomagnesaemia is not the only factor responsible for potassium wasting[532,533], and most of our patients had not presented with hypokalaemia *(Figure 10)*. The figure also visually suggests that those with a whole-gene deletion demonstrate lower levels of plasma magnesium than those with intragenic variants. The mixed linear models also support the interpretation. However, some patients have occasionally used magnesium supplements, which is not incorporated in the models.

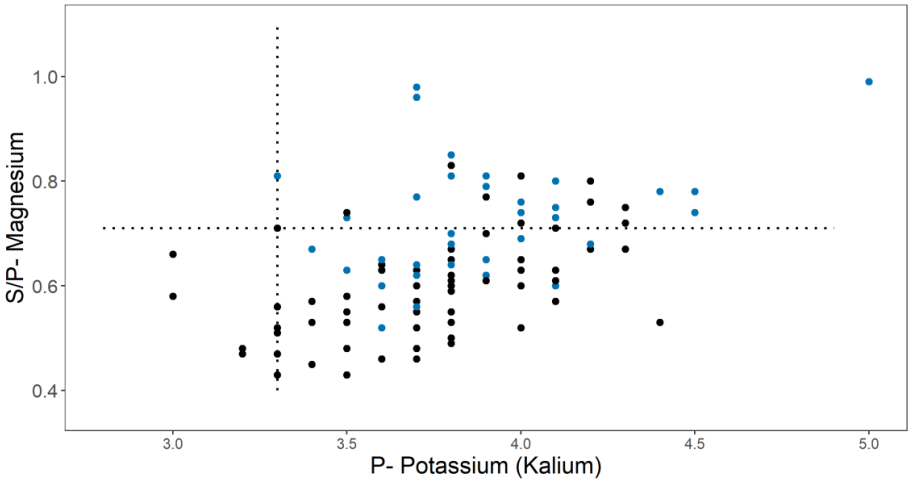

Figure 10. Plasma potassium (mmol/l) plotted against plasma or serum magnesium (mmol/l) in our patients with HNF1BD during the follow-up. The figure includes all measurements available for each patient. Dotted lines express the lower limit of the reference ranges of both potassium and magnesium. Black dots represent those with a whole-gene deletion, and blue dots those with intragenic variants.

4.1.2 Discussion and a dilemma of the BDCs among patients with HNF1BD

A high prevalence of BDCs in Study I indicated a novel biliary phenotype associated with HNF1BD. The overall phenotype associated with HNF1BD was heterogenous, as expected, and we could replicate several previously reported phenotypes.

BDCs are cystic dilatations of intrahepatic or extrahepatic bile ducts, with a population prevalence range of 1:13,000–1:150,000 (in Western populations) to 1:1,000 (in Asian, especially Japanese populations)[534–539]. The symptoms include a classic triad with abdominal pain, jaundice and palpable abdominal mass, although manifestation with a complete triad is somewhat uncommon. In a patient series published in 1994, 85% of paediatric patients, but only 25% of adult patients, demonstrated (at least) two of the three symptoms[540]. Incidental and asymptomatic BDCs have become increasingly common, representing up to 36% of the reported BDCs, assumedly due to an increased availability and use of imaging studies[537,541].

A risk of malignancies has been a classic concern associated with BDCs. In adults, 10–20% of the BDCs identified transform into cholangiocarcinoma

(or other biliary malignancies), but the rate is far lower in children (0.42–0.7%)[534,537,542]. Formerly, the treatment of extrahepatic BDCs involved cystoenterostomy, which left the extrahepatic cysts intact, but the procedure led to a high postoperative incidence of recurrent symptoms and malignancies in the follow-up. Therefore, a total excision of extrahepatic cysts and *Roux-en-Y* hepaticojejunostomy have become the gold standard of managing the BDCs, regardless of whether they present with or without symptoms[537,542,543].

BDCs are either acquired or congenital. Whether this distinction should influence the selection of the appropriate management and outcome has remained unclear. Most BDCs (50–80%) are believed to be acquired, and they co-occur with an anomalous pancreaticobiliary junction (APBJ) and/or other functional or structural strictures of the biliary tree (distal to the BDC). Consequently, the impaired flow in the bile system assumedly predisposes to a reflux of the pancreatic juice, hence increasing the risk of malignancies[537–539]. The acquired BDCs could partially explain the high prevalence of BDCs and cholangiocarcinoma in Asia and Japan[537].

Congenital BDCs might arise from ductal plate malformations[539]. In theory, these BDCs are less susceptible to deleterious effects from the pancreatic reflux. Considering that none of our patients had APBJs and that a few previous studies have identified ductal plate malformations in patients with neonatal HNF1BD[274,276–278,539], future research on BDCs and their management algorithms should involve evaluating the mechanism to drive cyst formation.

Although *HNF1B* plays a role in tumorigenesis and has been associated with numerous tumours, such as hepatocellular, pancreatic, ovarian, renal, endometrial, colorectal and prostate cancers[544,545], the clinical implications of the biliary findings in Study I remain unclear. On the one hand, the insufficiency of *HNF1B* as a tumour suppressor gene might predispose to malignancies, but on the other, the absence of APBJs might represent a favourable predictive factor. No case reports on patients with HNF1BD and bile duct carcinomas exist.

First, because of a relatively low prevalence of HNF1BD in the general population and a consequently small number of participants enrolled by Study I, we call for an international collaboration to further replicate the biliary phenotype in larger series of patients and to refine its prevalence. Second, whether the biliary findings remain unchanged or whether they progress during a follow-up study remains an unanswered question. A follow-up study is therefore crucial. Third, a genetic study in the patients with biliary neoplasms could reveal whether germline variants in *HNF1B* act as a risk factor for biliary malignancies.

4.2 Study II

Study II, a multinational collaborative effort between the British, Belgian and Finnish counterparts, concluded that individuals heterozygous for protein-truncating variants (PTVs) in *RFX6* are responsible for MODY but with a reduced penetrance (55% at 25 years of age). In addition, the heterozygosity was associated with higher levels of FPG, lower levels of plasma GIP, and an increased risk of diabetes compared to a population control group.

4.2.1 Discovery of a new form of MODY

Figure 11 elucidates the design of Study II. In short, monoallelic (heterozygous) PTVs in *RFX6* were enriched in a discovery cohort of 38 individuals whose phenotype had strongly resembled MODY (2 individuals accounted for 5.3% of the cohort). A prevalence of *RFX6* PTVs was also high in two replication cohorts (1.1% and 7.5%), comprising patients referred for genetic testing for MODY. Owing to PTV enrichment and clinical characterization in patients, the clinical spectrum associated with *RFX6* should also include MODY (see 2.4).

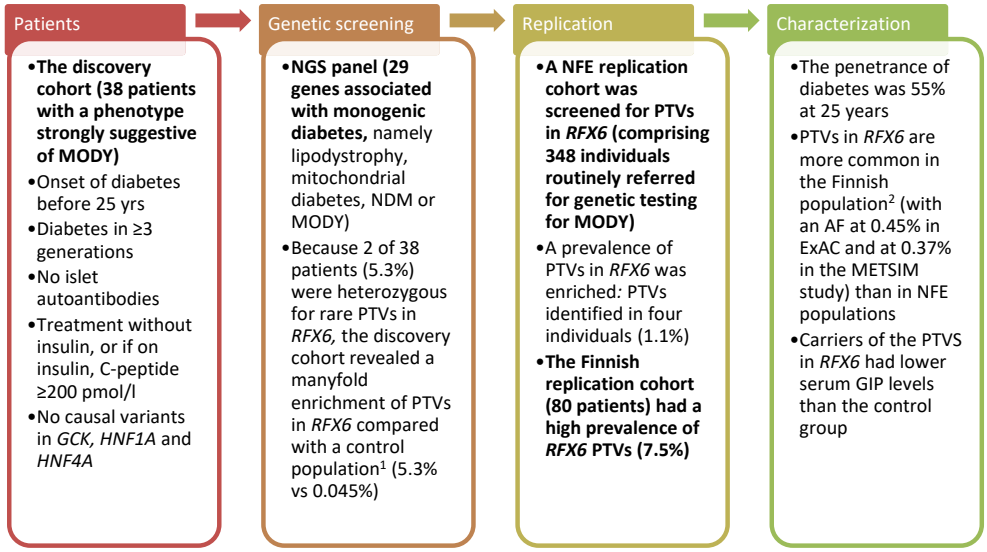

Figure 11. The approach used in Study II to identify novel genes responsible for MODY. NDM, neonatal diabetes mellitus; AF, allele frequency; PTV, protein-truncating variant; NFE, Non-Finnish European.

[1] *Non-Finnish individuals in the ExAC database for AFs*

[2] *In Finland, the enrichment is attributable to variant p.(His293Leufs) (rs762966411), which is possibly of an Eastern Finland origin (the METSIM study from Eastern Finland had a higher AF than the Botnia PPP study in Western Finland, where we identified the variant in only 2 out of 5180 participants, data not shown).*

4.2.2 Discussion

4.2.2.1 Does diabetes arise from β cell dysfunction or incretin failure?

Clinical details were available for 27 patients with RFX6-MODY, 8 (30%) of which were initially, and 18 (68%) currently, on insulin (with or without adjacent oral therapy; for further information, see *Table 2* of Study II[72]). Insulin deficiency, as defined by hyperglycaemia co-occurring with low levels of serum C-peptide, was manifest in some patients (Supplementary figure 4 of Study II[72]). Because *RFX6* regulates the foetal development of the pancreas and maintains adult β cell identity (see 2.4.1), β cell dysfunction is one of the most obvious candidates for the pathophysiology of RFX6-MODY. However, serum C-peptide and insulin were typically higher in the heterozygotes than in the control group (whether or not they reach statistical significance; Supplementary table 6 of Study II[72]). The evidence on insulin deficiency is therefore neither conclusive nor consistent.

On the other hand, could heterozygous PTVs in *RFX6* demonstrate a dual phenotype – presenting as insulin deficiency in some but predisposing to insulin resistance with compensatory hyperinsulinemia in others? The rare Finnish PTV in *RFX6* [p.(His293Leufs), rs762966411] is associated with an increased risk of both type 2 and type 1 diabetes in the FinnGen, which is a database for population genetics in Finland (its latest public freeze is available at r4.finngen.fi). This might imply a complex genetic architecture associated with *RFX6*.

On the other hand, disrupted islet function (and possibly decreased β cell survival) could be secondary to *RFX6* in the pancreatic islets. For example, the individuals with *RFX6* PTVs presented with low circulating levels of GIP. GIP is an insulinotropic and glucagonotropic incretin hormone secreted from intestinal K cells and under the transcriptional regulation by *RFX6*[440,546]. As GIP further contributes to various extrapancreatic tissues such as adipose tissue[547], vasculature[548] and bone marrow[549], GIP deficiency might affect insulin sensitivity and glucose metabolism throughout the body.

To delineate the exact pathophysiology of RFX6-MODY, iPSCs and other cell lines as well as animal models might provide future insights.

4.2.2.2 Would WES have been inferior to screening a list of candidate genes?

To identify novel genes responsible for a monogenic disease, a candidate gene approach provides both advantages and disadvantages as compared with larger screening efforts, namely whole exome sequencing (WES) or whole genome sequencing (WGS). Study II adopted the candidate gene approach, targeting 29 genes previously associated with monogenic diabetes *(Figure 11),* to uncover a novel association with MODY. After all, several genes (*GCK, KCNJ11, ABCC8* and *INS*) had already been associated both with MODY and neonatal diabetes, and other factors also favoured the candidate gene approach in Study II.

Commonly, candidate genes are those with a certain or putative function associated with human biology, as first unravelled, for example, by *in vitro* studies, animal models, phenotype–genotype associations in population databases, and *in silico* methods that predict DNA, RNA or protein folding and interactions. Because some gene-specific mechanistic studies might have already been conducted before the identification of a putatively pathogenic gene variant in the candidate gene, the requirement of further mechanistic studies after the gene discovery might be minimal. The candidate gene approach also produces far less data than WES/WGS and, therefore, requires far less data storage.

The number of candidate genes to screen has been highly critical as the candidate gene approach used to rely on the time-consuming Sanger method. Currently, however, the Sanger method has largely been displaced by the NGS methods, which can efficiently and rapidly cover arbitrary sets of candidate genes. The candidate gene approach by the NGS methods requires designing customized probes (baits) that cover the genetic region of interest. Predesigned commercial probes for WES and WGS are commercially widely available, which might favour using these techniques. Researchers and geneticists might also prefer first performing WES/WGS and then using "virtual probes" to prefilter a specific region out of the data.

Exhaustive screening by WES or WGS would also detect far more false positive findings than a target candidate gene approach. Should several persons from one affected family all undergo WES/WGS (*e.g.* trio studies), however, a robust exclusion of several false positive findings might often be possible. Unfortunately, the study cohorts of patients with rare diseases often lack samples from family members. WES and WGS techniques might also inadequately map short reads and sequences with challenging composition (such as repetitive elements)[550].

Nevertheless, WES and WGS might be necessary to identify novel genes associated with monogenic diabetes, as the hunt for new subtypes of monogenic diabetes has been vigorous ever since the 1990s, and only the subtypes with exceptionally low prevalence might have remained unidentified to date. To discover a new genetic aetiology for MODY, an extremely high number of MODY-like patients without a known genetic diagnosis would be needed. Alternatively, linkage studies in large families

affected by familial diabetes without a known genetic diagnosis could uncover a novel subtype of monogenic diabetes.

4.3 Study III

By the late 1990s, the Botnia study had already identified the first Finnish families with HNF1A-MODY and HNF4A-MODY[215,304]. In the three large multigenerational families with HNF1A-MODY (families B, C and D in [551]), the variant responsible for MODY was the protein-truncating variant (PTV) *HNF1A*, now annotated as p.(Gly292fs). This PTV ranks as the most common variant identified among those with HNF1A-MODY worldwide[305]. Therefore, research on *HNF1A* p.(Gly292fs) can provide valuable knowledge for wide audiences of scientists, clinicians and patients.

Study III invited new individuals from families B–D, as well as individuals from other families with the same pathogenic PTV, regardless of participants' previous levels of plasma glucose, and aimed at evaluating clinical and metabolic features associated with *HNF1A* p.(Gly292fs). By recruiting participants irrespective of previous levels of glycaemia, we should reduce the risk of ascertainment bias associated with phenotype-based recruitment. In addition, confounding environmental factors, on average, are rather similar in carriers and non-carriers from the same families. Study III included 12 families with 145 individuals heterozygous for the p.(Gly292fs), *carriers*, and 131 first-degree relatives without the variant, *non-carriers*, and integrated cross-sectional metabolic characterization of all families into the follow-up data of the families (B, C and D[551]). We also assessed whether the polygenic risk score for T2D (T2D-PRS) modified the age at onset of diabetes.

Study III concluded that carriers were leaner than non-carriers, and they demonstrated enhanced lipolytic activity (as reflected by higher levels of serum free fatty acids). Plasma glucose levels were higher in carriers than in non-carriers throughout the OGTT and were suggestive of insulin deficiency, and serum insulin levels were lower in carriers than in noncarriers during the OGTT response. Against our expectations, glucagon levels were similar in carriers and noncarriers. Although most carriers developed diabetes at a young age, one-third remained free of diabetes at 33 years. Probably owing to the increased awareness and the active screening by routine OGTTs, the age at onset was earlier among the carriers born after 1975 than among those born before 1975. T2D-PRS was a moderate and significant modifier of the onset of diabetes. The families in this study demonstrated a higher T2D-PRS than a population-based control cohort from Western Finland, PPP Botnia[552], which might contribute to the high background prevalence of diabetes among the non-carriers.

4.3.1 On the origin of spectacular families

An international collaboration had determined an association between MODY3 (now HNF1A-MODY) and *HNF1A* in late 1996[44]. The next year, the Botnia study group finalized its first characterization project in Finland on MODY3[551]. This effort also initiated the follow-up at the heart of Study III and its large families (B–D). The geographical roots of these large families trace back to a region in the southern parts of the historic province of Ostrobothnia (in Finnish, Pohjanmaa) located in Western Finland, roughly around the modern regions of Central and Southern Ostrobothnia *(Figure 12)*.

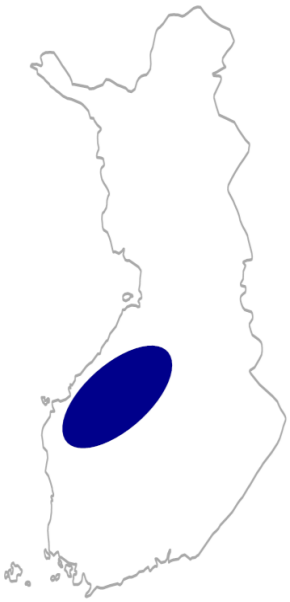

Figure 12. On the map of Finland, a data ellipse (as defined by the method by John Fox and Sandford Weisberg, 2011, using a level of 0.99) visualizes the affected parents' birthplaces in Study III.

But the Ostrobothnian families with MODY were hardly stunned to hear about the genetic diagnosis in the late 1990s. The personal discussions between the participants and our research nurse Leena Sarelin had also suggested that the families had long been aware of a particular young-onset form of diabetes that had manifested in numerous family members. Therefore, among the eldest carriers in the project (*e.g.* those born before 1975), the diabetes diagnosis had typically preceded the genetic diagnosis of p.(Gly292fs)[551]. However, among the carriers born more recently (*e.g.* after 1975), most carriers in the three large families of Study III had remained free of diabetes at the first assessment *(Figure 13)*.

On the other hand, and likely owing to an increased awareness of MODY and the systematic screening already performed at a young age, the carriers born after 1975 have been diagnosed earlier than those born before 1975. The difference appears most manifest during adolescence and early adulthood, as visualized in our recent review article[111].

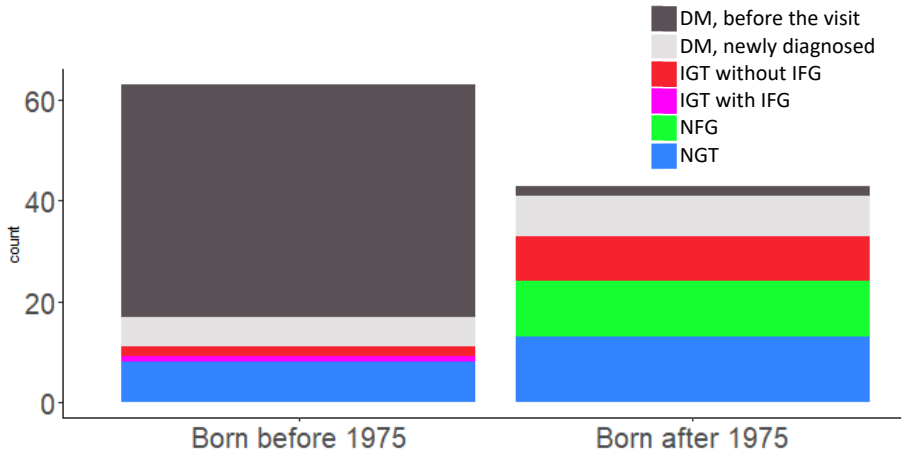

Figure 13. The number of carriers in the three large families of Study III stratified by glucose tolerance at the first study visit. The left bar represents the number of those born before 1975 and the right bar those after 1975.

DM, diabetes (mellitus); IGT, impaired glucose tolerance; IFG, impaired fasting glucose; NFG, normal fasting glucose (no OGTT performed); NGT, normal glucose tolerance.

4.3.2 Participation rate

In a family with a dominant monogenic condition (not arising from *de novo* variants), classical or reverse cascade screening[553] should generally yield a ratio of heterozygous carriers to non-carriers roughly at 1:1 (50–50%). Obviously, HNF1A-MODY should also not violate this assumption. Ascertainment bias might also occur if participants are *e.g.* healthier ("healthy user bias")[554] or more educated[555,556] than a general population. In addition, even the family-based studies might be subject to a bias related to geographical locations[557].

Study III presumably involved a rather low risk of ascertainment bias, as evidenced by a 53–47% ratio of carriers and non-carriers, a similar polygenic risk for type 2 diabetes in both groups and a satisfactory participation rate among families B–D participating in the study since the 1990s. In these families, the proportion of those participating in a combined sum of those participating and their siblings not participating was 85% *(Figure 14, panel A)*. If excluding those born before 1975 and those after 2000, the rate became 94%. Alternatively, the participation rate could be defined as a proportion of a number of carriers' offspring in the study to a number of the offspring whether in the study or not; this definition yielded a somewhat lower but still a satisfactory participation rate at 77% *(Figure 14, panel B)*. If excluding those born after 2000, the participation rate was at 92%, which suggests a low participation rate among the youngest. Regardless of the finding, even those born after 2000 typically participated in the study if any of their siblings had *(Figure 14, panel A)*.

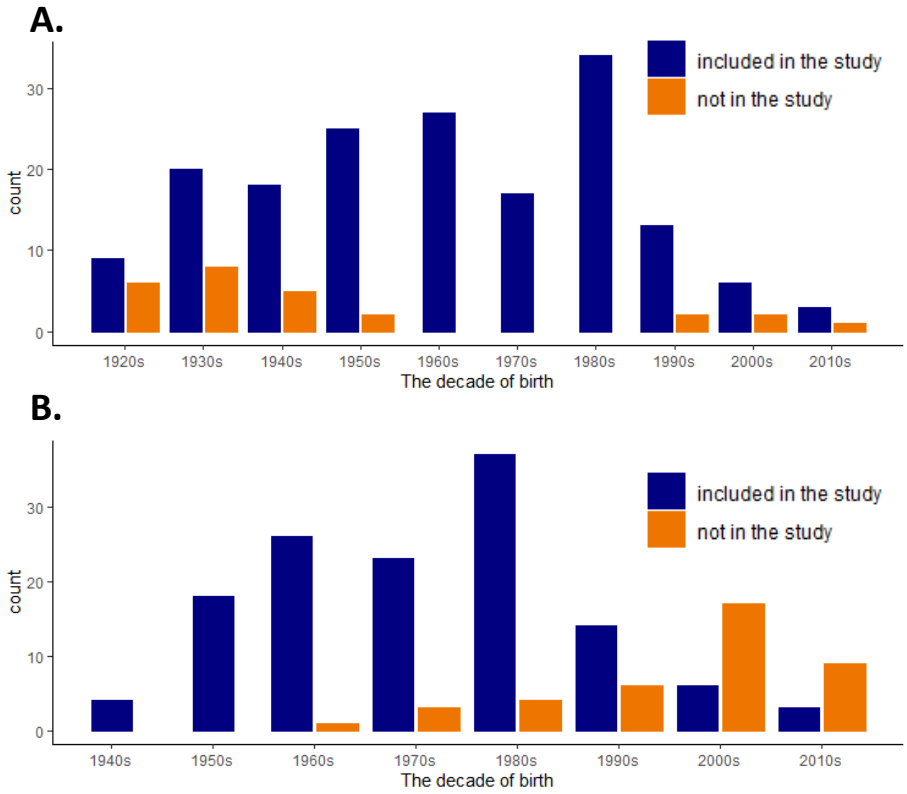

Figure 14. Panel A summarizes the number of participants from families B-D (blue bars) and the number of siblings not in the study (orange bars), grouped by the decade of birth since the 1920s, whereas panel B visualizes the number of the carriers' offspring, grouped by whether they are participating in the study (blue bars) or not (orange bars).

4.3.3 Modifications to medical treatment

Although Study III did not formally involve therapeutic interventions, the therapeutic insights (such as discontinuation of insulin treatment) are among the typical implications for the genetic testing for MODY. In Study III, we contacted participants as necessary to revise their current treatment after the study visits. To evaluate whether this actually affected the treatment, we later collected data on the electronic health record (EHR) systems, which are used in hospitals and health care centres to store patients' medical history and data on drugs prescribed. These data, after being sent to the national statistical authority, are further available for *e.g.* research purposes.

Although some EHR systems fail to store or report medication data, and although these systems lack information regarding actual dispensations of the drugs prescribed, the data from the most recent years of Study III uncovered that the participation demonstrated a moderate effect on the treatment among heterozygous carriers of *HNF1A* p.(Gly29fs) *(Table 6)*. A few carriers could discontinue their insulin. Interestingly, the number of participants using repaglinide clearly increased (91 carriers without repaglinide before the study visit and 77 after it, p=0.0025 by Fisher's test).

antihyperglycaemic drug	before the study	after the study
metformin	12	10
metformin and gliptin (FDC)	3	7
gliptin (DPP4i)	9	10
repaglinide	4	18
insulin aspart/lispro/glulis (fast-acting)	19	13
insulin detemir/glargine (long-acting)	24	18
other	3	6
NA	**54**	**55**

Table 6. The number of individuals prescribed any class of antihyperglycaemic drug during the time period of one year before and after the FINNMODY study (2014–2021) by using EHR data from heterozygous carriers of the familial HNF1A variant in Study III. Some patients had used multiple classes of drugs.

FDC, fixed-dosed combination; NA, no medication in use or EHR systems had failed to retrieve data.

4.3.4 Post hoc *analyses on glucagon*

Although glucagon levels for a cross-sectional subgroup in Study III were similar in carriers and noncarriers, we further performed a few *post hoc* analyses. Carriers displayed a moderate positive correlation between FPG and 120-min glucagon (linear model: est. 0.58 with p=0.042, model R^2=0.12, N=28; after the outlier exclusion: est. 0.47 with p=0.022, model R^2=0.19). No such correlation was identified in noncarriers, however. On the other hand, noncarriers' fasting C-peptide (but not that of carriers) demonstrated a positive correlation with glucagon at fasting (linear model: est. 4.89 with p=0.0084, model R^2=0.21) and at 120 minutes (linear model: est. 3.16 with p=0.022, model R^2=0.16). However, these *post hoc* analyses call for extreme

caution, considering the unequal proportion of those with diabetes among the two groups and a low number of patients included in the analyses.

4.3.5 The polygenic risk score for type 2 diabetes

The T2D-PRS was a modest modifier of the age at onset of diabetes. For example, a Cox model with additional covariates for *HNF1A:*p.(Gly292fs) status and the year of birth, every unit increase in standard deviation of T2D-PRS had HR of 1.4 (p = 0.00021), the *HNF1A* status had an HR of 22 ($p < 2\times10^{-16}$), and every unit increase in birthyear had an HR of 1.03 (p=9.4×10^{-9}). There was no difference in the T2D-PRS between the carriers and the non-carriers, but the families in our study had high T2D-PRS compared with the control population from the PPP Botnia study. The high T2D-PRS probably also contributed to the high-prevalent diabetes among the non-carriers.

4.3.6 Discussion

4.3.6.1 The fight against ascertainment bias

Undeniably, no human study on MODY – including Study III – can fully eliminate the risk of ascertainment bias. The patients diagnosed with MODY are often those with a clinical presentation suggestive of MODY, whereas those with atypical or relatively mild MODY might remain without a diagnosis or might be misdiagnosed with another type of diabetes, which skews the observed phenotype of MODY towards the most classic presentation. To detect early phases of asymptomatic diabetes across the whole population, massive and repetitive screening efforts would be necessary, but with the methods available today, a systematic population-level approach would be stupendously expensive and unreasonable. In the future, non-invasive devices[558] might permit less labour-intensive means to intensify the prospective research on MODY and other rare types of diabetes.

Although registry-based studies and biobanks might lack sensitivity to identify early stages of diabetes[111], population-level databases can identify individuals and families for more in-depth phenotyping. A careful analysis on ascertainment bias is crucial nevertheless.

In Study III, the balanced ratio between carriers and non-carriers (53–47%) and the similar polygenic risk score for type 2 diabetes in both groups, as well as the high participation rate in the large families B–D, all imply that the risk of ascertainment was low. However, even in Study III, the participants demonstrated higher T2D-PRS than the control population, which might

indicate suboptimal recognition of the patients and families with an average or protective T2D-PRS and, at the same time, with MODY.

4.3.6.2 On glucagon

Glucagon (once named *GLUC*ose *AGON*ist) can elicit a counterregulatory response to insulin by promoting hepatic glucose production, which consequently increases plasma glucose levels. Glucagon (secreted from pancreatic α cells) and insulin (secreted from pancreatic β cells) counteract with each other to maintain the physiological levels of plasma glucose, which is referred to as the bihormonal hypothesis. However, beyond this hypothesis, glucagon also widely contributes to several other aspects of human physiology and pathophysiology[559–565], including implications in the pathophysiology of diabetes.

Type 1 and type 2 diabetes, as well as HNF1A-MODY[559,566,567], have previously been associated with relative hyperglucagonaemia – *i.e.* a presence of seemingly high levels of circulation glucagon coinciding with high levels of plasma glucose. Suggested explanations include a disordered architecture in pancreatic islets and disorganized regulation between the pancreatic cells (possibly of paracrine, autocrine or endocrine nature).

In Study III, carriers' and noncarriers' glucagon levels were surprisingly similar on average. Considering the pronounced between-person variance, however, there must be factors that modulate glucagon secretion independent of the variant carrier status.

4.3.6.3 Body composition

As hypothesized in the manuscript, the difference in BMI could be secondary to insulin deficiency or to energy loss through glucosuria (see 2.3.3.4.2 *Renal phenotypes / HNF1A*), or both. To distinguish the relative proportion of these hypotheses, future research is crucial to estimate the long-term energy loss caused by glucose excreted into the urine and to compare the estimated energy loss with the weight difference. However, glucosuria in patients with a pathogenic *HNF1A* variant is still partially understood. The postprandial peak in insulin secretion might promote renal reabsorption of glucose (refer to a review by Ferrannini[454]), and therefore, glucosuria might occur secondarily to insulin deficiency or as a direct consequence of *HNF1A,* or both.

As Study III found no difference in the carriers' and non-carriers' adult height, the *HNF1A* variant hardly interferes with physical growth during childhood or adolescence. However, future research should elucidate whether *HNF1A* modulates body composition in children or teenagers.

4.3.6.4 Recommendations falling into oblivion?

Patients in Study III have been contacting their own health care centres or hospitals for annual diabetes care check-ups. However, guidelines for the common forms of diabetes are not fully in agreement with the specific recommendations for MODY. Yet the personalized approaches triggered by a genetic diagnosis of MODY must also persist for years and decades after the initial diagnosis[29,120,568]. As highlighted by the previous studies[29,120,568] and Study III *(4.3.3 Modifications to medical treatment)*, the management of MODY calls for relevant clinical expertise.

In Finland, the Rare Diseases Center of the Helsinki University Hospital could organize a future project on creating national clinical guidelines for MODY. Also, the Finnish university hospitals, which have joined The European Reference Network on rare endocrine conditions (Endo-ERN), could possibly deepen the cooperation across all Europe.

5 GENERAL DISCUSSION

5.1 Emerging demands for gene and variant curation

Current and innovative genetic technologies have massively expanded our knowledge on human and non-human physiology. Genetic testing has become ever more affordable and accessible for an increasing number of medical and non-medical[569] professionals – and directly for consumers[570]. Whereas the first reference for the human genome, published only 18 years ago, ended as an intensive multinational collaborative effort with an impressive price tag of 2.7 billion US dollars, whole genome sequencing (WGS) may cost less than 1000 euros (or 1000 US$) per sample today[571,572].

As genetic data progressively accumulate, some previously accepted variant-disease or gene-disease associations have become debatable or even turned out to be false[530]. Therefore, a number of variants once deemed pathogenic, *i.e.* responsible for a distinctive clinical presentation, have been re-classified as benign after an appropriate re-evaluation[29,573]. Moreover, a few genes once associated with MODY, namely *BLK, PAX4* and *KLF11,* now fail to meet the general definitions of Mendelian diseases (*i.e.* monogenic conditions)[530,574,575].

In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) introduced general guidelines for classifying and reporting variants identified in Mendelian genes[530]. To integrate the general guidelines with individual genes, the ClinGen consortium later launched Variant Curation Expert Panels (VCEPs), who comprise medical and genetic professionals aimed at curating gene–disease and variant–disease associations[576]. The Monogenic Diabetes Expert Panel (MDEP) chaired by Dr Toni I. Pollin, is responsible for the monogenic forms of diabetes[575,577]. In August 2021, ClinGen approved the MDEP's specifications to the ACMG/AMP guidelines regarding *HNF1A* and HNF1A-MODY (https://clinicalgenome.org/affiliation/50016/).

Before the 2015 ACMG/AMP guidelines, the interpreted clinical significance of the variants involved inconsistent reasoning. Occasionally, interpretation might have overestimated the pathogenicity of variants identified[29]. To classify variants in genes associated with Mendelian diseases, the ACMG/AMP guidelines have allowed a relatively reliable tool primarily for commercial laboratories but also for a researcher who might avoid exhaustive study protocols by applying the guidelines.

However, novel gene discoveries for monogenic diabetes cannot rely on ACMG/AMP guidelines. Instead, the gene discoveries require comprehensive evidence for clinical presentation and genetic segregation in humans, functional *in vitro* data and, possibly, animal models[111]. Recent suggestions for genes responsible for monogenic diabetes include *MANF*[578] and *YIPF5*[579], both associated with syndromic forms of early onset diabetes, as well as *KCNK16*[580,581], which is associated with MODY.

5.1.1 Fundamental concepts behind the ACMG/AMP guidelines

Most gene variants, with no effect or only a moderate effect on the gene function, are called benign. These variants also include risk variants for type 1 and type 2 diabetes, and they used to be referred to as polymorphism[530]. In contrast to benign variants, some variants can markedly disrupt gene function and are therefore called pathogenic ("a pathogenic variant" is currently preferred over "a mutation"[530]). To be classified as pathogenic, the variant must convincingly contribute to a distinctive clinical presentation of a monogenic condition, whose association with the affected gene is definitive and recognized (a definitive gene–disease association).

The general ACMG/AMP criteria apply to variants in genes with an established gene–disease association. In these genes, variants fall into one of five categories: benign, likely benign, likely pathogenic, pathogenic or a variant of unknown significance[530]. A likely pathogenic variant should reach >90% certainty of being pathogenic. Although the threshold is obviously arbitrary, 83.8–99.1% of the variants once classified as likely pathogenic could eventually have been re-classified as pathogenic[573].

The ACMG/AMP classification depends on combined evidence for *e.g.* functional consequences, allele frequencies in a population, clinical information and relevant family history, as well as *in silico* predictions and genetic impact, using methods described in the original publications[530,575–577,582]. Every appropriate piece of evidence converts into a criterion with a standardized acronym and a designated level of evidence *(Figure 15)*. The first letter of the acronym for criteria, either P or B, indicates the direction of the evidence (a *p*athogenic or *b*enign variant) and the next letter(s) the relative strength of the evidence. The final digit is only for indexing purposes (criterion PM1, where M is for *m*oderate, is not stronger nor weaker than PM5).

Occasionally, the strength of the evidence might be modified. For example, PP1 generally represents supporting-level evidence for cosegregation of a variant and a disease, whereas a modified criterion PP1_Strong conveys strong-level evidence because of more conclusive data on cosegregation to justify the modification. The modified strength criteria, however, are not available for personal judgement, as the definitions of the modified criteria are also an intrinsic part of the standardized guidelines. The combination of all applicable criteria ("criteria met") predetermines the final classification *(Table 7)*.

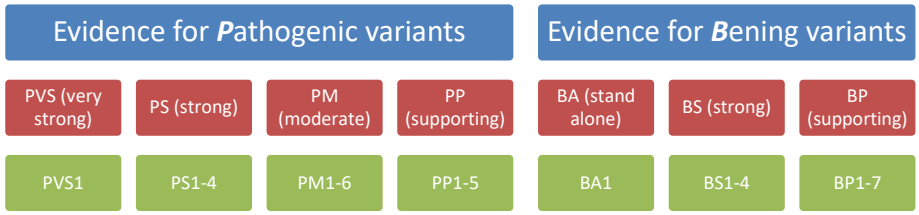

Figure 15. The first and the second rows in this figure include a verbal description for the acronyms included in the ACMG/AMP criteria found in the bottom row. For example, BS4 is one of four equally strong pieces of evidence for a benign variant.

Pathogenic	Likely pathogenic	Benign	Likely benign	VUS
• PVS *and* • PS *or* • 2 x PM *or* • 2 x PP *or* • PM + PP • 2 x PS • PS *and* • 3 x PM *or* • 2 x PM + 2 x PP *or* • PM + 4 x PP	• PVS/PS + PM • PS + 2 x PP • 3 x PM • 2 x PM + 2 x PP • PM + 4 x PP	• BA • 2 x BS	• BS + BP • 2 x BP	• other criteria not met *or* • contradicting evidence

Table 7. The number of criteria met determines the final classification by the ACMG/AMG guidelines[530].

5.2 Specific caveats of the ACMG/AMP guidelines considering monogenic forms of diabetes

Considering monogenic forms of diabetes, this section introduces a few caveats with possible relevance to the ACMG/AMP guidelines.

5.2.1 The dilemma of cosegregation

The ACMG/AMP guidelines have introduced the PP1 criterion to evaluate the cosegregation between a gene variant and a Mendelian disease. Therefore, in the context of MODY, a gene variant cosegregating with diabetes in affected families is required to use PP1. Possible pitfalls are numerous.

Unlike the Mendelian diseases with a distinctive clinical manifestation, MODY and several other forms of diabetes can develop without symptoms. The non-diabetic family members can therefore represent family members completely unaware of their asymptomatic diabetes. Among familial controls, the glycaemic status should be definitive and preferably verified by an OGTT. Therefore, we invited participants in Study III to undergo an OGTT irrespective of previous evidence of dysglycaemia or an absence of it.

Patients with GCK-MODY can demonstrate characteristic fasting hyperglycaemia (with FPG persisting above 5.5 mmol), but many of the affected do not meet the formal diagnostic criteria for diabetes. The data for families with a variant in *GCK* must therefore include exact data on FPG.

On the other hand, patients with other subtypes of MODY might have normal glucose tolerance (NGT) before progressing to impaired glucose tolerance (IGT) and, eventually, diabetes. Owing to the continuum related to the clinical presentation, the detailed assessment of cosegregation requires exact definitions for an impact of a variant[583] (roughly, "what does it mean to be affected or unaffected?"), and possibly an expected level of the penetrance. For example, Study II defined RFX6-MODY as a monogenic form of diabetes with a reduced penetrance.

Individuals with a monogenic form of diabetes can also have relatives with a more common form, such as T2D. The presence of different forms of diabetes in one family could distort the analysis on cosegregation, but to exclude the patients with common forms of diabetes is occasionally rather complicated due to the phenotypic overlap between different forms of diabetes.

Some families with an extremely high risk for type 1 or type 2 diabetes can manifest with a high prevalence of diabetes. The ratio between those with diabetes and those without it can reach a level that is characteristic to MODY, and a high prevalence of familial diabetes can further motivate genetic testing for MODY. Should a gene test identify a variant of unknown significance in these families, the analysis for cosegregation might also need to assess the evidence for "non-cosegregation", *i.e.* an absence of the familial variant in those who nevertheless have developed diabetes. Whereas the

simple methods only evaluate cosegregation between the variant and a disease[584], relevant analytic methods[585,586] to address non-cosegregation are, unfortunately, rather complex.

The future versions of easily accessible computational tools might address most of the concerns above. Statistical analyses, adjusted for age and polygenic risk for diabetes, along with the exact measurements of plasma glucose (or, possibly, HbA1c), could uncover reliable results on cosegregation and its relative magnitude.

5.2.2 The individuals behind the population databases

Rare Mendelian diseases arise from rare variants, whereas relatively common variants in a general population cannot be responsible for a high-penetrant disease. Therefore, a disproportionally high allele frequency provides strong evidence for a benign variant using the ACMG/AMP classification (BA1, BS1). Population databases for allele frequencies, such as gnomAD[587], aim to exclude the individuals with severe childhood-onset diseases and their first-degree relatives. However, patients with MODY are not always aware of their diabetes, or diabetes develops after childhood. These patients might end up in these databases in small numbers.

A founder effect in small subpopulations (the Finnish and the Ashkenazi Jewish in gnomAD) might interfere with the variant interpretation by the ACMG/AMP criteria. Therefore, the criteria might need to define statistical methods to address the caveat (*e.g.* the PopMax filtering to identify the highest credible population AF frequency[588,589]). For example, the AF of the *RFX6* p.(His293Leufs) in Study II is higher in the Finnish population than in other populations probably due to the founder effect.

5.2.3 The functional studies and their relevance

Although functional studies *in vitro* hardly mimic the complex human physiology *in vivo,* they provide valuable evidence for both pathogenic and benign variants. In short, the functional studies for transcription factors include evaluation of transcriptional activity (usually by a luciferase reporter assay that identifies the promoter activity of genes regulated by HNF1α, *e.g.* albumin gene in HeLa cells or other cells not expressing HNF1α), DNA binding (typically by an electrophoretic mobility shift assay [EMSA] to assess the ability of HNF1α to bind to a radiolabelled target gene), cellular localization (usually by immunofluorescence staining) and protein expression levels[229,590]. The functional studies for GCK investigate enzymatic activity, protein stability, and interaction with the glucokinase regulatory protein (GKRP)[591–593].

The ACMG/AMP criteria might include thresholds for interpreting the results from functional studies and their significance. For example, transcriptional activity over 80% could be defined as evidence for a benign variant. Such a dichotomous approach, however, is problematic because the functional consequences of variants are not discrete but continuous (see also 5.3).

Engineered human cell line models could provide novel mechanistic insights into the response to treatment in monogenic diabetes[198]. Moreover, if a class of antihyperglycaemic drugs displayed protective *in vitro* effects on β cell function, this drug could also speculatively affect the prognosis of the human disease.

5.2.4 Issues arising from the lack of a specific phenotype

The general and gene-specific ACMG/AMP guidelines contain a criterion for the disease-associated phenotype (PP4) and for an absence of the phenotype (BS2). Although it is nearly impossible to define a phenotype that accurately distinguishes patients with one form of monogenic diabetes from other patients with diabetes, all definitions contribute to what is eventually considered a monogenic form of diabetes (or a monogenic disease in general)[594,595]. Previously identified phenotypes are crucial to understand the clinical significance of newly identified pathogenic gene variants. For example, those with *RFX6* PTV in Study II presented with low serum levels of GIP, and those affected by HNF1BD in Study I presented with biliary anomalies, which both might represent phenotypes with clinical implications. Obviously, these findings warrant more in-depth research. In summary, the integration of phenotypes in the ACMG guidelines strengthen the clinical impact of the genetic diagnosis (the author's suggestion for the PP4 criterion regarding MODY can be found in *Figure 16*).

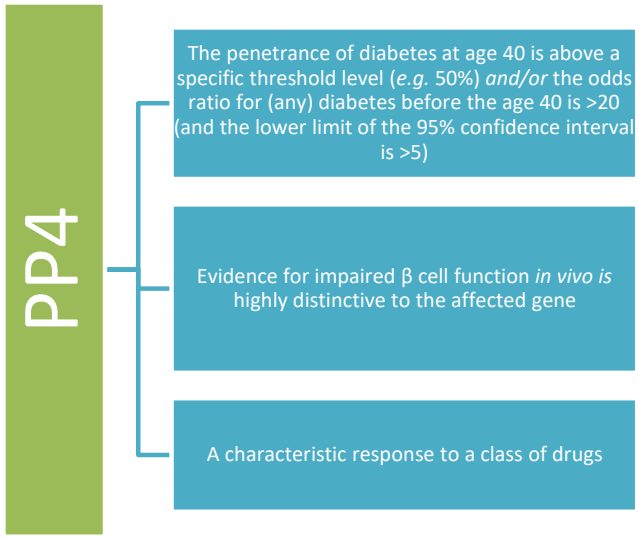

Figure 16. A conceptual summary of the author's suggestion for the PP4 criterion of the ACMG/AMP guidelines concerning monogenic forms of diabetes with high penetrance and a dominant pattern of inheritance, especially MODY.

A few gene–disease associations are overly heterogenous, as exemplified by the *HNF1B*-related disease (HNF1BD) (see 2.3.3 *Clinical characteristics in humans with HNF defects*). Whether each phenotype associated with *HNF1B* (such as diabetes, the renal phenotype, or the biliary phenotype discovered in Study I) requires its own phenotype-specific rules or whether all phenotypes associated with the same gene could be assessed by the same gene-specific set of rules could be open to discussion.

5.3 Future insights

Most study projects have historically engaged either with polygenic forms or monogenic forms of diabetes, but only rarely in unison[86,194,201,596–600]. As a result, different forms of diabetes have triumphed as discrete conditions *(Figure 17)*. However, variants in functional studies (see 5.2.3) have not aligned as discrete groups but as a continuum.

Patients with monogenic diabetes also possess an extensive number of non-Mendelian gene variants conferring the risk of complex polygenic diseases. Patients with MODY can additionally develop type 1 or type 2 diabetes[111,194,601,602]. In Study III, we assessed how the polygenic risk for diabetes modified the age at onset of MODY. Indeed, a combination of rare and common variants predetermine the genetic burden in individuals, and

the combined effect predisposes to disturbances across different glycaemic and metabolic pathways (trajectories)[194,603,604].

At the same time, few variants *in vivo* have evidenced a variant-specific phenotype. Such variants include *GCK* p.(Gly261Arg) with a relatively severe clinical presentation[110], homozygous hypomorphic *HNF1A* variants responsible for HNF1A-MODY[43], and *HNF4A*, p.(Arg76Trp) associated with Fanconi syndrome[269].

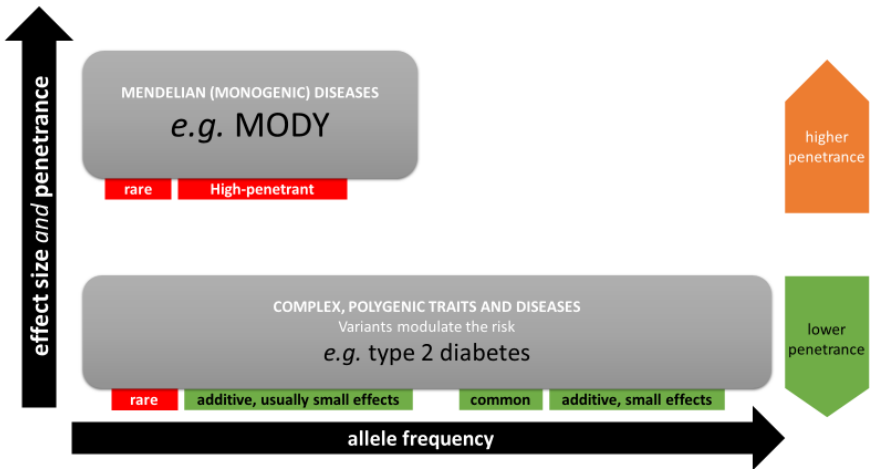

Figure 17. Gene variants associated with diabetes have conventionally been classified either as those responsible for monogenic diseases or those modulating the risk of polygenic diabetes, as illustrated by the two separate categories in this figure.

The conceptual division into polygenic diabetes and monogenic diabetes might become obsolete. All rare and common gene variants act in concert with environmental (such as medication) and intrinsic factors (such as age) to determine the susceptibility to specific metabolic and glycaemic trajectories[605]. Future studies on distinct trajectories might provide insights into detailed disease mechanisms that affect individual patients and allow for tailoring of personalized medicine.

The structure of this thesis has intentionally and even provocatively highlighted an emerging paradigm shift in medicine. While the review of the literature is portrayed as a network of numerous highly in-depth studies that strive to answer deliberate research questions, the general discussion is not

about questions to be answered but about those to be asked. In the future, bioinformatics and other computational methods might be able to perform complex analyses, model diverse biological phenomena, and produce various high-quality predictions, but without the purposeful questions and premises, the results can be deceptive. Asking the right questions will be more important than ever.

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8 ORIGINAL PUBLICATIONS