REVIEW

Identifying subtypes of monogenic diabetes

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Practice Points

- Monogenic diabetes is often misdiagnosed as Type 1 or 2 diabetes and should be considered in phenotypically atypical cases.
- A classical patient with maturity-onset diabetes of the young presents in the second to third decade of life, maintains endogenous insulin secretion regardless of diabetes duration and has a family history including at least two generations of diabetes.
- Patients with *HNF1A* and *HNF4A* mutations have progressive β-cell failure, but are very sensitive to sulfonylureas, which should be offered at an initial low dose.
- Presentation of diabetes in the first 6 months of life suggests monogenic diabetes and a causative gene can be found in 75% of babies.
- Neonates with mutations in ATP-sensitive K^+ channel subunit genes (*KCNJ11* and *ABCC8*) can be treated with high-dose sulfonylureas rather than insulin.
- Patients with GCK-maturity-onset diabetes of the young have mild fasting hyperglycemia and do not require treatment.
- Cases with features of two different types of diabetes are more frequent and remain a challenge to clinicians.

SUMMARY Monogenic diabetes is estimated to account for 0.5–1.2% of all diabetes cases and remains underdiagnosed. It consists of a variety of subtypes associated with mutations in more than 25 genes. The main groups of monogenic diabetes include maturity-onset diabetes of the young (MODY), permanent and transient neonatal diabetes and mitochondrial diabetes. MODY is the most common and is caused most frequently by mutations in transcription factors (HNF1A, HNF4A and HNF1B) or the enzyme GCK. The characteristics of MODY include young-onset nonautoimmune diabetes, usually with a family history, maintenance of endogenous insulin secretion and absence of features of insulin resistance. Correct molecular diagnosis allows personalized treatment and, in types sensitive to sulfonylureas, discontinuation of insulin allows improved diabetes control.

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Monogenic diabetes is caused by single gene mutations, and comprises an increasing number of entities, with a prevalence of approximately 0.5–1.2% in the diabetes population [1–3]; although many cases still remain misdiagnosed as more common forms of diabetes. The biggest groups belonging to this category are maturity-onset diabetes of the young (MODY), neonatal diabetes and mitochondrial diabetes. The prevalence of MODY in the UK was calculated by the UK MODY diagnostic center as a minimum of 68–108 cases per million [3], with 84 cases per million in a population-based cross-sectional study in Oxfordshire (UK) [1]. Three recent independent studies of pediatric diabetic populations from Norway, the USA and Poland estimated that MODY accounts for 1.1–4.2% of cases in this group, which gives a prevalence of 2.1–4.6 per 100,000 of the general population [4–6].

MODY was first described by Tattersall in 1974 as an insulin-independent form of diabetes inherited in an autosomal dominant pattern [7]. MODY is a heterogeneous group of monogenic causes of β-cell dysfunction. The causal genes for MODY subtypes started emerging in the 1990s [8–10], and this was followed by the development of diagnostic tests and therapeutic recommendations [11]. The classification of monogenic diabetes is constantly evolving and, while the term 'MODY' remains in common use, it is important to use the genetic classification to report results, for example 'MODY subtype HNF1A', shortened to 'HNF1A-MODY'. This reflects the fact that it is possible to make a specific diagnosis using genetic testing. More than 25 genes have been identified in patients with monogenic diabetes (13 among those with the MODY phenotype); although most of them are very rare (<1% of MODY cases) and some have been reported in fewer than five families, with some doubt over whether they are truly pathogenic [12]. Establishing a genetic diagnosis is crucial as this is likely to be followed by a change in the treatment approach; for example, identification of *HNF1A, HNF4A, ABCC8* and *KCNJ11* mutations often results in improved diabetic control. In GCK-MODY, treatment is likely to be stopped. A recent study based on health economic modeling suggests that screening for MODY is cost effective [13].

Data from the UK MODY diagnostic center published in 2010 demonstrate that 62% of MODY patients have pathogenic mutations in transcription factors, where *HNF1A* predominates and *GCK* mutations were responsible for 32% of cases [3]. The reported prevalence of MODY subtypes varies, reflecting the routine screening for diabetes performed in some countries. GCK-MODY predominates in Germany, Italy, France and Spain [14,15].

This review will concentrate on clinical aspects of different subtypes of monogenic diabetes, with most attention being paid to MODY.

Known subtypes of monogenic diabetes

■ Presentation in adolescence & early adulthood

Patients presenting with diabetes in adolescence or early adult life, particularly those with a strong family history of diabetes and who do not match Type 1 diabetes mellitus (T1DM) or Type 2 diabetes mellitus (T2DM) in phenotype should be considered for genetic testing, starting with *HNF1A*, *HNF4A*, *GCK*, *HNF1B* and mitochondrial genes, and if there is still strong suspicion of monogenic diabetes test for *INS*, *ABCC8* and *KCNJ11* (especially when there is a family history of neonatal diabetes). Additional clinical features such as deafness or renal disease will guide testing priorities.

HNF1A-MODY

Mutations in *HNF1A* are the most common cause of MODY in adults, accounting for 52% of patients with monogenic diabetes in the UK [3]. More than 400 mutations have been reported in excess of 1200 families [16]. *HNF1A* encodes a transcription factor that is crucial for β-cell differentiation and function and, therefore, affected cases have progressive β-cell defects. Mutations in this gene are highly penetrant as 96% of affected individuals develop diabetes by the age of 55 years [17].

Children with the *HNF1A* mutation generally have normal glucose and usually present with hyperglycemia after 10 years of age. *HNF1A* mutation carriers initially have normal fasting glucose and develop impaired glucose tolerance during an oral glucose tolerance test with 2 h glucose levels usually increased by more than 4.5 mmol/l from baseline [18]. With time, their fasting glucose levels gradually increase. Subjects also have a lower renal threshold for glucose excretion, resulting in glycosuria [19]. This phenomenon is linked to decreased expression of SGLT2 in the proximal renal tubule [20].

Patients with HNF1A-MODY were noted to be very sensitive to sulfonylureas (SUs), and this

clinical observation was confirmed by Pearson *et al.* in a small randomized controlled trial comparing matched cases of HNF1A-MODY and T2DM [21]. Pearson *et al.* reported a 5.2-times greater fall in fasting glucose when using gliclazide versus metformin in subjects with *HNF1A* mutations and a 3.9-times greater response to gliclazide when patients with HNF1A-MODY were compared with T2DM. Thus, SUs are recommended as a first-line treatment for patients with HNF1A-MODY, who will characteristically be well controlled on 20–40 mg of gliclazide or 1.25–2.5-mg glibenclamide daily. A patient initially misdiagnosed with T1DM can safely stop insulin, provided C-peptide levels remain in the normal range, and a low-dose SU should be offered instead. In the study by Shepherd *et al.*, 79% of patients with an initial label of T1DM and a subsequently confirmed *HNF1A* mutation successfully stopped their insulin, which was replaced by gliclazide [22]. Out of those patients, 71% remained off insulin 39 months later with a median HbA1c of 6.9%. Many patients with HNF1A-MODY maintain good control on this therapy for many years; although as the *HNF1A* mutation causes progressive β-cell dysfunction, at some stage, a secondary SU failure will occur and other oral/injectable agents or insulin will be required. There is no evidence base for a particular second-line therapy.

Micro- and macro-vascular complications of HNF1A-MODY are reported with a similar frequency to T1DM or T2DM, and increase with poor glycemic control [23,24]. Although HNF1A-MODY patients have been shown to have a better lipid profile compared with T2DM, with increased HDL-cholesterol and decreased triglycerides in gender- and BMI-matched subjects [25], they should be considered for lipid-lowering agents and those with hypertension should be managed to the target used for patients with T1DM and T2DM.

HNF4A-MODY

Inactivating mutations in *HNF4A* also cause slowly progressing β-cell failure and, thus, these patients share many common clinical features with HNF1A-MODY. It is the third most frequently diagnosed type of MODY, occurring in 10% of patients in the UK and 10–29% of patients with a high clinical suspicion of MODY who are negative on *HNF1A* testing [26]. The penetrance of pathogenic mutations is high, so most patients develop diabetes by middle age.

Similar to HNF1A-MODY, HNF4A-MODY patients are very sensitive to SUs and are at risk of vascular complications, especially if poorly controlled. Patients with HNF4A-MODY have lower HDL-cholesterol, apoA2 and A1 compared with mutation-negative family members and normal triglyceride levels [26]. Another feature distinguishing HNF4A- from HNF1A-MODY is the patient's normal renal threshold for glucose. Therefore, glycosuria before presentation of diabetes is not a feature.

Neonatal hypoglycemia and macrosomia have been noted to be a feature of *HNF4A* mutations. In a study of 108 HNF4A-MODY cases, birth weight was increased by a median of 790 g compared with family members without a mutation, and macrosomia (>4000 g) was present in 56% of neonates [27]. Transient hypoglycemia was reported in 14% of children. Neither of these findings was present in 134 patients with *HNF1A* mutations (although Stanescu *et al.* later reported that two patients with neonatal hyperinsulinemic hypoglycemia were found to have pathogenic mutations in *HNF1A* [28]). To date, these observations remain unexplained.

HNF1B-MODY

HNF1B-MODY is caused by heterozygous mutations in *TCF2*/*HNF1B*, which encodes HNF1B. Although *HNF1B* is closely related to *HNF1A*, its mutations give rise to a distinctive phenotype owing to differences in timing and the tissue specificity of its expression. It has a role in the development of the pancreas, renal tract and Mullerian tract; explaining the range of associated abnormalities in affected patients.

Coexistence of diabetes and extrapancreatic features involving congenital genitourinary abnormalities should prompt testing for HNF1B-MODY. The most commonly reported abnormalities involve renal cysts (renal cysts and diabetes syndrome), renal dysplasia, familial hypoplastic glomerulonephritic kidney disease, collecting system anomalies and, less frequently, female genital tract abnormalities [29]. In a systematic review of patients with diabetes and/or renal abnormalities, the detection rate of *HNF1B* mutations in MODY patients was 1.4% (13 out of 917), 21.4% in patients with the renal phenotype only and 41.2% if both were present [29]. Most patients present first with renal disease and then develop diabetes. Pancreatic structural abnormalities (mainly atrophy) were reported in 10.4%. There was an increased

incidence of hyperuricemia and abnormal liver function.

When compared with BMI-matched HNF1A-MODY, patients with HNF1B-MODY had fasting hyperinsulinemia with a 2.4-fold lower insulin sensitivity, lower HDL-cholesterol and increased triglycerides, which were all at similar levels compared with T2DM [30]. Furthermore, HNF1B-MODY patients had β-cell responses to glucose and SUs similar to T2DM. Interestingly, insulin requirements in HNF1B-MODY tend to be low (<0.45 U/kg) despite insulin resistance. Hyperinsulinemia was also present in patients with normal renal function, so it is not a consequence of accumulation [30].

Metformin or pioglitazone have been suggested as first-line oral agents in HNF1B-MODY [30] (if renal function allows), as patients are not sensitive to SUs. If satisfactory control is not achieved, insulin should be started.

Other rare forms of MODY

Heterozygous mutations in genes known to cause neonatal diabetes (*ABCC8, KCNJ11* and *INS*) have been shown to cause MODY in several case studies. *ABCC8* encodes the SUR1 subunit and *KCNJ11* the Kir6.2 subunit of the β-cell ATP-sensitive potassium channel. Initially, *ABCC8* mutations were found in older relatives of children with neonatal diabetes [31] and subsequently by screening young adults with a MODY phenotype, where mutations were often sporadic [32]. Similarly, some family members of babies with neonatal diabetes with the *KCNJ11* mutation were found to have adult-onset diabetes caused by the same gene alteration [33]. Based on experience from neonatal diabetes secondary to both mutations, treatment with SU should be attempted.

IPF1 (*PDX1*), *NeuroD1* (*BETA2*) and *PAX4* genes encode three other transcription factors that control the development of the endocrine pancreas. Although very rare, mutations in these genes have been reported to cause MODY in a handful of families each.

Homozygous mutations of *IPF1* from consanguineous parents were reported to cause pancreatic agenesis and neonatal diabetes in three infants [34,35]. In families with pancreatic agenesis, heterozygous carriers had young-onset diabetes. In these families, the mutations may be pathogenic, but the missense mutations that have been reported in other pedigrees have less genetic evidence for pathogenicity.

Two families from Norway have been described with *CEL* gene mutations, young adult-onset diabetes and exocrine pancreatic dysfunction [36].

It is possible that for some of the rare causes of MODY, where genetic evidence for pathogenicity is not compelling, the variants are increasing susceptibility to young-onset diabetes within the families, but have a low penetrance.

A number of families with a classic MODY phenotype (previously estimated to make up ~10–30% of cases) are negative for mutations in currently known associated genes. Efforts to identify additional causative genes, either through linkage [37] or, more recently, next-generation sequencing (NGS) [38] have been disappointing, often finding missed mutations in known genes rather than new pathologies. One problem is that it is hard to distinguish what is truly a 'MODY' phenotype from young-onset, lean, familial T2DM.

■ GCK-MODY: mild fasting hyperglycemia

GCK catalyses glucose phosphorylation in pancreatic β cells. By 2009, more than 600 *GCK* mutations had been reported in over 1400 families [39]. Inactivating heterozygous mutations in *GCK* reset the glucose threshold for insulin secretion, resulting in mild fasting hyperglycemia of 5.5–8 mmol/l from birth. HbA1c usually does not exceed 8% (64 mmol/mol) [40,41], the glucose increment on oral glucose tolerance tests does not exceed 4.5 mmol/l [18] and there is no deterioration of diabetes control over time.

Patients with GCK-MODY are asymptomatic and usually diagnosed either incidentally or as part of routine screening, for example during pregnancy. In the cohort from the ATLANTIC DIP study, 1.6% of women with fasting plasma glucose >5.1 mmol/l were diagnosed with GCK-MODY. This gives a population prevalence of 0.11% (one per 1000) [42]. It is easy to imagine that a child presenting with hyperglycemia, even if apparently healthy, will probably be diagnosed with T1DM and started on insulin inappropriately. Approximately 50% of children with incidentally found hyperglycemia have GCK-MODY [43]. When mild fasting hyperglycemia is diagnosed in a young adult it is likely to be labeled and treated as early T2DM.

After a *GCK* mutation is confirmed, it is recommended that patients should stop all antiglycemic treatments as observation data suggest that medications do not improve hyperglycemia or HbA1c [44] and vascular complications of

diabetes are rarely reported [41,45]. One exception remains in the case of pregnancy, where the outcome depends on the interaction of maternal and fetal mutation status. Where the mother has GCK-MODY and the baby does not, macrosomia can develop as in any diabetic pregnancy, owing to increased fetal insulin production. However, when the *GCK* mutation has been inherited by the fetus, growth is normal as the mild maternal hyperglycemia is at the appropriate level for fetal insulin secretion. Usually, fetal mutation status is not known during pregnancy, so fetal growth is monitored; if an acceleration in abdominal circumference is noted, the mother is usually treated with insulin, which is stopped after delivery [46,47]. If the baby has inherited a *GCK* mutation from an affected father then the birth weight tends to be lower [48].

Another situation when treatment with metformin could be considered in patients with GCK-MODY is when HbA1c rises from the normal baseline as a result of obesity, suggesting that the patient has developed T2DM in addition to GCK-MODY.

As *GCK* mutations are inherited in an autosomal-dominant pattern, a child of a parent with GCK-MODY has a 50% chance of having a mutation. Genetic testing should be performed in relatives with diabetes as they also have the potential to stop treatment.

■ Maternally inherited diabetes & deafness

Maternally inherited diabetes and deafness (MIDD) caused by mitochondrial DNA mutations affects approximately 1% of patients with diabetes and most commonly results from an A to G substitution at position 3243 (m.3243A>G) of the mitochondrial DNA [49]. Cosegregation of early-onset diabetes and sensorineural deafness with a maternal family history should trigger testing for mitochondrial diabetes.

Patients with MIDD have progressive β-cell dysfunction with normal insulin sensitivity [50]. Most patients are lean and present insidiously in the third to fifth decade of life; although approximately 8% present with ketoacidosis [51]. Sensorineural hearing loss occurs in more than 75% of affected patients and often precedes the diagnosis of diabetes. Neurological involvement in carriers of the m.3243A>G mutation may vary from deafness only to rare syndromes such as mitochondrial diabetes, encephalopathy, lactic acidosis and stroke-like episodes, myoclonal epilepsy, ragged red fibers in the muscles or Kearns–Sayre syndrome (ophthalmoplegia, retinopathy, proximal myopathy and cerebellar ataxia) [52]. Patients with MIDD have an increased prevalence of renal impairment (28%) most commonly secondary to focal segmental glomerular sclerosis, which often manifests with proteinuria [53]. Cardiomyopathy is also associated with MIDD and should be screened for.

It is likely that the MIDD phenotype varies so widely owing to varying levels of mutant mitochondria in different tissue types – termed heteroplasmy. Initial treatment of diabetes depends on the presentation, but patients usually progress through diet and oral agents to insulin more rapidly than in T2DM. There is some controversy over the use of metformin owing to the theoretical risk of lactic acidosis, but it is used in some centers. There is a consensus that metformin should not be used when patients have a neurological disease.

■ Presentation in the first 6 months from birth

Neonatal diabetes is very rare and accounts for approximately one in 100,000–260,000 births [54]. Studies have shown that if a baby presents with hyperglycemia in the first 6 months of life, it is most likely not T1DM but monogenic diabetes [55]. Neonatal diabetes is divided into permanent neonatal diabetes mellitus (PNDM) and transient neonatal diabetes mellitus (TNDM).

Permanent neonatal diabetes mellitus

PNDM affects approximately 45% of patients with neonatal diabetes, and mutations in *KCNJ11* and *ABCC8* are the most common. *KCNJ11* mutations are found in 53% of Caucasian patients with PNDM [56,57]. *KCNJ11* and *ACCB8* encode the Kir6.2 and the SUR1 subunits, respectively, of the ATP-sensitive K^+ channel in pancreatic islets.

Heterozygous activating mutations in *KCNJ11* and *ABCC8* prevent the K-ATP channel from closing in response to ATP, leading to markedly reduced insulin secretion, and often ketoacidosis, at diagnosis [56]. This also affects the K-ATP channels in extrapancreatic tissues and is associated with neurological features, known as developmental delay, epilepsy and neonatal diabetes syndrome in 30% of cases; although some may not have epilepsy or have muscle weakness and/or moderate developmental delay (intermediate form) [58,59]. Mutations are frequently sporadic. Affected babies have a

low mean birth weight of 2500 g at a mean gestational age of 39 weeks. Children with mutations in *ABCC8* have a similar phenotype to patients with *KCNJ11* mutations; although neurological features are generally absent or mild [60].

Establishing mutations in *KCNJ11* and *ABCC8* in children with PNDM has significant implications for their treatment, as most respond well to SUs. This is because SUs can close the K-ATP channel in an ATP-independent manner with subsequent insulin secretion. Therefore, in these patients, insulin therapy can usually be stopped and a SU started, with a resultant improvement in glycemic control [61,62]. Typically, glibenclamide is used in a high dose of 0.4–0.8 mg/kg/day orally. This treatment may also result in some improvement of neurological symptoms [63]. Good glycemic control (HbA1c: 6.5%) was maintained 2 years after discontinuation of insulin [63]. Some patients, especially those with associated delay, epilepsy and neonatal diabetes syndrome, do not respond to SUs and require insulin or combination treatments to maintain adequate glycemic control.

Heterozygous mutations in the insulin gene (*INS*) occur in 15–20% of neonates with PNDM [64,65]. Children with an *INS* mutation present with hyperglycemia and often associated ketoacidosis at a median of 13 weeks from birth. Approximately 80% of cases are *de novo* mutations [64], which are subsequently inherited in an autosomal dominant pattern. They are treated with insulin from diagnosis and median birth weight of neonates is reduced to 2800 g.

The number of genes found to be associated with PNDM is continuously growing and pathogenic mutations in *PTF1A, PDX1, HNF1B, EIF2AK3, PTF1A, GLIS3, PAX6, RFX6, GATA6, SLC2A2, SLC19A2, IER3IP1, NEUROD1* and *NEUROG3* have been reported **(Table 1)** [54]. Many mutations are associated with multisystem syndromic features and/or pancreatic agenesis.

Transient neonatal diabetes mellitus

TNDM accounts for 50–60% of neonatal diabetes cases [66]. It is typically diagnosed in the first week of life with hyperglycemia and negative urinary ketones. The insulin requirement is lower in children with TNDM compared with PNDM [67], and decreases with time, leading to insulin independence by a median age of 12 weeks [68]. Between 50 and 60% of patients have a relapse of their diabetes in later life at an average age of 14 years (range: 4–25 years). At that point, a SU [69] or other oral agents could be tried, but if satisfactory glycemic control is not achieved insulin should be started.

Alterations at an imprinted locus on chromosome 6q24 are found in 70% of patients with TNDM, and these result in overexpression of two paternally expressed genes, *PLAGL1* and *HYMA1* [70,71]. Only paternal copies of those genes are expressed in offspring. In a study by Temple *et al.*, the majority of patients with 6q24 alterations had sporadic mutations (23 out of 30) and 23% of children had a family history of T2DM [68]. Children with TNDM secondary to a 6q24 alteration have lower mean birth weights compared with neonates with PNDM (2100 g at a mean gestational age of 39 weeks), which suggests insulin insufficiency *in utero* during the third trimester of pregnancy. Mutations in the *ZFP57* encoding a zinc-finger transcription factor linked to DNA hypomethylation of multiple imprinted loci were reported to be associated with TNDM in several cases [72].

Activating mutations of K-ATP channel genes (*KCNJ11* and *ABCC8*) are also known to cause TNDM; and a study of children with TNDM and PNDM by Vaxillaire *et al.* reported mutations in *ABCC8* in 53% of patients with TNDM and in only 14% of children with PNDM [60]. *KCNJ11* mutations are rarely found in TNDM.

All children diagnosed with diabetes before 6 months of age should receive genetic testing. Mutations in *ZFP57*, which encodes a zinc-finger transcription factor, were reported to be associated with TNDM in several pedigrees [73]. The mutations were also linked to DNA hypomethylation of multiple imprinted loci in the genome.

Differential diagnosis, investigations & diagnostic algorithm

Currently, it is estimated that approximately 80% of patients with monogenic diabetes are misdiagnosed as T1DM or T2DM, and this percentage is likely to be underestimated [3].

Patients with confirmed MODY characteristically present in the second to forth decade of life, maintain endogenous insulin secretion (proven by C-peptide level in the reference range) and have a significant family history of diabetes, often involving more than two consecutive generations. Typically, they do not have features of insulin resistance, such as acanthosis nigricans or central obesity, and the majority are negative for β-cell antibodies.

Table 1. Genes associated with monogenic diabetes.

Gene	Protein	Type of diabetes	Characteristic features
HNF1A	HNF-1α	MODY	Progressive β-cell dysfunction, low renal threshold for glucose, low hsCRP, increased HDL and decreased TG, sensitive to SU
HNF4A	HNF-4α	MODY	Progressive β-cell dysfunction, neonatal macrosomia/hypoglycemia, low HDL and normal TG, sensitive to SU
GCK	Glucokinase	MODY	Stable mild hyperglycemia, HbA1c <8% (64 mmol/mol); no treatment required, complications rare
HNF1B	HNF-1β	MODY	Progressive β-cell dysfunction, associated genitourinary abnormalities, pancreatic exocrine dysfunction/pancreatic atrophy, abnormal LFTs
ABCC8	SUR1	PNDM, TNDM, MODY	Common form of PNDM, less frequent that TNDM; decreased birth weight, treated with high-dose SU
KCNJ11	IRK channel, subunit Kir6.2	PNDM, MODY	Most common PNDM, decreased birth weight, associated developmental delay and epilepsy, treated with high-dose SU
INS	Insulin	PNDM, MODY	10–15% of PNDM, decreased birth weight, requires insulin treatment
ZFP57 (6p22), 6p24 alterations	Zinc finger protein 57	TNDM	Most common form of TNDM, diagnosed in first few weeks of life, resolves by median age of 12 weeks and recurrent later in life in 50–60%, decreased birth weight, associated macroglossia
Mitochondrial genome		MIDD	Progressive β-cell dysfunction, 75% sensorineural deafness, other neurological features (MELAS, MERRF); myopathy, cardiomyopathy, renal impairment
KLF11	KLF11	MODY	Rare, pathogenicity not fully established
PAX4	PAX4	MODY	Rare, pathogenicity not fully established
BLK	B-lymphoid tyrosine kinase	MODY	Rare, pathogenicity not fully established
CEL	Carboxyl ester lipase	MODY	Rare, pancreatic exocrine dysfunction/pancreatic atrophy
IPF1	Insulin promoter factor 1	MODY, PNDM	Rare, pancreatic agenesis in homozygous mutation
NEUROD1	Neurogenic differentiation factor 1	MODY	Rare, associated cerebellar hypoplasia, sensorineural deafness, developmental delay and visual impairment
EIF2AK3	EIF2α kinase 3	PNDM	Rare, AR, associated renal failure, mental retardation, recurrent hepatitis and spondyloepiphyseal dysplasia (Wolcott–Rallison syndrome)
WFS1	Wolframin	PNDM, recent report of MODY [79]	DIDMOAD
RFX6	Regulatory factor X6	PNDM	Rare, associated hypoplastic pancreas and gall bladder, intestinal atresia
PAX6	PAX6	PNDM	Association with brain malformations, microcephaly, microphthalmia, cataract

AR: Autosomal recessive; DIDMOAD: Diabetes insipidus, diabetes mellitus, optic atrophy and deafness; hsCRP: High-sensitivity CRP; LFT: Liver function test; MELAS: Mitochondrial diabetes, encephalopathy, lactic acidosis and stroke-like episodes; MERRF: Myoclonal epilepsy, ragged red fibers in the muscles; MIDD: Maternally inherited diabetes and deafness; MODY: Maturity-onset diabetes of the young; PNDM: Permanent neonatal diabetes mellitus; SU: Sulfonylurea; TG: Triglyceride; TNDM: Transient neonatal diabetes mellitus.
Data taken from [12,54].

Different subtypes of MODY have additional clinical features that can guide the subsequent investigations **(Table 1)**. Renal or genitourinary tract abnormalities are associated with HNF1B-MODY, exocrine pancreatic dysfunction occurs with *HNF1B*, *CEL* and homozygous *IPF1* mutations; and deafness or neurological features in the proband or family members should trigger testing for mitochondrial diabetes.

Current guidelines for genetic testing for monogenic diabetes include patients with diabetes diagnosed before 25 years of age, a strong family history of diabetes of any type and insulin independence/C-peptide in the normal range more than 3 years after diagnosis [11]. However, we know that when using these criteria, approximately 50% of patients will be missed [3,74]. In the Young Diabetes in Oxford study, 8% (20 out of 247) of cases initially labeled as T1DM had maintained endogenous insulin secretion out of the 'honeymoon period' and 10% of those subjects had genetically confirmed HNF1A-MODY [75]. In the subjects initially given a diagnosis of T2DM, 4% (12 out of 277) had a mutation in *HNF1A* or *HNF4A*.

As professional awareness of MODY is limited, there is also a significant delay (>10 years) from initial diagnosis of diabetes to confirmation of a monogenic pathology [75]. Such a delay is likely to jeopardize clinical management of those patients.

Therefore, it is important to consider monogenic diabetes in patients diagnosed as young adults – we suggest investigating those diagnosed before 45 years of age, as most cases will present by this age, while T2DM becomes increasingly common thereafter. **Figure 1** proposes a diagnostic algorithm for clinicians, based on practice in our center. **Table 2** offers help with the differential diagnosis. Using an online MODY probability calculator (see below) may also help [76].

■ Patients initially diagnosed with T1DM

The phenotype of patient with T1DM is often similar to the patient with MODY as both are likely to be lean and young. The typical patient with T1DM will present with hyperosmotic symptoms resulting from hyperglycemia: polyuria and polydipsia with subsequent weight loss. However, some, particularly later-onset T1DM may have a more gradual onset and fewer marked symptoms. Diabetic ketoacidosis is extremely rare in MODY; although it can occur in any kind of diabetes.

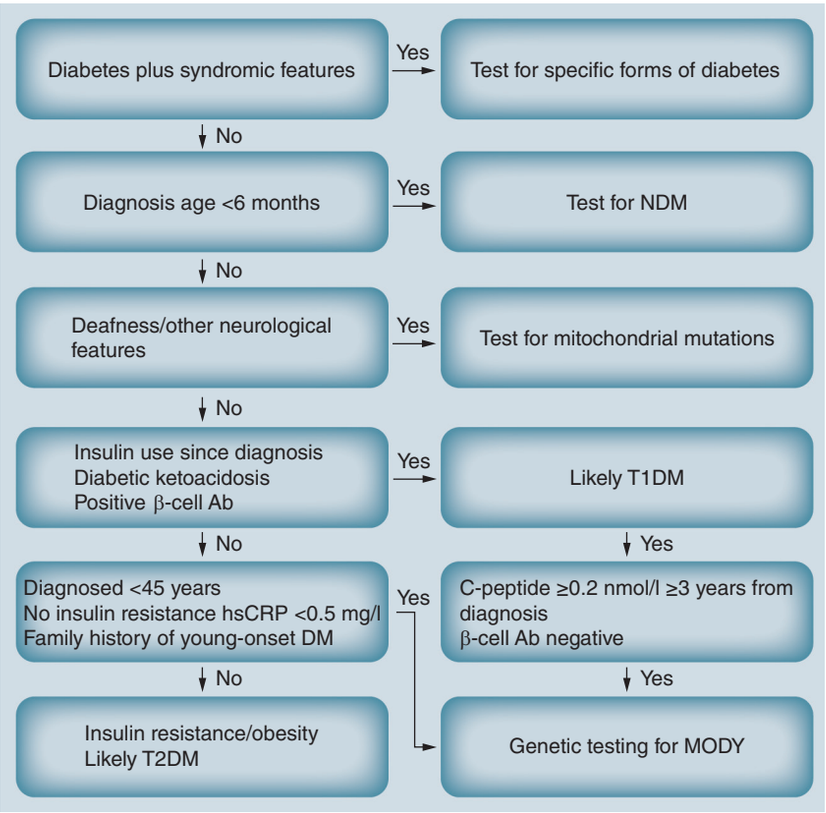

Figure 1. Investigation algorithm in young-onset diabetes.
Ab: Antibody; DM: Diabetes mellitus; hsCRP: High-sensitivity CRP; MODY: Maturity-onset diabetes of the young; NDM: Neonatal diabetes mellitus; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.

When working out the diagnosis, it is important to check for β-cell-specific antibodies: anti-GAD, ICA (islet cell antibodies) or IA2 antibodies. These antibodies will be strongly positive at diagnosis in the majority of patients with autoimmune diabetes [77].

Patients with MODY are generally negative for β-cell antibodies. A cross-sectional study of 508 patients from the UK with genetically confirmed monogenic diabetes reported <1% of participants with β-cell autoimmunity [78]. This differed markedly when compared with a pediatric group of patients with MODY from Germany, where positive antibodies to pancreatic β-cells were found in 17% of cases [79]. Therefore, some MODY patients will have positive antibodies, but it is not known whether this modulates the phenotype. Pragmatic advice is to exclude those with positive antibodies from testing unless the suspicion of MODY is very strong for other reasons.

Persistent C-peptide production (>0.2 nmol/l), especially outside the 'honeymoon period', in a patient previously diagnosed with T1DM should prompt further investigations for monogenic diabetes. Other signs of continued endogenous insulin secretion are low insulin requirements (<0.5 unit/kg/day) and absence of ketoacidosis during periods of low compliance with insulin injections.

Urinary C-peptide:creatinine ratio in a spot sample can be a noninvasive alternative to serum C-peptide in patients with normal renal function. In the study by Besser *et al.*, a urinary C-peptide:creatinine ratio >0.2nmol/mmol differentiated well between HNF1A/4A-MODY and T1DM of more than 5 years duration with a sensitivity of 97% and specificity of 96% [80]. The urine sample should ideally be postprandial and collected with a boric acid preservative. It is stable for up to 3 days and, thus, can be posted.

An autosomal dominant inheritance pattern of diabetes is less likely to be seen in patients with T1DM and only 2–4% of them will have a parent with T1DM [81].

■ Patients with an initial diagnosis of T2DM

It is characteristic for patients with T2DM to be overweight with features of metabolic syndrome and insulin resistance. This includes central obesity, acanthosis nigricans, hypertension, elevated triglycerides, low HDL-cholesterol, polycystic

Table 2. Comparison of main types of monogenic diabetes with Type 1 and 2 diabetes.

Feature	HNF1A/4A-MODY	GCK-MODY	PNDM with *ABCC8*/*KCNJ11* mutation	MIDD	T1DM	T2DM
Most common age at diagnosis	10–45 years	Birth onwards	Birth to 6 months	20–40 years	1–30 years	After 25 years
Insulin dependence	No	No	Not if treated with SU	No	Yes	No
Diabetic ketoacidosis	Rare	Would not be predicted to occur	Can present with diabetic ketoacidosis	Rare	Common	Rare except in ketosis-prone subtype
Family history of any DM	60–90% depending on testing criteria	One parent will usually have IFG if tested	<15%	Maternal relatives	2–4%	50–65%
Insulin resistance/obesity	Same as population	Same as population	Same as population	Same as population	Same as population	Common
β-cell antibodies	Rarely positive	Rarely positive	Rarely positive	Rarely positive	Positive in majority	Negative – would be redefined as LADA if positive
Endogenous insulin production 3 years from diagnosis	Yes	Yes	Yes, but C-peptide is undetectable without SU treatment	Yes	Low or undetectable C-peptide in majority	Yes
First-line treatment	Low-dose SU	None	High-dose SU	Oral antiglycemic	Insulin	Metformin

DM: Diabetes mellitus; IFG: Impaired fasting glucose; LADA: Latent autoimmune diabetes of adulthood; MIDD: Maternally inherited diabetes and deafness; MODY: Maturity-onset diabetes of the young; PNDM: Permanent neonatal diabetes mellitus; SU: Sulfonylurea; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus.
Data taken from [73].

ovarian syndrome and elevated fasting insulin levels [82].

Most patients with MODY have a normal BMI and no features of metabolic syndrome. Patients with mutations in *HNF1A* are reported to have low or normal triglyceride levels and high or normal HDL-cholesterol [25,83].

A multicenter study including patients from seven European countries established that high-sensitivity CRP (hsCRP) is a good biomarker for HNF1A-MODY, and hsCRP levels of <0.5 mg/l distinguish HNF1A-MODY from young-onset T2DM with a sensitivity and specificity of approximately 80% [84]. Sensitivity was improved further to 90% when hsCRP was combined with age at diagnosis of <25 years with minimal loss in specificity. hsCRP was significantly lower in patients with *HNF1A* mutations than in *HNF4A*- or *GCK*-mutated subjects. It is less useful in distinguishing HNF1A-MODY from T1DM patients, as their CRP levels are lower compared with T2DM. Another disadvantage of using this marker is potential coexisting infection/inflammation; therefore, it is recommended to repeat the test after several weeks if CRP is initially >10 mg/l.

A family history of diabetes is less helpful in distinguishing T2DM from MODY as prevalence of parental diabetes is high in T2DM, particularly with younger ages of onset. A case–control study of 44 patients with HNF1A-MODY versus 44 age-matched patients with T2DM did not report any significant difference in parental history of diabetes (65.9 vs 63.6%; p = 0.92) [83]. Similarly, in the SEARCH study of 586 diabetic patients younger than 20 years of age, parental history of diabetes did not differ between participants with MODY and those selected for monogenic diabetes testing who were found negative (50 vs 51%) [4]. Selection included patients with C-peptide ≥0.8 ng/ml and negative β-cell antibodies and, thus, the majority of patients found negative on testing for MODY had young-onset T2DM.

Sensitivity to SU could be another clinical feature suggestive of MODY with hepatocyte transcription factor mutations.

Conclusion & future perspective

Recent research on MODY has concentrated on two areas: first, defining clinical algorithms using biomarkers to improve the selection of patients for traditional genetic testing and increase the pick-up rate of positive tests; and, second, the development of diagnostic sequencing platforms based on NGS. Collaborative efforts, such as the EU-funded CEED3 study [101], have been essential in advancing knowledge

of rare forms of diabetes and encouraging the uptake of diagnostic genetic testing.

A further novel biomarker for HNF1A-MODY is based on changes in plasma glycans. Most plasma proteins are post-translationally modified by adding oligosaccharides chains known as glycans. A genome-wide association study identified that HNF1A regulates fucosylation of plasma proteins and follow-up work showed major alterations in plasma glycans in HNF1A-MODY subjects. A glycan index based on a ratio of core to antennary fucosylation ('DG9 index') was able to discriminate HNF1A-MODY from other forms of diabetes with high discriminative accuracy (C statistic >0.9) [85]. We suggested a diagnostic threshold of the DG9 index of ≤0.16 as a screening test for HNF1A-MODY, which has 88% sensitivity and 81% specificity to discriminate HNF1A-MODY from T2DM and 88% sensitivity and specificity to differentiate HNF1A-MODY from T1DM. This test appears to perform better than hsCRP, but has the disadvantage of not being an easily available commercial test, which limits immediate translation.

The UK MODY diagnostic laboratory at Exeter University has created a MODY Probability Calculator for patients with onset of diabetes younger than 35 years of age based on a European Caucasian population [76, 102]. It takes into account current age and age at diagnosis, treatment, family history, BMI and HbA1c, and produces a pretest positive predictive value of a patient having MODY. Further iterations are planned with the addition of biomarkers.

Whole-exome sequencing enables screening the whole exome for all potentially causative mutations of genetic diseases. Those methods are currently being evaluated as a research and diagnostic tool for sequencing DNA of patients with MODY phenotype who are negative for common gene mutations on Sanger sequencing. Johansson *et al.* reported the whole-exome sequencing of 111 candidate genes linked to monogenic diabetes, glucose metabolism or insulin resistance in nine such patients. Initially, they identified >14,000 substitutions or indels per targeted exome and after using multiple selection criteria found three new mutations to be potentially pathogenic [86]. Using whole-exome sequencing also enabled a causative relationship between *KCNJ11* mutations and MODY to be established for the first time in an adult without a history of neonatal diabetes [87]. Although exome sequencing shows a great potential to identify new mutations causing monogenic diabetes, it can be difficult to credibly establish pathogenicity.

NGS can also use gene panels to simultaneously analyze multiple genes that have already been shown to be linked with a particular disease. Ellard *et al.* designed NGS for 29 genes previously reported to cause monogenic diabetes and analyzed DNA of patients with a phenotype of monogenic diabetes and negative on Sanger sequencing for the most common genes causing MODY, TNDM or PNDM [38]. They identified new mutations in 14 patients (17%), but it is worth noting that in eight of those identified the affected gene had not been analyzed efficiently by traditional sequencing methods. The error rate for NGS is reported to be 1% so multiple reads are needed [38]. A further example of NGS is provided by the Genetic Services of University of Chicago (IL, USA), which offers a panel for testing 26 genes [103].

Although the two approaches presented above may seem to be at odds with each other in their aims (novel biomarkers are very selective for MODY subtypes, while NGS panels are not selective at all), actually defining the phenotype of MODY subjects with specific biomarkers might be very important in interpreting the results of NGS, which represents a great challenge.

Monogenic diabetes comprises of many entities, with a wide variety of phenotypes and a constantly growing number of pathogenic mutations. New methods of identification of monogenic diabetes mean that a systemic approach to diagnosis can now be taken. Despite this, many patients with young-onset diabetes remain misdiagnosed as more common types of diabetes. It is important to increase awareness of those rare types of diabetes, as establishing a molecular diagnosis allows personalized management of the disease, and may lead to treatment changes and genetic testing of relatives.

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