Monogenic diabetes refers to diabetes mellitus (DM) caused by a mutation in a single gene and accounts for approximately 1%–5% of diabetes. Correct diagnosis is clinically critical for certain types of monogenic diabetes, since the appropriate treatment is determined by the etiology of the disease (e.g., oral sulfonylurea treatment of HNF1A/HNF4A-diabetes vs. insulin injections in type 1 diabetes). However, achieving a correct diagnosis requires genetic testing, and the overlapping of the clinical features of monogenic diabetes with those of type 1 and type 2 diabetes has frequently led to misdiagnosis. Improvements in sequencing technology are increasing opportunities to diagnose monogenic diabetes, but challenges remain. In this Review, we describe the types of monogenic diabetes, including common and uncommon types of maturity-onset diabetes of the young, multiple causes of neonatal DM, and syndromic diabetes such as Wolfram syndrome and lipodystrophy. We also review methods of prioritizing patients undergoing genetic testing, and highlight existing challenges facing sequence data interpretation that can be addressed by forming collaborations of expertise and by pooling cases.
Monogenic diabetes: a gateway to precision medicine in diabetes

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Introduction
Monogenic diabetes is caused by a single defect in one of over 40 genes (1, 2). Since maturity-onset diabetes of the young (MODY) was named by Fajans for the type 2 diabetes–like presentation in young people with an autosomal dominant pattern of inheritance (3, 4), our understanding of phenotypic and genetic heterogeneity in monogenic diabetes has increased. The major monogenic diabetes categories are MODY, neonatal diabetes mellitus (NDM), and syndromic diabetes (5). Misdiagnosis is frequent because of overlapping of phenotypes with type 1 diabetes (T1D), such as young onset and leanness, and with type 2 diabetes (T2D), such as preserved β cell function and family history. Tailored treatment of some monogenic diabetes depends on the disease’s underlying etiology — e.g., oral sulfonylurea treatment of HNF1A/HNF4A-MODY — and requires genetic testing to diagnose. Here we will describe monogenic diabetes types, etiologies, diagnosis, management, and strategies to improve diagnosis.

Monogenic versus polygenic diabetes
Monogenic and polygenic diabetes are traditionally considered distinct, with monogenic diabetes resulting from one highly penetrant variant in one gene in a given individual and polygenic diabetes resulting from the contribution of several variants with smaller effects in the context of environmental/lifestyle factors. In T1D, autoimmune dysfunction is the prominent mechanism, with variation in the major histocompatibility locus and other genomic factors combining with apparent environmental triggers to result in β cell loss and diabetes. In monogenic diabetes, highly penetrant variants, mostly causing extremely impaired β cell development and insulin secretion, cause diabetes regardless of other risk factors. T2D, sometimes considered a diagnosis of exclusion, is a heterogeneous group of disorders involving smaller genetic effects on multiple mechanisms, including insulin secretion and insulin sensitivity, combining with environmental and lifestyle factors, mostly impacting insulin sensitivity. While this distinction is important both scientifically and clinically, emerging studies of the genetic architecture of diabetes reveal more of a spectrum with respect to the penetrance of genetic variants and their relative role in diabetes. For example, the HNF4A variant p.R114W, found in 0.02% of non-Finnish Europeans, has been shown to be overrepresented in patients with MODY (OR = 30.4 vs. public variant databases) but to have a distinct clinical phenotype (including lack of sulfonylurea response) and much lower penetrance than other HNF4A MODY mutations (54% vs. 71% by age 30; ref. 6). In Mexican Americans, HNF1A variant p.ES08K (NM_000545.8, rs483353044) was associated with T2D with a much greater effect size (OR = 4.2) than most polygenic T2D variants, with diabetic carriers and noncarriers having similar onset age and BMI (7). T2D polygenic risk scores have also shown evidence of modifying age at diagnosis of monogenic diabetes (8). Finally, while lack of features of either autoimmunity or obesity/metabolic syndrome raises the likelihood of monogenic diabetes, these features can coexist with monogenic diabetes — particularly obesity, given its high prevalence especially in youth. In the Treatment Options for Diabetes in Adolescents and Youth (TODAY) clinical trial, in which overweight or obesity was required for enrollment of newly diagnosed youth with T2D, at least 4.5% were identified as having MODY. Those with HNF4A-MODY had poor response to metformin, representing a previously missed opportunity for optimal
In summary, monogenic and polygenic forms of diabetes exist along more of a continuum than previously appreciated. Therefore, knowledge about monogenic diabetes not only provides opportunities for etiology-based treatment of the minority of individuals with highly penetrant variants, but also informs etiology-based treatment of the minori-

## Types of monogenic diabetes

**Maturity-onset diabetes of the young**

MODY comprises most monogenic diabetes cases, with classical characteristics of young diagnosis age, family history of diabetes in an autosomal dominant pattern of transmission, and insulin independence, with some types having additional features (Table 1). While 14 genes have now been designated as MODY genes in OMIM and/or the literature, three of these (BLK, PAX4, and KLF11) have been proposed for elimination based on a recent study (10) (see Table 1 for the remaining 11 along with RXF6, recently proposed as an additional MODY gene; ref. 11). Variants in GCK, HNF1A, and HNF4A are responsible for most MODY cases, followed by HNF1B (12). Given the known genetic etiology of most MODY cases and the increased frequency of pediatric T2D due to increased childhood overweight and obesity prevalence, it has been suggested that this term be abandoned in favor of terms describing the etiology of the type of diabetes, such as transcription factor diabetes for MODY caused by mutations in the transcription factor genes HNF1A, HNF4A, HNF1B, and others (13). Moreover, it can be argued that any diabetes designation is unsuitable for the usually benign condition of heterozygous GCK deficiency, which is characterized by only mildly elevated glucose levels often not reaching the diabetic range and, more to the point, generally does not lead to diabetic micro- and macrovascular complications (14).

### Common types of MODY-classified monogenic diabetes

**HNF1A-MODY and HNF4A-MODY** are caused by variants in genes encoding HNF1 homeobox A and hepatic nuclear factor 4a, respectively. These transcription factors play essential roles in transcription of genes related to β cell development and insulin secretion. **HNF1A** variants decrease expression of HNF1A target genes (15). Among patients diagnosed with diabetes, **HNF1A-MODY** is the most common MODY. To date, over 400 HNF1A variants and 100 HNF4A variants have been discovered from MODY families (16).
HNF1A/HNF4A-MODY is usually diagnosed in adolescence or early adulthood. Compared with T2D, HNF1A-MODY and HNF4A-MODY occur at younger ages with lower BMI, lower hemoglobin A1c and triglycerides, and similar risk for microvascular complications. Approximately 50% of patients with HNF4A-MODY are macroscopic, which is attributed paradoxically to transient neonatal hyperinsulinemic hypoglycemia at birth (17). Hyperinsulinemic hypoglycemia was also recently observed in some patients with HNF1A-MODY (18).

Individuals with HNF1A- and HNF4A-diabetes have increased sensitivity to sulfonylureas, an insulin-stimulating class of drug (19, 20), such that low doses are effective, and more typical T2D doses cause hypoglycemia. Sulfonylureas bind to the subunit of the K<sub>ATP</sub> channel to depolarize the β cell and release insulin. In a randomized clinical trial, low doses of sulfonylureas (e.g., 20–40 mg gliclazide daily) produced better glucose control than metformin in HNF1A- and HNF4A-MODY (21). In an observational study, most patients with presumed T1D who were subsequently found to have HNF1A-diabetes gained glycemic control when treatment was changed from insulin to sulfonylureas (19). Glucagon-like peptide-1 receptor agonist monotherapy (22) or sulfonylurea in combination with dipeptidyl peptidase-4 inhibitor (23) was recently demonstrated to achieve good glycemic control in HNF1A-MODY with reduced or no hypoglycemia, suggesting possible utility as a first-line HNF1A/HNF4A-diabetes treatment.

GCK encodes glucokinase, an enzyme catalyzing glucose phosphorylation at glycolysis initiation. GCK is a pancreatic β cell glucose sensor; genetic defects change the glucose-stimulated insulin secretion threshold (24, 25). In the United Kingdom, the prevalence of GCK-hyperglycemia was estimated at 0.1% among White Europeans (26) — higher than that of HNF1A-diabetes because the lack of symptoms keeps many cases from coming to medical attention. Further studies are needed in other populations. GCK-hyperglycemia has limited phenotypic heterogeneity; most patients have lifelong mild, persistent, and asymptomatic fasting hyperglycemia within the prediabetes range (27), with hemoglobin A1c values not exceeding 7.5% (60 mmol/mol; ref. 28), though some have glucose levels that meet the diabetes mellitus (DM) criteria, and a few have T2D and related complications, likely due to additional genetic and environmental risk factors (29, 30). Glucose levels are resistant to lowering by insulin or oral agents. Moreover, since GCK-hyperglycemia does not appear to be associated with significant microvascular and macrovascular diabetes complications (14, 31), patients with GCK-hyperglycemia usually do not require glucose-lowering medication, except possibly during pregnancy. Maternal GCK mutations increase risk for macrosomia and associated perinatal complications similar to gestational or pre-gestational diabetes of any type owing to the excess insulin secretion in response to a hyperglycemic intrauterine environment. Fetal GCK mutations decrease birthweight as a result of poor insulin response. A paternally inherited fetal mutation places the fetus at risk for low birthweight in a normoglycemic intrauterine environment. A maternal mutation creates a hyperglycemic intrauterine environment for fetal insulin secretion needed for normal growth of a GCK-deficient fetus, and thus attempts at normalizing maternal glucose may result in harm. In pregnant women with GCK mutations, it is recommended that, at minimum, fetal growth be monitored by serial ultrasound to guide treatment, but it is ideal to know the fetal mutation status early in pregnancy (32). A noninvasive technique for determining fetal GCK mutation status from cell-free DNA in maternal circulation is being developed that will enable women with a mutation-positive fetus to be discharged from high-risk antenatal care (33).

HNF1B variants are estimated to account for less than 1% of MODY (34). Patients with HNF1B defects may exhibit early-onset DM only; diabetes with renal, pancreas, or liver phenotypes (renal cysts and diabetes [RCAD] syndrome); or other features with or without diabetes, such as neurodevelopmental disorders (35, 36) and hypomagnesemia. HNF1B genotype-phenotype correlation is currently unclear, with clinical heterogeneity even among family members with the same variant. However, renal outcome as measured by estimated glomerular filtration rate has been reported to be better in deletion versus nondeletion variants (36, 37); this is hypothesized to result from a dominant-negative effect (38, 39). Some HNF1B-MODY initially responds to sulfonylurea or repaglinide (36) but may ultimately require insulin.

Rare MODY-classified monogenic diabetes types

ATP-sensitive potassium channel (K<sub>ATP</sub> channel) diabetes. Pathogenic variants in ABCC8 and KCNJ11, the genes encoding sulfonylurea receptor 1 (SUR1) and the inward rectifier potassium channel 11 (Kir6.2), subunits of the ATP-sensitive potassium channel (K<sub>ATP</sub> channel) found in β cells (Figure 1), are common causes of NDM (either permanent or transient; see below) but also can occasionally cause diabetes with later childhood or young adult onset (sometimes referred to as MODY12 [ref. 40] and MODY13 [ref. 41], respectively). K<sub>ATP</sub> diabetes is discussed further in the NDM section below.

The prominent and rarer types of MODY and their genetic and clinical features are summarized in Table 1. Emerging findings obtained through next-generation sequencing (NGS) to identify new causes of MODY have suggested potential roles of APPL1 (42) and PCBD1 (43) in MODY.

Neonatal DM

NDM is defined as diabetes diagnosed within the first 6 months of age and can be either permanent (PNDM) or transient (TNMD). Clinical features of NDM also include intrauterine growth retardation, failure to thrive, polyuria, and severe dehydration (44, 45). Depending on the genetic etiology, some patients can also have birth defects and neurological disorders (46). It affects 1 in 90,000 to 260,000 live births (47, 48), 50% being PNDM and 50% being TNMD (44).

The diabetes phenotype in TNMD results from inadequate insulin production presenting at the first week of life and resolving by 18 months (44), but 50% of patients relapse during early adulthood (46). Approximately 60%-70% of TNMD is caused by overexpression of paternally expressed imprinted genes on chromosome 6q24 (hereafter referred to as 6q24-TNMD) resulting from paternally inherited duplications or paternal disomy for the region or chromosome (both copies inherited from the father; ref. 49). The remaining cases mostly result from mutations in K<sub>ATP</sub> channels, KCNJ11 (50) and ABCC8 (51), which tend to be functionally less severe than those causing PNDM (52). There are also
It is recommended that patients diagnosed with diabetes in the first 6 months of life obtain immediate genetic testing to identify the subtype, since T1D is extremely rare in this subgroup. Approximately 80%–85% of NDM cases have an identifiable genetic cause (63), half of these being KATP-diabetes caused by \( \text{KCNJ11} \) or \( \text{ABCC8} \) mutations, treatable with high-dose sulfonylureas rather than insulin (50, 65, 66). The benefit of identifying patients with KATP-diabetes is thus considerable, and many studies have attempted to establish genotype-phenotype correlation (67) to facilitate the prediction of patients’ clinical courses based on genetic data.

NDM caused by pathogenic variants in K\(_{\text{ATP}}\) channels. Activating K\(_{\text{ATP}}\) channel gene variants cause NDM by decreasing ATP’s ability to achieve channel closure in multiple ways (68). Whether variants will cause PNDM or TNDM (or, rarely, MODY) is determined in part by the functional severity of the mutation as well as which gene is involved, with \( \text{KCNJ11} \) variants mainly associated with PNDM and most \( \text{ABCC8} \) variants linked to TNDM (51, 69). Diabetes severity could be partially explained by the extent to which the variant impacts ATP sensitivity (70); however, the same variant in one family could cause both NDM and MODY in different patients (e.g., the \( \text{KCNJ11} \) C42R variant; ref. 71), suggesting that other mechanisms influence the development of clinical presentation. Loss-of-function (LOF) mutations in both genes cause an increase in insulin secretion and present as congenital hyperinsulinemic hypo-
glycemia when found in the homozygous or compound heterozygous state (72, 73) and when dominant LOF mutations are found in the heterozygous state. In addition, paternally inherited recessive state (72, 73) and when dominant LOF mutations are found in the homozygous or compound heterozygous state (74).

Some individuals with NDM have neurological features in addition to DM (77), as K ATP channels (60%–84%) arise de novo (75, 76). Some individuals with NDM variants with high-dose sulfonylureas has proven safe and effective for both short-term and long-term glycemic control and may resolve CNS features (79–82). Ninety percent of K_{ATP}-NDM patients could switch from insulin therapy to sulfonylurea successfully (79, 83), with mutation severity (77, 84, 85) and diabetes duration before the transition (86) predicting the likelihood of success.

For patients who cannot completely transfer to sulfonylurea, combining insulin and sulfonylurea has shown favorable results (87).

Table 2. Genetic causes of neonatal diabetes with current ISPAD testing guidelines

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>Inheritance</th>
<th>Other features</th>
<th>Pathophysiology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA4</td>
<td>PNDM, TNDM</td>
<td>AD</td>
<td>Developmental delay, congenital cardiac defects, developmental delay, neurocognitive defects</td>
<td>Abnormal pancreatic development</td>
<td>155</td>
</tr>
<tr>
<td>GATA6</td>
<td>PNDM</td>
<td>AD</td>
<td>Pancreatic agenesis, congenital cardiac defects, congenital biliary tract anomalies</td>
<td>Abnormal pancreatic development</td>
<td>156, 157</td>
</tr>
<tr>
<td>GLK</td>
<td>PNDM</td>
<td>AR</td>
<td>β Cell dysfunction</td>
<td>β Cell dysfunction</td>
<td>25, 158–160</td>
</tr>
<tr>
<td>GLIS3</td>
<td>PNDM</td>
<td>AR</td>
<td>Congenital hypothyroidism, IUGR, polycystic kidney disease</td>
<td>Abnormal pancreatic development</td>
<td>161–163</td>
</tr>
<tr>
<td>HNF1B</td>
<td>PNDM, TNDM</td>
<td>AD</td>
<td>Pancreatic hypoplasia and renal cyst</td>
<td>Abnormal pancreatic development</td>
<td>55, 56</td>
</tr>
<tr>
<td>KIF3P1</td>
<td>PNDM</td>
<td>AR</td>
<td>Microcephaly, simplified gyral pattern, severe epilepsy</td>
<td>β Cell destruction</td>
<td>164</td>
</tr>
<tr>
<td>INS</td>
<td>PNDM, TNDM</td>
<td>AD, AR</td>
<td>β Cell destruction</td>
<td>β Cell destruction</td>
<td>53, 150–152, 166–170</td>
</tr>
<tr>
<td>KCNJ11</td>
<td>PNDM, TNDM</td>
<td>AD</td>
<td>Developmental delay, epilepsy (DEND)</td>
<td>β Cell dysfunction</td>
<td>76, 153, 154</td>
</tr>
<tr>
<td>MNT1</td>
<td>PNDM</td>
<td>AR</td>
<td>Developmental delay, sacral agenesis, imperforate anus, IUGR</td>
<td>Abnormal pancreatic development</td>
<td>170</td>
</tr>
<tr>
<td>NEUROD1</td>
<td>PNDM</td>
<td>AR</td>
<td>Developmental delay, cerebellar agenesis, sensorineural deafness, and visual impairment</td>
<td>β Cell dysfunction</td>
<td>145</td>
</tr>
<tr>
<td>NEUROG3</td>
<td>PNDM</td>
<td>AR</td>
<td>Malabsorptive diarrhea</td>
<td>Abnormal pancreatic development</td>
<td>171–173</td>
</tr>
<tr>
<td>NRK2-2</td>
<td>PNDM</td>
<td>AR</td>
<td>Developmental delay, hypotonia, short stature, deafness, constipation</td>
<td>Abnormal pancreatic development</td>
<td>170</td>
</tr>
<tr>
<td>PAX6</td>
<td>PNDM</td>
<td>AR</td>
<td>Brain anomalies, microphthalmia</td>
<td>Abnormal pancreatic development</td>
<td>174–176</td>
</tr>
<tr>
<td>PDX1</td>
<td>PNDM</td>
<td>AR</td>
<td>Pancreatic agenesis (common)</td>
<td>β Cell dysfunction</td>
<td>141, 142, 177–182</td>
</tr>
<tr>
<td>PLAG1/</td>
<td>TNDM</td>
<td></td>
<td>Macroglossia, umbilical hernia</td>
<td>Abnormal pancreatic development</td>
<td>183</td>
</tr>
<tr>
<td>HYMAI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIT1A</td>
<td>PNDM</td>
<td>AR</td>
<td>Pancreatic agenesis, cerebellar agenesis</td>
<td>Abnormal pancreatic development</td>
<td>184–186</td>
</tr>
<tr>
<td>RXF6</td>
<td>PNDM</td>
<td>AR</td>
<td>Pancreatic hypoplasia, intestinal atresia, and gallbladder aplasia or hypoplasia (Mitchell-Riley syndrome)</td>
<td>Abnormal pancreatic development</td>
<td>187–190</td>
</tr>
<tr>
<td>SLC2A2</td>
<td>PNDM</td>
<td>AR</td>
<td>Fanconi–Bickel syndrome</td>
<td>β Cell dysfunction</td>
<td>191</td>
</tr>
<tr>
<td>SLC1B2</td>
<td>PNDM</td>
<td>AR</td>
<td>Rogers syndrome</td>
<td>β Cell dysfunction</td>
<td>192</td>
</tr>
<tr>
<td>WFS1</td>
<td>PNDM, AD, AR</td>
<td></td>
<td>Wolfram syndrome</td>
<td>β Cell destruction</td>
<td>94, 95</td>
</tr>
<tr>
<td>ZFSP57</td>
<td>TNDM</td>
<td>AR</td>
<td>IUGR, microglossia, facial dysmorphism, cardiac anomalies, umbilical hernia, and developmental delay</td>
<td>Abnormal pancreatic development</td>
<td>193</td>
</tr>
</tbody>
</table>

The Exeter Genomics Laboratory (Royal Devon and Exeter NHS Foundation Trust and University of Exeter Medical School, Exeter, United Kingdom) maintains an up-to-date, annotated list of genes sequenced for monogenic diabetes (Diabetes Genes website; https://www.diabetesgenes.org/tests-for-diabetes-subtypes), which currently includes 71 genes, including some with putative/research status, of which 35 have been evaluated for neonatal diabetes, including those listed here. In addition to MODY and NDM genes, the list also contains genes for syndromic subtypes diagnosed outside of the neonatal period. AD, autosomal dominant; AR, autosomal recessive; IUGR, intrauterine growth restriction; PNDM, permanent neonatal DM; TNDM, transient neonatal DM; XLR, X-linked recessive.
sulfonylureas and insulin (88, 89). Compared with $K_{ATP}$-TNDM patients, patients with 6q24-TNDM were observed to have lower birthweight and earlier presentation (90). Some patients with 6q24-TNDM may also experience hyperinsulinemic hypoglycemia following diabetes remission (91). Certain congenital abnormalities, such as macroglossia, are characteristic of 6q24-TNDM and thus could help to distinguish this type of TNDM from other types in considering testing strategies.

**Syndromic diabetes**

In addition to RCAD syndrome due to *HNF1B* variants as described above, other monogenic syndromes include DM as one of the clinical features. We describe the best-characterized of these syndromes below.

**Wolfram syndrome.** Two types of Wolfram syndrome (WS) corresponding to two causative genes have been identified to date. Wolfram syndrome 1 (WS1), characterized by diabetes insipidus, DM, optic atrophy, and deafness, is a rare autosomal recessive disease caused by variants in wolframin ER transmembrane glycoprotein (*WFS1*). Severe cases with dominant heterozygous variants are also reported (92). Often, patients’ first manifestation is DM at an average age of 6 years. Though most WS1 patients require daily insulin as therapy, the high morbidity and mortality rates as well as low average age of death make an accurate and timely diagnosis essential. Recently, a presentation similar to WS1 in many *WFS1* mutation–negative patients was linked to variants in CDGSH iron sulfur domain 2 (*CISD2*) and thus named Wolfram syndrome 2 (WS2). Clinical features of WS2 resemble those of WS1 but without diabetes insipidus and with the addition of peptic ulcer bleeding and defective platelet aggregation (93). In addition, there are some *WFS1* mutations that cause isolated diabetes with significantly reduced penetrance or nonpenetrance for other WS-related features (94, 95).

**Insulin resistance due to insulin receptor defects.** Genetic defects in the insulin receptor gene (*INSR*) result in several insulin resistance syndromes, which are distinguished from typical insulin resistance not only by their severity but by normal lipid profiles because the etiology is directly due to defects in insulin receptor signaling rather than obesity and its sequelae (96). The most common type is type A insulin resistance syndrome, which has autosomal dominant and autosomal recessive forms. Type A insulin resistance syndrome affects predominantly nonobese females and presents with extreme insulin resistance, acanthosis nigricans, hirsutism, and polycystic ovarian disease (97, 98). Rabson-Mendenhall syndrome (RMS) is an intermediate form of insulin resistance with autosomal recessive inheritance. Patients with RMS have clinical features of extreme insulin resistance, acanthosis nigricans, hirsutism, dental precocity, thick nails, pineal hyperplasia, genital enlargement in both males and females, abdominal distension, and other distinctive dysmorphic features (99, 100). The most severe form is Donohue syndrome (DS), an autosomal recessive disorder in which patients present with failure to thrive, severe hyperinsulinemia, and fasting hypoglycemia. Patients with DS seldom survive infancy (101). LOF variants in the fibronectin type III (FnIII) domain are proposed to be associated with more severe DS, and there are genotype-phenotype and structure-phenotype correlations of *INSR* variants (102).

**Lipodystrophy.** Monogenic lipodystrophy is a group of diseases featuring a complete or partial lack of adipose tissue and adipose tissue–derived hormones, which results in insulin resistance and other metabolic complications. Unlike insulin receptor defects, the lack of adipose tissue in lipodystrophy leads to dyslipidemia and insulin resistance due to spillover of fat into ectopic areas, paradoxically similar to the consequences of obesity (96). Based on the loss of adipose tissue, this disease can be divided into congenital generalized lipodystrophy (CGL) and familial partial lipodystrophy (FPLD). CGL is an autosomal recessive disease; pathogenic variants in genes encoding 1-acylglycerol-3-phosphate O-acetyltransferase 2 (*AGPAT2*) and Berardinelli-Seip congenital lipodystrophy 2 (*BSCL2*) account for most CGL cases, with rare cases being caused by pathogenic variants in *CAV1* and *PTRF*. CGL patients show common features, such as generalized lipodystrophy, muscular appearance, DM, and dyslipidemia; however, patients with pathogenic *BSCL2* variants display lower serum leptin levels than patients with pathogenic *AGPAT2* variants (103) but a higher rate of developing intellectual disability (104). The majority of FPLD cases are caused by pathogenic variants in lamin A/C (*LMNA*) or PPAR$\gamma$ (*PPARG*), and there are also other rarer forms caused by pathogenic variants in *PLINI*, *AKT2*, *LIPE*, *CIDEC*, and *PCYT1A*. Body fat deficiency in FPLD is found on limbs, buttoks, and hips. Patients with pathogenic variants of either *LMNA* or *PPARG* appear to benefit similarly from leptin replacement therapy with metreleptin (105) in terms of improved glycaemia and cardiometabolic outcomes.

**Mitochondrial diabetes.** Mitochondrial diabetes, also known as maternally inherited diabetes and deafness (MIDD), is caused by pathogenic variants in mitochondrial DNA, mostly tRNA variant m.3243A>G. Patients often present with diabetes in adulthood, but a greater proportion of mutated mitochondrial genomes in the affected tissues is associated with a younger age of diagnosis of diabetes in some studies (106). The hearing loss associated with m.3243A>G is bilateral, sensorineural, and progressive, typically preceding the diagnosis of diabetes (107, 108). Other clinical features such as macular pattern dystrophy, nephropathy, and neurological symptoms are more common in rarer forms of mitochondrial diabetes than the classical form (109). The penetrance of mitochondrial diabetes is estimated to be nearly 100% by the age of 70 years. The disease etiology determined that patients have impaired insulin secretion, and insulin treatment is eventually required for most patients. The effects of other treatments, such as coenzyme Q$_\text{10}$ and PPARY agonists, were only evaluated in single cases, thus requiring caution for application. To better screen patients suspected to have mitochondrial diabetes, clinical features including diabetes and hearing loss on the maternal side are key. Tian et al. established a mitochondrial diabetes score system with good performance (100% sensitivity, 69.9% specificity) to select patients suspected to have mitochondrial diabetes, clinical features such as macular pattern dystrophy, nephropathy, and neurological symptoms are more common in rarer forms of mitochondrial diabetes than the classical form (109). The penetrance of mitochondrial diabetes is estimated to be nearly 100% by the age of 70 years. The disease etiology determined that patients have impaired insulin secretion, and insulin treatment is eventually required for most patients. The effects of other treatments, such as coenzyme Q$_\text{10}$ and PPARY agonists, were only evaluated in single cases, thus requiring caution for application. To better screen patients suspected to have mitochondrial diabetes, clinical features including diabetes and hearing loss on the maternal side are key. Tian et al. established a mitochondrial diabetes score system with good performance (100% sensitivity, 69.9% specificity) to select patients diagnosed with T2D for genetic testing in a Chinese cohort (110), although this system needs validation in other populations.

**Challenges in identifying and diagnosing monogenic diabetes**

The broad application of personalized medicine to patients with monogenic diabetes faces challenges in two aspects: detecting patients suspected of having monogenic diabetes to pursue
etiology-based therapies and accurately interpreting sequence variants of monogenic diabetes genes.

Monogenic diabetes detection methods

At present, practical guidelines for systematic screening for monogenic diabetes have been limited. The International Society for Pediatric and Adolescent Diabetes (ISPAD) has recommended testing for NDM in all patients diagnosed with diabetes before the age of 6 months as well as in patients diagnosed with diabetes before the age of 12 months with negative islet antibodies. This recommendation not only has the potential to dramatically improve care at the individual level when K ATP-diabetes is diagnosed but has been shown to be cost-effective in this population (111). However, adult and pediatric T1D and T2D populations, which also include misdiagnosed patients with monogenic diabetes (112), are more challenging to screen routinely for MODY (111) and can be challenging especially for clinicians with limited experience diagnosing MODY. More complex screening criteria based on age of onset, family history, endogenous insulin secretion, non-obesity, and absence of pancreatic autoantibodies are needed to achieve cost-effectiveness and an ideal balance of sensitivity and specificity (113–115). The American Diabetes Association (ADA) recommends some scenarios for considering testing individuals who do not fit into the T1D or T2D classifications (2). A proposed algorithm to increase sensitivity is shown in Figure 2. Clinicians are referred to the primary source (116) as well as current ADA (2) and ISPAD guidelines (1) for further guidance; additional development is needed and is ongoing in this area.

Biomarkers or derived scores avoid reliance on clinical judgments and arbitrary cutoffs and establish a quantitative evaluation that could be validated and replicated across cohorts. The Swedish Better Diabetes Diagnosis (BDD) study showed that absence of glutamic acid decarboxylase (GAD), islet antigen-2, zinc transporter 8 antibodies, and insulin autoantibodies could be a good discriminator, since in this study, MODY patients were only identified from the antibody-negative group, and 15% of antibody-negative patients had MODY (115). However, other studies have shown that 1%–2% of patients diagnosed with MODY are GAD antibody positive (117), reducing the antibody’s sensitivity as a screen. Meanwhile, the types of autoantibodies tested on each patient may vary depending on the clinic; thus, using negative antibodies as a screening method may not be practical without standardization. Table 3 summarizes published biomarkers other than pancreatic antibodies that have been utilized to distinguish monogenic diabetes subtypes from T1D or T2D. Limited by the low prevalence of monogenic diabetes, these biomarkers were developed in selected populations to differentiate the most common types of MODY.

In addition to biomarkers, Shields et al. established a MODY calculator predicting the possibility of testing positive for MODY given a set of common clinical criteria (118). In the initial cohort of White European patients who were diagnosed before the age of 35, the cutoff of probability at 40% yields sensitivity of 96% and specificity of 91% in differentiating MODY from T2D, and yields 87% sensitivity and 88% specificity for MODY versus T1D. Validations in other cohorts with different ethnic backgrounds show variable outcomes, suggesting room for improvement, including the need for a more ethnically diverse reference database.

Selection of method and genes for testing

Previously, molecular diagnosis of monogenic diabetes was usually performed through Sanger sequencing of one or several common-cause genes based on clinical suspicion. With the development of NGS, all known monogenic diabetes genes or even a patient’s whole exome can be analyzed simultaneously. Targeted panels typically include all the MODY genes, or at least the most common ones, as well as the NDM and syndromic forms of diabetes genes. There are both advantages and disadvantages to using NGS gene panels. The low price of massively parallel sequencing enables the analysis of additional genes that were reported to be associated with syndromic forms of diabetes. This is useful because patients with syndromic forms of diabetes may lack or appear to lack the clinical features that would lead to testing of a single syndromic gene (119). However, it is important that diagnostic panels not include genes with weak or disputed associations with monogenic diabetes, or, if they are included for surveillance purposes, that they not be reported (120). The yields of these panels will not only facilitate molecular diagnosis but also add rare or novel variants to the knowledge base for future studies. Sanger confirmation is sometimes needed after variant discovery in NGS
panels, though increasingly less so except in difficult regions of the genome. Regardless of methodology, it is becoming increasingly clear that evaluating only exonic regions will overlook some causal variants, as variants in the noncoding regulatory and deep intronic regions and 5′- and 3′-UTRs have also been implicated in monogenic diabetes (16, 121).

Searching for monogenic diabetes using exome or genome sequencing enables novel gene discovery and also requires caution. The coverage of exome sequencing may not be complete, and the extent to which rare but benign genetic variation exists in the general population was not known and was thus probably underestimated. As NGS has begun to boom, the problem of large quantities of genetic data for interpretation has arisen. The genetic and phenotypic heterogeneity of monogenic diabetes, and its overlapping features with T1D and T2D, together increase the difficulty of interpreting the pathogenicity of variants found in patients suspected to have monogenic diabetes. On the other hand, NGS emergence has led to the availability of exome and genome sequences of over 100,000 individuals of diverse ancestries in the gnomAD database, dramatically improving the ability to assess variant frequency in the general population. Additional resources have emerged, including computational predictive tools (126–128), and other sources of data, including phenotype specificity, familial

<table>
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<th>Table 3. Biomarkers for monogenic diabetes detection</th>
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<td>Standard biomarkers</td>
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<tr>
<td>Fasting C-peptide</td>
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<tr>
<td>Random or glucagon-stimulated C-peptide</td>
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<tr>
<td>Autoantibodies</td>
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<tr>
<td>GADA &lt; 99th percentile</td>
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<tr>
<td>IA-2 &lt; 99th percentile</td>
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<td>GADA and IA-2 &lt; 99th percentile</td>
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<td>GADA and/or IA-2 and/or ZnT8A &lt; 99th percentile</td>
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<td>Proposed biomarkers</td>
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<tr>
<td>Serum 1,5-anhydroglucitol</td>
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<td>Highly sensitive C-reactive proteins (standard in UK)</td>
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<td>Urinary C-peptide/creatinine ratio</td>
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<td>HDL-cholesterol</td>
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Variant classification

Key to diagnosing monogenic diabetes and other genetic conditions is not only identifying the variant but also distinguishing disease-causing variants from normal variation. Previous approaches to determine whether a variant identified in a patient was disease-causing involved sequencing a group of matched controls (usually 100–200 people) to assess the variant’s presence in the general population. This approach was limited because the sample size was too small to rule out population prevalence being too high for the disease; e.g., HNF1A-diabetes has an estimated population prevalence of 1 in 10,000. Moreover, the extent to which rare but benign genetic variation existed in the population studied was not known and was thus probably underestimated. As NGS has begun to boom, the problem of large quantities of genetic data for interpretation has arisen for genetic diseases in general. The genetic and phenotypic heterogeneity of monogenic diabetes, and its overlapping features with T1D and T2D, together increase the difficulty of interpreting the pathogenicity of variants found in patients suspected to have monogenic diabetes. On the other hand, NGS emergence has led to the availability of exome and genome sequences of over 100,000 individuals of diverse ancestries in the gnomAD database, dramatically improving the ability to assess variant frequency in the general population. Additional resources have emerged, including computational predictive tools (126–128), and other sources of data, including phenotype specificity, familial
functional studies, are also used. However, there is subjectivity in assigning pathogenicity to variants, and in the early 2010s, a lack of consistency of variant interpretation across laboratories became apparent.

In 2015, the ACMG and the Association for Molecular Pathology (AMP) jointly published a consensus recommendation on standards and guidelines for clinical genomic variant interpretation (129). The guidelines were developed through data sharing by a large number of American Board of Medical Genetics and Genomics–certified clinical molecular geneticists and pathologists from Clinical Laboratory Improvement Amendment/College of American Pathologists–accredited laboratories. The recommendations suggested that variants could be assigned to a five-tier system of classification: (a) pathogenic, (b) likely pathogenic, (c) uncertain significance, (d) likely benign, or (e) benign. The proposed sets of criteria include population data, computational and predictive data, clinical data, functional data (in vitro studies), and pedigree segregation. Each criterion is weighted by different levels of strength based on observed evidence and combined with other collected criteria to reach a conclusion. Since the publication of the initial ACMG/AMP guidelines, additional refinements have been published to improve rigor, including recommendations for evaluating the strength of evidence for LOF (130), standards for assessing functional studies (131), and application of a Bayesian quantitative point system (132).

**Value of establishing gene-specific rules**

The aim of the ACMG/AMP guidelines is to provide a universal set of criteria for interpreting variants for Mendelian disease. Additionally, each gene-disease pair requires further specification to reflect the specific disease frequency, clinical features, and genotype-phenotype relationships. In 2013, the Clinical Genome (ClinGen) Resource was founded by the National Human Genome Research Institute to serve as a knowledge base that defines gene-disease relationships, curate variants of genetic disease using a standardized approach, and distribute information about the variants to researchers and clinicians. Since then, dozens of expert panels and working groups have been formed to examine specific gene or disease groups for determining clinical significance and constructing gene-specific standardizations. The Monogenic Diabetes Expert Panel (MDEP), established in 2017, brings together experts and data to adapt the ACMG/AMP variant interpretation guidelines for monogenic diabetes genes and classify variants using these gene-specific rules, thereby improving the accuracy of variant classification in these genes and in turn improving the ability to accurately diagnose monogenic diabetes (133).

**Value of data sharing**

The establishing of guidelines is fundamental to standardized and concordant interpretation of monogenic diabetes gene variants. This process calls for expertise in endocrinology, molecular genetic testing, genetic counseling, and biochemistry. To reach the full potential of precision medicine in monogenic diabetes, centralization of case-level data is important. For instance, when the variant being evaluated is not observed in the general population but is observed in affected individuals, a higher number of occurrences leads to a higher level of evidence supporting pathogenicity. However, the uncommonness of monogenic diabetes often makes it difficult for individual laboratories to acquire enough cases. By pooling case data, expert panels can achieve levels of case-based evidence for pathogenicity not possible for any single laboratory or clinic.

**Value of functional evidence**

Well-established functional studies on variants boost the understanding of disease mechanisms and provide evidence supporting or disputing the pathogenicity of the variants. Studies have shown that functional analyses clarify variant interpretation in HNF1A-MODY variants, especially when family segregation data or phenotype data are not available (134). Caution is needed in using these data, because not all functional assays reflect the disease mechanism and not all variants impact the function in the same way. Full inspection of the consequences of a variant may require multiple assays to reach a conclusion (135). Systematic validation and statistical quantification of the level of strength of pathogenicity or benignity in functional assays are recommended (131). This approach encourages high-throughput mutation screenings, such as saturation mutagenesis (136) and systematic functional profiling of variants identified in the population (137, 138), which consist of pathogenic and benign variants. The MDEP is currently developing standards for evaluating evidence from luciferase assays for transactivation, which assess transcriptional activity of HNF1A and HNF4A variants, along with assays of DNA binding activity and protein expression (138, 139). For GCK variants, similar work is focused on the relative activity index of glucokinase as a measure of enzyme kinetic characteristics (140). In the longer term, multiplexed assays of variant effect (MAVEs) could provide comprehensive catalogs of allelic effects that can be interrogated to aid variant interpretation. This approach is particularly well suited for transcription factors such as HNF1A. It is important to note that functional evidence does not single-handedly implicate a variant in disease; the functional data must be evaluated in concert with the population and clinical data to make a pathogenicity determination.

**Conclusion**

Accurate genetic diagnosis of monogenic diabetes is crucial for patients, since it helps optimize treatment, especially for some patients switching from insulin or metformin to low-dose sulfonylureas (HNF1A-MODY and HNF4A-MODY) or no treatment (GCK-MODY) or from insulin to high-dose sulfonylureas (K_{ATP}-diabetes). Additionally, accurate monogenic diabetes diagnosis leads to better familial risk management and clinical course prediction. Advancement in genetic testing technology has increased the capacity of genetic diagnosis while decreasing sequencing cost. However, until we can offer genetic testing to every patient with diabetes, prioritizing patients with high suspicion of monogenic diabetes through assessment of their biomarker profiles or probability score is more practical. Monogenic diabetes provides an example of translating research findings into clinical practice that improves diagnosis and quality of life. Multidisciplinary expert collaboration and case sharing combined with incorporation of basic science into sequence variant...
interpretation will lead to improved diagnosis. Establishing clear guidelines for evaluating the causality of individual variants by this process is essential for widespread diagnosis of monogenic diabetes; more broadly, routine incorporation of emerging genomic data into the care of diabetes and disease in general is needed to realize the full potential of personalized and precision medicine. And as we celebrate the 100th anniversary of insulin’s discovery, it seems fitting to now celebrate and disseminate our more recently discovered ability to identify individuals who can make their own insulin once they have received the appropriate genomic diagnosis and treatment.

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Docherty LE, et al. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in


