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PEDIATRIC TYPE 2 AND MONOGENIC DIABETES (O PINHAS-HAMIEL, SECTION EDITOR)

How Recent Advances in Genomics Improve Precision Diagnosis and Personalized Care of Maturity-Onset Diabetes of the Young

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Abstract

Purpose of Review Non-autoimmune monogenic diabetes (MD) in young people shows a broad spectrum of clinical presentations, which is largely explained by multiple genetic etiologies. This review discusses how the application of state-of-the-art genomics research to precision diagnosis of MD, particularly the various subtypes of maturity-onset diabetes of the young (MODY), has increasingly informed diabetes precision medicine and patient care throughout life.

Recent Findings Due to extended genetic and clinical heterogeneity of MODY, diagnosis approaches based on next-generation sequencing have been worthwhile to better ascribe a specific subtype to each patient with young-onset diabetes. This guides the best appropriate treatment and clinical follow-up.

Summary Early etiological diagnosis of MD and individualized treatment are essential for achieving metabolic targets and avoiding long-term diabetes complications, as well as for drastically decreasing the financial and societal burden of diabetes-related healthcare. Genomic medicine–based practices help to optimize long-term clinical follow-up and patient care management.

Keywords Genetic diagnosis · Insulin secretion · MODY · Monogenic diabetes · Next-generation sequencing · Precision medicine

Introduction

Maturity-onset diabetes of the young (MODY), a dominantly inherited familial form of diabetes typically diagnosed before 25 years of age in non-obese subjects, represents the most frequent subgroup of early-onset non-autoimmune diabetes [1, 2]. MODY is a monogenic disease but with a high clinical and genetic heterogeneity, although always caused by a primary inherited or de novo genetically induced defect in insulin secretion responsible for chronic hyperglycemia. This pathophysiological feature common to all MODY cases arises from a functional impairment of one of the diverse pancreatic β-cell expressed key regulators of insulin biosynthesis and secretion [2, 3]. More than fifteen MODY genetic subtypes have been characterized raising the issue of an accurate etiological genetic diagnosis at an early age enabling a genuine personalized medicine of diabetes. MODY patients are usually diagnosed under the age of 25–30 years, but overt diabetes or moderate chronic hyperglycemia can happen at any age from childhood to young adulthood or at later age. The broad range of phenotypic features and variability in the clinical presentations are largely dependent on the underlying genetic defect that actually determines both pathophysiology and long-term progression of diabetes.

MODY may account for about 2–5% of patients diagnosed with diabetes (as from estimates in European patient cohorts, mainly from childhood diabetes registries), with a global prevalence of ~ 100 per million in white European populations

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[4–9]. However, the true prevalence of MODY in the general population may be largely underestimated in many countries due to a poor access to genetic testing (such testing is even unworkable in some areas). A high frequency (> 20%) of clinically suspected MODY was reported in South India among diabetic patients younger than 25 years, not resembling type 1 diabetes (T1D), although no genetic characterization was done [10].

MODY is often misdiagnosed as either T1D or type 2 diabetes (T2D), notably because some clinical patterns at diagnosis may overlap with both types of diabetes [11•]. Moreover, the presence of autoimmunity markers in MODY cases has been documented in several patient cohorts with accurate genetic diagnosis [12, 13], which may impact the genetic screening strategies based on standard clinical criteria of MODY.

In this review, we highlight the recent advances in the field of genomics of monogenic diabetes (MD) with the current challenges of accurately defining and recognizing the various MODY subtypes and of translating molecular diagnosis into personalized care over the lifetime.

A Global View on MODY Genetics

Clinical Heterogeneity and Genetic Subtypes of MODY

More than 25 years of comprehensive investigation of MODY genetic components, through the study of patient cohorts and multiplex families, have provided great advances in the knowledge and functional characterization of major MODY genes with mostly various protein-coding changes. So far, at least 15 genes causing MODY, involving different mutation types, have been formally identified (details on these genes are given in Table 1). In these genes, a single, mostly highly penetrant, rare mutation is sufficient to cause a MODY phenotype. The major MODY genes encode pancreatic β-cell expressed proteins involved in developmental processes, in the maturation and maintenance of β-cell function (through transcription factors regulating the transcriptional network of pancreatic β-cells), in the control of β-cell glucose sensing (through the glucokinase enzyme), in β-cell signaling, and in insulin production and secretion [2]. From our current knowledge of the underlying pathogenic mechanisms, it is well substantiated that MODY-causing mutations cluster into key genes and interconnected biological pathways that represent core regulatory networks for pancreatic β-cell identity and function (as for β-cell transcriptional network, or regulatory proteins of reticulum endoplasmic homeostasis) [14••]. Along the same line, β-cell dysfunction is the main driver of MODY, together with decreased β-cell mass and cellular death.

Among the known MODY genetic subtypes, mutations in four genes (*GCK*, *HNF1A*, *HNF4A*, and *HNF1B*) are the most frequent causes of MODY in Europeans, accounting for more than 75% of MODY diagnoses in several European cohorts [6, 9, 15]. A recent survey of MODY patients from South India showed *HNF1A* and *ABCC8* are the most frequently mutated genes (although together accounting for only 11% of MODY cases) [16•]. Other genes (e.g., *KLF11*, *BLK*, *APPL1*; Table 1) ascribed to a MODY phenotype still lack reproducibility through multiple families worldwide.

Importantly, the different mutations in one of the MODY genes such as *GCK*, *ABCC8*, *KCNJ11*, *INS*, or *WFS1*, or even a specific mutation in these genes, can lead to a large spectrum of diabetes presentations from neonatal and childhood-onset diabetes to MODY and young-adult diabetes between and within MODY families (Fig. 1), which strongly complicates the attribution of a clinical diagnosis by physicians [17–20]. While heterozygous loss-of-function *GCK*-MODY mutations usually lead to a moderate, non-progressive fasting hyperglycemia from birth (fasting glucose levels of 5.5–8.0 mmol/l), homozygous inactivating *GCK* mutations cause neonatal diabetes requiring life-long insulin treatment, and heterozygous activating *GCK* mutations cause persistent hyperinsulinemic hypoglycemia of infancy (PHHI). *GCK*-MODY is a frequent cause of incidental hyperglycemia in children, and the mild clinical phenotype in these patients is associated with a low prevalence of microvascular and macrovascular complications despite the life-long hyperglycemia [21]. A population prevalence of *GCK* mutations has been estimated to 1.1 in 1000 in a white European population ($n = 5500$), with about 2% of women diagnosed with gestational diabetes mellitus (GDM) carrying a heterozygous *GCK* mutation in the Atlantic Diabetes in Pregnancy study [22], and to 2.4 in 1000 in the Chinese population–based study of the Pinguu cohort ($n = 3345$), with approximately 1.3% of *GCK*-MODY in the group of diabetic patients [23]. A high frequency of pathogenic *GCK* mutations (all in heterozygote state) was also reported in a patient cohort from Poland (6.88/100,000) [9] and in non-obese Russian women with GDM (accounting for 23.4% of GDM patients) [24].

A paradoxically dual phenotype, characterized by fetal macrosomia, increased birth weight, and neonatal hypoglycemia with hyperinsulinism usually remitting during infancy and diabetes occurring from adolescence, is a specific feature of *HNF4A*-MODY patients and in a few rare cases of *HNF1A*-MODY [25–28]. In contrast, *HNF4A* mutation carriers with less severe presentations and later-onset diabetes may be misdiagnosed as T2D due to the delayed age of diagnosis like those carrying the *HNF4A*-p.R114W mutation, for which the penetrance has been estimated to only ~ 10% from the UK Biobank meaning that incidental finding of this variant in normoglycemic individuals would not be ineluctably predictive of diabetes [29, 30•]. Another example of variable clinical

Table 1 Genes causing maturity-onset diabetes of the young (MODY) and the different genetic subtypes

Gene name (locus)	Protein (function)	Phenotypes/syndromes	OMIM[a]	Low-frequency or common variants involved in T2D[b]
Established causes of MODY (both common and rare genetic subtypes)				
HNF4A (20q12)	HNF-4α (transcription factor)	Diabetes in adolescence or early adulthood (and neonatal hyperinsulinism)	125850, 600281	Yes
GCK (7p13)	Glucokinase (glycolytic enzyme)	Mild hyperglycemia (onset in early childhood and life-long) (frequent)	138079, 125851	Yes
HNF1A (12q24.2)	HNF-1α (transcription factor)	Diabetes in adolescence or early adulthood (frequent)	600496, 142410	Yes
PDX1 (13q12.1)	IPF1 (transcription factor)	Diabetes in early adulthood (rare form)	606392, 600733	Yes
HNF1B (17q21)	HNF-1β (transcription factor)	Diabetes in early adulthood, renal cysts, and diabetes (RCAD)	137920, 189907	Yes
NEUROD1 (2q31.3)	NeuroD1 or Beta2 (transcription factor)	Diabetes in early adulthood (rare form)	606394, 601724	Not substantiated
PAX4 (7q32)	Paired box gene 4 (transcription factor)	Diabetes in early adulthood (rare form)	167413, 612225	Yes
INS (11p15.5)	Preproinsulin, insulin (hypoglycemic hormone, effect on anabolism)	Diabetes in childhood and early adulthood	613370, 176730	Not substantiated
ABCC8 (11p15.1)	SUR1 (sulfonylurea receptor; K_{ATP} channel regulatory subunit)	Diabetes in childhood and early adulthood	600509	Yes
KCNJ11 (11p15.1)	Kir6.2 (K_{ATP} channel pore-forming subunit)	Diabetes in childhood and early adulthood	600937	Yes
WFS1 (4p16)	Wolfram syndrome 1 (Wolframin)	Diabetes in early adulthood, non-syndromic or mild form of sensorineural hearing impairment (SNHI)	606201, 222100	Yes
Other rare or controversial causes of MODY (some lacking evidence of replication through multiple families/cases in independent cohorts)				
APPL1 (3p14)	Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (binding to Akt, insulin-signaling pathway)	Diabetes in early adulthood	604299, 616511	Not substantiated
BLK (8p23)	B Lymphocyte kinase (non-receptor tyrosine kinase)	Diabetes in early adulthood	191305, 613375	Yes
CEL (9q34)	Carboxyl-ester lipase enzyme	Diabetes in early adulthood; pancreatic exocrine insufficiency, pancreatic atrophy, and lipomatosis	114840, 609812	Not substantiated
KLF11 (2p25)	Krüppel-like factor 11 (transcription factor)	Diabetes in childhood and early adulthood	603301, 610508	Insufficiently substantiated
MAFA (8q24)	MAF bZIP transcription factor A	Diabetes in early adulthood or insulinomatosis (also associated with congenital cataract or glaucoma)	610303, 147630	Yes
PCBD1 (10q22)	Pterin-4 α-carbinolamine dehydratase, dimerization cofactor of HNF-1α	Diabetes with early-onset, antibody-negative, and mild hyperphenylalaninemia	126090	Not substantiated
RFX6 (6q22)	Regulatory factor X, 6 (transcription factor)	Diabetes in early adulthood	612659	Not substantiated
SLC19A2 (1q23)	Solute carrier family 19 (thiamine transporter), member 2	Diabetes in childhood and early adulthood	603941	Not substantiated

[a] The Online Mendelian Inheritance in Man (OMIM) numbers depict the phenotype and/or gene MIM numbers

[b] This is based on the latest and largest meta-analysis of low-frequency and common variants (including either coding or non-coding variants) associated with type 2 diabetes mellitus (T2DM) [68••]

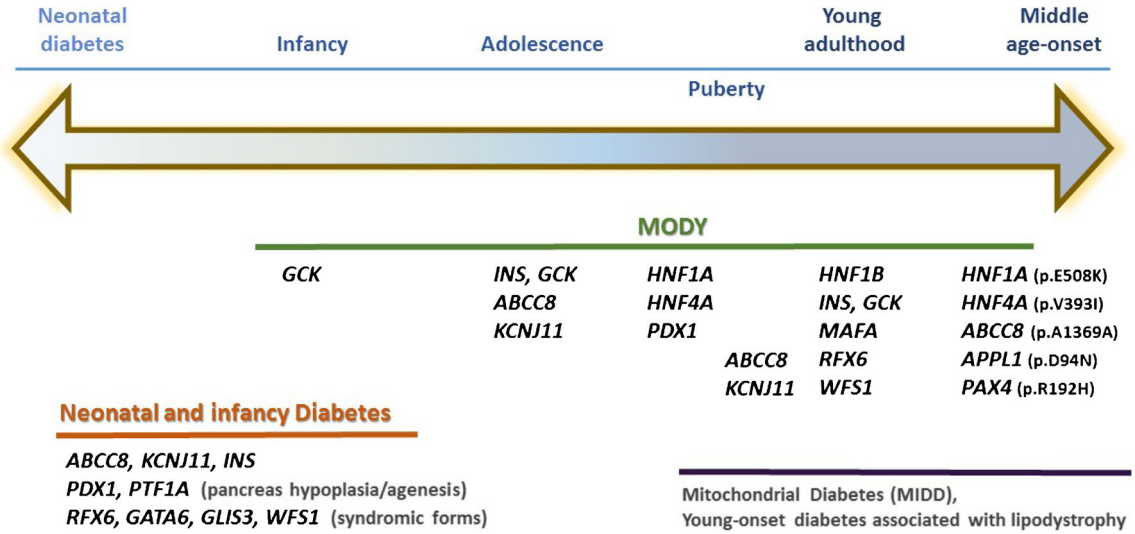

Fig. 1 Spectrum of monogenic diabetes genetic subtypes. The figure depicts the spectrum of monogenic diabetes along the life course, including MODY and neonatal- or infancy-onset diabetes, with major causal genes according to the diabetes genetic subtype. Human gene symbols are indicated with specific diabetes-causing missense mutations in parenthesis

presentations of a MODY phenotype has been described in a French diabetic family with a *KCNJ11* mutation (p.Glu227Lys) shared by several patients with early-onset to adult-onset diabetes (range of ages at diagnosis 13–59 years) as well as by normoglycemic adult-related individuals [17]. A dual phenotype of hyperinsulinism in infancy and later-onset diabetes is also observed in some patients with an *ABCC8* mutation although another underlying mechanism arises from continuous overstimulation of insulin secretion and slow progressive loss of β-cell function with insulin deficiency and diabetes in early adulthood [31].

In some MODY cases, there is no clear dominant inheritance that means the classic definition of MODY is ineffective to recognize all MODY patients. Indeed, numerous de novo mutations have been reported in children with insulin-requiring antibody-negative diabetes, and in European and Japanese cohorts, relatively high de novo rates of *GCK*, *HNF1A*, and *INS* mutations have been reported [32–34].

New Insights on Pathways to Early-Onset Non-Autoimmune Diabetes Through Next-Generation Genomics Approaches

The known genetic causes of MODY have pointed out major pancreatic β-cell expressed genes regulating insulin secretion, such as alterations in GCK and a network of transcription factors important for the control of β-cell function. Recent works have further provided new clues for better understanding specific functional mechanisms related to MODY genetic defects.

Among the commonest causes of MODY, a large series of *HNF1A* mutations are the well-established cause in patients with MODY3 of several cohorts worldwide. A recent outstanding study, aiming to better understand the functional changes in the pancreatic islet of an individual with a clinical diagnosis of T1D, reported a pathogenic heterozygous *HNF1A*-p.Thr260Met mutation resulting in altered DNA binding and leading to β-cell dysfunction, impaired glucose-stimulated insulin secretion (GSIS), and insulin-deficient diabetes reminiscent of T1D [14••]. Furthermore, RNA sequencing and transcriptomic profiling of purified $HNF1A^{+/T260M}$ β-cells showed decreased expression of several transcription factors involved in mature β-cell function (like MAFA and RFX6) with the identification of yet unrecognized HNF1A-regulated targets and pathways, like protein synthesis and amino acid metabolism, and, interestingly, revealed a previously unknown role for HNF1A in α-cell dysfunction [14••]. This kind of study highlights how a physiological defect of insulin-deficient diabetes reminiscent of T1D can be unveiled at the molecular level, from isolated human islets of a single patient case, impacting β-cell transcriptional regulatory networks, and solved through rationale for a therapeutic alternative.

Another genome-wide analysis of induced pluripotent stem cells (iPSC) differentiating into human pancreatic progenitors (PPs) identified stage-specific target genes of PDX1 (also known as MODY4) during in vitro differentiation into PPs, strengthening that appropriate execution of a β-cell developmental program is essential for β-cell function and homeostasis [35••].

A recent study highlighted the role of the β-cell-enriched MAFA transcription factor in GSIS and its oncogenic capacity in the β-cell by identifying a missense mutation (*MAFA*-pSer64Phe) in two pedigrees as the cause of early-onset diabetes resembling MODY and insulinomatosis [36•]. The p.Ser64Phe mutation was found to enhance MAFA protein

stability, transactivation potential, and activity in β-cell lines, which points out MAFA both as an oncogene and a key regulator of β-cell activity [36•].

Specific, or Hotly Debated, Genetic Etiologies of MODY

Heterozygous nonsense *RFX6* mutations have been reported to cause MODY in European cohorts, whereas biallelic mutations of the β-cell expressed transcription factor RFX6 are responsible for a rare form of neonatal diabetes associated with gastro-intestinal system defects (known as the Mitchell-Riley syndrome) [37•]. Compared with *HNF1A*-MODY and *HNF4A*-MODY, the *RFX6* protein truncating mutations showed a reduced penetrance (only 27% of heterozygotes developed diabetes by age 25 years and 78% by age 51 years) [37•]. RFX6 has a critical role in pancreatic endocrine cell development during prenatal life, in maintaining β-cell maturity, and it regulates insulin expression and secretion by modulating L-type Ca^{2+} channel activity [38, 39].

A recent study in young diabetic patients from South India has reported two new heterozygous mutations in the *NKX6-1* gene with functional impairment in in vitro assays, suggesting that they likely contribute to the development of MODY in mutation carriers [16•]. However, other mutations in independent patients have not yet been reported. Two loss-of-function mutations of *APPL1* have been reported in two large families showing co-segregation with a MODY phenotype, although with incomplete penetrance; however, no other families have been reported so far. APPL1 is implicated in the insulin-signaling pathway by enhancing insulin-induced AKT2 activation and thus preserving or regulating glucose homeostasis [40].

A missense mutation of *BLK* (p.A71T), encoding a non-receptor tyrosine kinase, was identified in several diabetic relatives and shown to blunt insulin secretion in vitro; thereby, the authors reported *BLK* as a potential cause of MODY [41]. However, the same mutation (*BLK*-p.A71T) was found as a low-frequency variant (rs55758736) in a French general population, potentially influencing diabetes risk in a context of obesity [42]. Furthermore, this missense variant is reported with a population frequency of 0.012 in the gnomAD browser, and no other *BLK* mutations have been reported to cause MODY in other families so far. All those findings question the assignment of *BLK* as a MODY gene.

Other genes like *KLF11* and *CEL* have been reported as causal for MODY, although genetic or functional evidence of obvious pathogenicity is not fully compelling (Table 1).

Despite these important advances in understanding the molecular pathogenesis of MODY, the genetic determinants in many patients with young-onset diabetes resembling a MODY-like phenotype remain unknown, suggesting additional locus heterogeneity and new pathogenic mechanisms to be yet discovered. This has particularly been observed in the US or the North African, Indian, and Asian populations, which opens avenues to discover new potential genetic causes and pathogenic defects in early-onset diabetes [5].

Clinical Translation and Precision Medicine in MODY Subtypes

A genuine clinical translation that takes into account precise knowledge of genetic etiology is illustrated by the offering of clear therapeutic recommendations according to the MODY subtype following an accurate genetic diagnosis. The most exemplary cases of precision medicine in MODY are described herein.

There is a shared consensus that antidiabetic therapy is ineffective and not recommended in GCK-MODY patients, as they maintain a good glycemic control with low-carbohydrate regimen alone; based on several clinical follow-up studies, when oral hypoglycemic treatment or low-dose insulin are stopped, there is no deterioration in HbA1c levels for decades in these patients [43]. However, GCK-MODY may have clinical implications in pregnancy. There are four possible situations.

1. When the diabetic mother carries a *GCK* mutation but not the fetus, maternal hyperglycemia causes fetal hyperinsulinemia and increased birth weight.
2. The mother and the fetus share a *GCK* mutation; as the glucose threshold to trigger insulin secretion is similar in the mother and fetus, fetal insulin levels remain normal and birth weight is normal. This is true providing the mother's hyperglycemia is not treated.
3. The mother is healthy and the father is the carrier of a *GCK* mutation; if the fetus carries the paternal mutation, the levels of maternal glycemia are insufficient to stimulate adequate fetal insulin secretion to sustain optimal growth, and birth weight will be low.
4. The mother is healthy and the father is the carrier of a *GCK* mutation, but the fetus does not carry the paternal mutation; his birth weight will be normal.

Based on these possibilities, it has been recommended to treat hyperglycemia in pregnant women carrying a *GCK* mutation only when there is ultrasound evidence of accelerated fetal growth (particularly in the third trimester, as elevated fetal insulin secretion leads to increased risk of fetal macrosomia and associated obstetric complications) [43]. Therefore, it is important to make a genetic diagnosis of GCK-MODY in women with gestational diabetes, as they require different managements that greatly depend on whether the fetus has inherited or not the *GCK* mutation rather than the degree of maternal glycemia [43, 44]. As this condition can be

complex, several issues on the clinical management of GCK-MODY in pregnancy and the outcomes in offspring are reviewed in the paper by Chakera et al. [43]. In contrast, patients with *HNF1A*- and *HNF4A*-MODY develop a more severe diabetes at diagnosis that usually quickly progresses, and they need to be treated with low-dose sulfonylurea (SU) drugs as the recommended first-line treatment. However, the response to SU treatment may greatly vary depending on the glycated hemoglobin levels, body mass index (BMI), and duration of diabetes at the time of SU transfer, which again advocates for an early genetic diagnosis [45]. When additional treatment is required because SU monotherapy fails, or in case of hypoglycemia, a combination therapy with dipeptidyl peptidase (DPP)-4 inhibitor, or glucagon-like peptide-1 receptor agonist, is recommended. These drugs have also been used as first-line therapy. Indeed, DPP-4 inhibitors that act by prolonging the activity of circulating incretins are an effective first-line treatment in patients with *HNF1A*-MODY or *HNF4A*-MODY. Some patients with the *HNF4A* p.R114W mutation presented a reduced sensitivity to low-dose SU treatment, as well as diabetic carriers of the *HNF1A* p.E508K variant that is specific to the Mexican population (as detailed below) [46•].

Otherwise, patients with MODY bearing a mutation in the pancreatic ATP-sensitive potassium (K_{ATP}) channel genes (i.e., *ABCC8* and *KCNJ11*) can also benefit from an oral SU treatment, as long-lasting effectiveness of SU in neonatal diabetes caused by a K_{ATP} channel gene mutation is well documented [47••]. The earlier the SU treatment is started, the better the in vivo efficiency on glycemic control [47••]. However, the response to SU treatment may be determined by the functional consequences on K_{ATP} channel activity according to the nature and position of mutations, especially whether it results in activating effect (with blunted insulin secretion/diabetes) or inhibitory effect (with hyperinsulinism/hypoglycemia).

A definite genetic diagnosis is also crucial to anticipate the long-term clinical course in young patients with MODY and the later risk of diabetes complications, and so how to ensure the most suitable health care is provided to a specific patient. Long-term cost-effectiveness of routine genetic screening for MODY has rigorously been estimated using Markov decision models based on population-based prevalence data [48, 49••]. Based on a model sensitivity analysis for mutations in *GCK*-MODY2, *HNF1A*-MODY3, and *HNF4A*-MODY1, or for high-throughput sequencing in a pediatric population with diabetes, it is now well demonstrated that routine genetic screening practices can reduce health system costs and improve patient quality of life (QoL) (with costs of screening fully offset within 10 years, at a MODY prevalence as low as ~1% and sequencing costs as high as 765 USD or 675 EUR; from the study by Johnson et al. [49••]). The cost-effectiveness and QoL benefits greatly resulted from the use of SU rather than insulin, so much so that individualization of therapy will save many children with diabetes from life-long insulin use. With regard to practical health applications, as the global costs of genetic testing are decreasing with the recent progress made in high-throughput sequencing technologies, a broader screening for all known MD subtypes should be provided, particularly in patients with infancy-onset diabetes for early and effective implementation of appropriate health care.

Moreover, genetic counseling from an index patient diagnosed with a specific MODY subtype can trigger molecular testing in other family members, particularly in children and young adults known to present with incidental hyperglycemia or in still undiagnosed patients; such follow-up diagnosis may also help reclassifying diabetes in patients misdiagnosed with T1D or T2D with greatly beneficial therapeutic adaptations.

Current and Prospective Challenges in Etiological Diagnosis of MODY

Consensus guidelines for the diagnosis and management of MD subtypes including MODY were published in 2018 [50]. Usually, when atypical diabetes is diagnosed in young patients and pregnant women, genetic testing is recommended to provide an etiological diagnosis and select the optimal treatment (if needed). On that matter, specific advice has been provided on how to identify patients most likely to have a diagnosis of MD and how to differentiate MODY cases from patients with type 1 or type 2 diabetes (based on specific clinical and biological criteria) [50, 51]. Furthermore, MODY genetic diagnosis must be widened beyond standard guidelines for diabetic patients diagnosed before age of 30 years, as it was highlighted in the Young Diabetes in Oxford (YDX) study showing that less than 50% of cases with a mutation met the commonly used MODY diagnostic criteria (with treatment changes in 27% of newly identified cases with MODY) [52].

Given the genetic heterogeneity of MODY underlying the broad range of clinical presentations, and the allelic heterogeneity with a continuum of phenotypes and/or specific clinical features, an accurate genetic diagnosis relies on comprehensive analysis of all known MODY genes based on next-generation sequencing (NGS) methods with integrated analysis pipelines. For almost 10 years, whole-exome sequencing (WES) and gene panel analyses through various NGS platforms have been routinely applied in diagnosis of MD, as a reliable, quick, and cost-effective means compared with previous screening methods [53–57]. Furthermore, WES enables both the mutation detection for all known MODY genes and identification (or confirmation) of novel putative causative genes for MODY, along with revealing possible genetic multi-causality [36•, 58, 59].

A recent report of NGS-targeted screening of 30 genes in 89 Japanese children with insulin-requiring antibody-negative

diabetes identified 11 pathogenic mutations in *INS* and *HNF1A/HNF4A/HNF1B* genes (but not in *GCK*) and also reported a relatively high de novo rates of MODY mutations in the studied patients (only two mutation carriers had affected parents) [33]. Another comprehensive exome analysis of 152 clinically diagnosed unrelated MODY cases from South India identified ~15% of cases carrying a relevant mutation in *HNF1A* (7%) or *ABCC8* (3%), but *GCK* in < 1% of the cases that is a lower rate than in Europeans and consistent with previous reports of Indian patients with MODY [16•]. Interestingly, this study also reported two new mutations in *NKX6.1*, which showed impaired transcriptional activity, suggesting a plausible role of NKX6.1 in MODY, with additional relevant variants in other genes like *RFX6*, *WFS1*, or *AKT2*, which require further validation [16•].

One important, and still challenging, issue in interpreting NGS data lies in mutation pathogenicity assignment and accurate classification, as rare pathogenic mutations causing MODY have to be distinguished from neutral mutations. Indeed, approved pathogenic mutations in the MODY genes do impact the treatment and clinical care of the patients (according to the genetic subtype as stated above). Usually, there are several levels of evidence for assessing the pathogenicity of mutations, including the following: (1) identification of a null variant or a missense mutation already reported as causing MODY, (2) assessing the presence of other mutations at the affected nucleotide position (via tools like the Human Gene Mutation Database [HGMD], ClinVar, or Alamut-Genova powered by SOPHIA GENETICS), (3) estimation of minor allele frequency in the general population via the publicly available "Genome Aggregation Database" (gnomAD, which is a multi-ethnic dataset of all genetic variants identified by NGS), (4) assessing in vitro or in vivo functional studies supportive of a damaging effect or by in silico functional prediction via multiple prediction software programs and database search, and (5) co-segregation analysis of the identified mutation among relatives with diabetes (when possible within multiplex families). To ensure best practices for mutation interpretation, the *American College of Medical Genetics and Genomics* (ACMG) established rules for variant classification and reporting guidelines which are commonly used and shared among the genetics research community and specialized health services [60]. Using these ACMG criteria for pathogenicity, the variants are usually classified ranging from "benign" and "variant-of-uncertain-significance" (VUS) to "pathogenic" or "likely pathogenic" [16•, 60].

The NGS-based genetic screening strategies have been shown to provide a better assessment of genetic subtype prevalence from nationwide population–based patient registries in specific ethnicities. For example, targeted NGS analysis of a nationwide Norwegian childhood diabetes registry suggested a prevalence of 6.5% for MODY in antibody-negative children, with one third of these MODY cases not having been recognized by clinicians [6]. Otherwise, the increasing number of genes tested through NGS may lead to identify digenic presentations of early-onset diabetes, like the co-inheritance of two pathogenic mutations in *HNF1A* and *GCK* reported in a few MODY families [55, 61].

Free-hypothesis NGS-based study of unelucidated cases is also a powerful mean to identify disease-causing mutations in unsuspected genes with the uncovering of new or specific diabetes pathophysiological mechanisms, such as the identification of novel mutations in *PCBD1* and *MAFA* genes in familial forms of diabetes with clues to their mechanistic impacts [36•, 59].

Beyond new gene discovery in the field of research, an important challenge in the next coming years is how to set up a more open population-level and high-quality genetic screening strategy aiming to improve etiological diagnosis in almost all of cases with early-onset diabetes.

Extensive Genetic Evidence for a Continuum of Diabetes Phenotypes

Several studies have now suggested a potential continuous spectrum of diabetes phenotypes encompassing rare monogenic forms and common T2D in adults, with an allelic series across several MD genes (as exemplified in *HNF1A* or *HNF4A*) (Fig. 1) [62•]. Large studies in general populations or T2D case-control cohorts sought out the spectrum and effect size of rare variants in MD-MODY genes by DNA sequencing (either targeted MD genes panel or WES) and assessed the frequency and phenotypic effects of such rare variants in common adult-onset T2D. From randomly selected individuals of the Framingham and Jackson Heart studies, Flannick et al. (2013) reported 1.5% and 0.5% of carriers of rare disease-causing mutations, respectively, and the vast majority of carriers remain euglycemic at middle-aged questioning the penetrance and expressivity of rare variants identified as deleterious mutations [63]. The SIGMA T2D consortium using WES in ~4000 Mexican and Latino-American individuals identified one low-frequency variant in *HNF1A* (p.E508K in 2.1% of carriers with T2D and 0.36% of carriers without T2D, which was shown to affect protein function) with a fivefold increased risk in patients with diabetes; this missense variant was shown to affect protein function and to be specific to the Latino population [64]. However, the study by Martagon et al., using a standardized pharmacogenetic protocol (SUGAR-MGH), assessed the treatment response in 46 carriers of the *HNF1A*-p.E508K variant compared with 50 age- and sex-matched non carriers and showed that the p.E508K carriers have a reduced insulin response rather than an increased response to SU (glipizide challenge), such as it is observed in most *HNF1A*/MODY3 patients [46•]; one hypothesis of this unexpected reduced SU

response might be a more aggressive β-cell failure rendering SU less effective in variant carriers over time.

The study by Bansal et al. based on targeted sequencing of 22 genes involved in MD in pooled DNA from 4016 cases with diabetes, including 1346 individuals diagnosed before age 40 years and 2872 controls, identified 40 individuals with T2D (accounting for 1.8% of early-onset and 0.6% of adult-onset subgroup) carrying known pathogenic missense variants in the *GCK*, *HNF1A*, *HNF4A*, *ABCC8*, and *INS* genes [65•]. Via NGS performed in ~6500 cases and ~6500 controls, the GoT2D and T2D-GENES consortia found a strong signal of association between the aggregation of rare deleterious variants in MODY genes and increased T2D risk [66•]. However, through gene-centric analyses, the authors found that this association was not explained by the most frequently mutated genes in MD (i.e., *GCK*, *HNF1A*), but partly by *BLK* (which is a controversial cause of MODY; Table 1). The latest study by Flannick and colleagues reports on whole-exome sequencing analyses in a much larger cohort of 45,000 individuals from five ancestries (with a sample and variant quality control workflow providing an analysis dataset of 20,791 patients with T2D and 24,440 non-diabetic controls, and of 6.33 million variants including 93.5% of rare variants with minor allele frequencies [MAF] of less than 0.5%). The authors confirmed the association between the aggregation of rare variants (with a MAF below 0.5%) in the genes implicated in MODY and increased T2D risk, in part explained by the rare mutations found in *PDX1* [67••]. Interestingly, the cluster of rare variants was also associated with lower body mass index and lower fasting insulin levels, consistent with MODY phenotypes [67••]. Furthermore, a posterior probabilities of true, causal association (PPA) method was applied to evaluate rare and low-frequency variants in the set of MODY genes (assuming that all MODY genes may be relevant to T2D), and 24 variants (combined MAF = 1.1%) were predicted with a PPA ≥ 40% (nine of them with estimated odds ratio > 3) [67••]; these rare non-synonymous variants were not previously reported as pathogenic MODY mutations and could be used as T2D risk prediction markers, although they might be false-positive rather than true associations. Additional investigations of the identified mutations and replication studies are still required to have a clearer view on this crucial question that would actually be a gateway for precision medicine in common T2D.

Conclusions

Genomics research in monogenic diabetes and the implementation of NGS-based approaches for precision diagnosis of MODY subtypes undoubtedly move the physicians and patients towards the era of precision genomic medicine that takes into account the individual genetic data. Specific issues are emerging such as the right estimate of variant pathogenicity and age-dependent penetrance, the multi-genic causality, and the composite phenotypes. Lessons learned from MD with recent findings in common T2D genetic architecture support a continuum of diabetes phenotypes from rare monogenic to common adult-onset diabetes which impacts the strategies for both diagnosis and longitudinal investigation of diverse clinical subtypes along the life course. Beyond facing young-onset diabetes, practitioners should systematically promote a comprehensive genetic testing of MD-MODY subtypes, with benefits of optimal patient care and of strong reduction of global medical costs.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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