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Monogenic diabetes: old and new approaches to diagnosis

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ABSTRACT – Up to 5% of young adults diagnosed with diabetes have a monogenic aetiology, the most common of which is maturity-onset diabetes of the young (MODY). A definitive molecular diagnosis is important, as this affects treatment, prognosis and family screening. Currently, however, rates of diagnosis are low due to a combination of lack of awareness of the benefits of making the diagnosis and the challenges of differentiating patients with MODY from those with common forms of diabetes. This article aims to introduce general physicians to the characteristics of monogenic diabetes and the clinical features that can be used to diagnose patients. Recently, genomewide association studies have resulted in the identification of C-reactive protein and glycan profile as specific biomarkers for the most common MODY subtype due to *HNF1A* mutations, and the potential translation of these findings are discussed.

KEY WORDS: Aetiology of diabetes, glycolytic enzyme glucokinase (GCK), *HNF1A*, maturity-onset diabetes of the young (MODY), monogenic diabetes

Introduction

Although most people with diabetes have type 1 or type 2 diabetes, the American Diabetes Association's classification of diabetes[1] reveals a long list of less common aetiologies. Maturity-onset diabetes of the young (MODY) is a heterogenous group of monogenic causes of β-cell dysfunction that leads to diabetes presenting in young adults. Those with MODY have diabetes characterised by:

- young age at onset (10–45 years)
- autosomal dominant family history
- continued production of endogenous insulin
- absence of β-cell autoimmunity
- absence of signs of insulin resistance.

Box 1 features the case history of a real patient with a very typical story.

Features of common forms of MODY

Mutations in around 12 different genes have been associated with a MODY-like phenotype.[2] However, only four genes are frequently involved in clinical practice. These code for the transcription factors hepatocyte nuclear factor 1-alpha (*HNF1A*), hepatocyte nuclear factor 4-alpha (*HNF4A*), hepatocyte nuclear factor 1-beta (*HNF1B*) and the glycolytic enzyme glucokinase (*GCK*).

Box 1. Case history of typical patient with maturity-onset diabetes of the young (MODY).

Mike, a keen sportsman, was diagnosed with diabetes at the age of 17 years. He was lean and symptomatic, and so was assumed to have type 1 diabetes and started on insulin. He had a strong family history of diabetes in his father, paternal grandfather and paternal uncle. His father, who was also slim and active, had glycosuria noted in his 20s and type 2 diabetes diagnosed at the age of 44 years.

Mike took his insulin regularly but struggled to control his diabetes around sports training. He was taking up to eight insulin injections a day at one point, but continued to experience both hyperglycaemia and post-exercise hypoglycaemia. When Mike was 26 years old, he attended a sports diabetes clinic. It was noted that he was not using any basal insulin, but despite this had a fasting glucose of 5 mmol/l and glycosylated haemoglobin (HbA_{1c}) of 5.7%/39 mmol/mol – both in the normal range. This was suggestive of continued secretion of endogenous insulin, which would be unusual in type 1 diabetes 9 years after diagnosis. C-peptide in the normal range confirmed this, and the combination of positivity for C-peptide, young-onset, familial diabetes led to referral for genetic testing. He was found to have an *HNF1A* mutation.

Insulin treatment was stopped and Mike transferred to gliclazide 20 mg/day (one-quarter of a tablet). On this treatment, his HbA1c remained at around 6%/42 mmol/mol, but he had no further problems during exercise and experienced infrequent hypoglycaemia.

HNF1A- and HNF4A-MODY

Mutations in these genes present a very similar clinical picture. Individuals are normoglycaemic in childhood but develop progressive β-cell dysfunction. Diabetes typically presents in the second to fourth decade of life. A low renal threshold for glucose is seen in HNF1A-MODY (but not HNF4A-MODY), so postprandial glycosuria is observed in non-diabetic carriers of mutations and used to screen family members. Poor control leads to the usual micro- and macrovascular complications of diabetes.

The most striking feature of HNF1A-/HNF4A-MODY is sensitivity to low-dose sulphonylurea (SU) drugs (eg gliclazide and glibenclamide) and the related class of prandial secretagogues (eg repaglinide and nataglinide). A small randomised controlled trial showed that those with HNF1A-MODY had a five-fold greater decrease in fasting glucose with gliclazide than matched type 2 controls,[3] while metformin was equally effective in the

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two groups. Low-dose SUs are thus the first-line treatment for HNF1A-/HNF4A-MODY. In those started on insulin because they were assumed to have type 1 diabetes, insulin can be stopped safely with maintenance of good control (see the case history in Box 1). Other oral agents should also be changed to an SU. Hypoglycaemia is frequently seen on standard doses of SUs, so one-half or one-quarter of a tablet (eg 20–40 mg gliclazide) is the starting dose.

Mutations in *HNF1A* are the most common form of MODY in adults, accounting for about 50% of cases in the UK. A further 10% of cases of MODY are HNF4A-MODY.

GCK-MODY

Mutations in the gene for the glycolytic enzyme GCK account for about 30% of cases of MODY in the UK and are the most common form of MODY in children. GCK-MODY presents with mild fasting hyperglycaemia (fasting plasma glucose 5.5–8 mmol/l) and small postprandial glucose excursions.[4] The underlying mechanism is an increase in the threshold for glucose-stimulated insulin secretion. Unlike other forms of dysglycaemia, insulin secretion remains regulated. Microvascular complications are not observed in patients with GCK-MODY and pharmacological treatment is not recommended for the slightly increased levels of glycosylated haemoglobin (HbA_{1c}).

HNF1B mutations – RCAD syndrome

Although the transcription factor HNF1B is closely related to HNF1A, the different pattern and timing of expression of HNF1B means that mutations lead to a distinct phenotype of developmental abnormalities involving cystic renal disease accompanied by pancreatic and genitourinary anomalies. This is termed renal cysts and diabetes (RCAD) syndrome, and diabetes alone is unusual. Patients are not sensitive to SUs and usually require treatment with insulin. Mutations in *HNF1B* account for 5–10% of cases of MODY in the UK.

Diagnosis

Why diagnose MODY?

The most important reason to arrive at a correct molecular diagnosis is the potential for treatment changes in those assumed initially to have type 1 or type 2 diabetes. As described above, low-dose SUs can be used in HNF1A-/4A-MODY and no treatment is needed in GCK-MODY. In our study in Oxford, about one-quarter of the patients diagnosed with MODY had a treatment change as a consequence of their diagnosis.[5] We are also able to give people information about the likely course and prognosis of their diabetes, which is particularly helpful for those with GCK-MODY. Finally, follow up and screening of family members is very important for a monogenic condition, as first-degree relatives will have a 50% risk of carrying the same mutation and should have diabetes screening with or without genetic testing. The network of genetic diabetes nurses and MODY clinics across the UK[6] can help to arrange follow up for family members.

Missed and misdiagnosis of MODY

Despite the advantages of making an accurate aetiological diagnosis, most individuals with monogenic diabetes are initially misdiagnosed as having type 1 or type 2 diabetes and it seems likely that many are never correctly identified. Regional rates of referral for genetic testing vary a great deal across the UK,[7] presumably due to local enthusiasm or knowledge about monogenic diabetes, as well as financial constraints in some areas (each gene sequenced costs about £350). From the areas of maximum prevalence (about 100 cases per million population), it was calculated that at least 80% of cases are being missed across the UK,[7] and there are long delays averaging >10 years from onset of diabetes until correct molecular diagnosis.[5,7]

Misdiagnosis of MODY arises because the phenotype of monogenic diabetes is not sufficiently distinctive to allow easy clinical differentiation from common forms of diabetes. For example, patients tend to be young and lean, as is the case in type 1 diabetes, but do not require insulin and are β-cell antibody negative, as is the case in type 2 diabetes. Family history of diabetes is common for both type 1 and type 2 diabetes and is not invariably reported in monogenic diabetes (due to de-novo mutations or uncertainty about parental diabetes). Table 1 compares the clinical features of monogenic, type 1 and type 2 diabetes.

Using clinical features to diagnose MODY

Clinical criteria for diagnosing MODY, which were proposed in the 1970s based on early families who clearly had a multigenerational form of young-onset, non-insulin-dependent diabetes, include age of onset at age <25 years, parental history of diabetes and evidence of endogenous secretion of insulin.[8] Although only about 50% of the diagnosed cases of MODY fit these criteria,[7,9] most probands referred for testing still have this classic history.

Using the Young Diabetes in Oxford study, we aimed to expand the criteria for selecting patients for genetic testing.[5] We chose characteristic features to distinguish MODY from common forms of diabetes: residual endogenous insulin secretion for at least 3 years after diagnosis in those labelled clinically as having type 1 diabetes and young age of onset and absence of metabolic syndrome in those assumed to have type 2 diabetes. Within these selected groups, 10–20% had MODY, showing that it is possible to select cases on the basis of simple clinical features and achieve reasonable rates of positive tests. Fewer than half of the cases would have been identified if the selection had been done on the basis of the 'classic criteria'. Overall, we found that 1% of patients clinically labelled as having type 1 diabetes and 4% of those with type 2 diabetes diagnosed when they were younger than 45 years had MODY.

Table 1. Features of the most frequent types of maturity-onset diabetes of the young (MODY) compared to common forms of diabetes.

Feature	Type 1 diabetes	Type 2 diabetes	GCK-MODY	HNF1A- and HNF4A-MODY
Typical age of onset (years)	10–30	>25	Fasting hyperglycaemia from birth	10–45
β-cell antibodies	>90% at diagnosis	Negative by definition	Rare	Rare
Diabetic ketoacidosis	Common	Rare	Not observed	Rare
Parental diabetes	10–15%	Common	Not always reported, but one parent has impaired fasting glucose (IFG) if tested	60–90%, depending on ascertainment criteria
C-peptide levels	Undetectable/low	Normal/high	Normal	Normal
Features of insulin resistance	Infrequent	Common	Infrequent	Infrequent
hsCRP levels	Normal	Often chronically elevated	Normal	Suppressed in HNF1A-MODY Normal in HNF4A-MODY
First-line treatment	Insulin	Metformin	Nil	Low-dose sulphonylurea

GCK = glucokinase; HNF1A = hepatocyte nuclear factor 1-alpha; HNF1β = hepatocyte nuclear factor 1-beta.

Using biomarkers to diagnose MODY

As clinical features are not a perfect way to select cases for genetic testing, it would be extremely advantageous to find a biomarker specific for a MODY subtype that could be used as a screening test. The transcription factors HNF1A and HNF4A are expressed in a number of other tissues, including the liver, gut and kidney. There could therefore be extrapancreatic features unique to a MODY subtype that are not seen in other forms of diabetes.

Candidate biomarkers were initially derived from animal models, human mutation carriers and bioinformatics approaches.[10] These approaches were largely unrewarding, with difficulties in terms of both replicating and finding a biomarker that was sufficiently sensitive and specific enough to identify cases of MODY.

C-reactive protein and HNF1A-MODY

A new avenue for generating candidate biomarkers came with the advent of genomewide association studies (GWAS) in 2007. A GWAS examines the common genetic variation associated with a phenotype of interest without any assumptions about candidacy. In 2008, two independent GWAS in 2008 reported that a common variation close to *HNF1A* caused small changes in levels of highly sensitive C-reactive protein (hsCRP) in serum. We hypothesised that an inactivating mutation such as that seen in MODY would be associated with much greater changes in hsCRP and that this might be used as a diagnostic marker.

There was biological support for this hypothesis, as HNF1A was known to regulate expression of CRP, which is downregulated in the liver of the Hnf1a knockout mouse. We tested this theory initially in a small number of local patients with MODY[11] and then the results were replicated in two large European studies,[12,13] including nearly 700 cases of HNF1A-MODY. The results showed that levels of hsCRP were significantly lower in those with *HNF1A* mutations compared with those with all other forms of diabetes and also compared with those without diabetes. This finding was reproducible across four common CRP assays and showed clinically valid discrimination from type 2 diabetes using receiver operating characteristic (ROC) curve analysis (C-statistic: 0.80 to 0.97). Low hsCRP can differentiate HNF1A-MODY cases from type 2 diabetes with high sensitivity and specificity: 70–80% of HNF1A-MODY cases will have an hsCRP <0.5 mg/l compared to <20% of cases of type 2 diabetes. A great advantage of hsCRP as a diagnostic screening tool is that it is widely available and cheap to measure and so has the potential to be translated rapidly into diagnostic pathways. The main downsides of hsCRP are that it is affected by concurrent inflammation and that differentiation from type 1 diabetes is less good than from type 2, because those with type 2 diabetes tend to have a higher CRP due to the presence of chronic, low-grade inflammation.

Glycan profile

Glycosylation is an important and ubiquitous process of post-translational modification of proteins via addition of sugar moieties. A GWAS performed in 2010 revealed that HNF1A was a key regulator of fucosylation – the attachment of fucose to glycoproteins.[14] In particular, HNF1A increased the availability of the fucose donor and upregulated the addition of fucose to the outer or antennary section of the glycoprotein. We hypothesised that those with *HNF1A* mutations might therefore have changes in the plasma glycan profile. In a preliminary study we showed that there was widescale alteration of plasma glycans and, as predicted, a decrease in the ratio of antennary to core fucosylation.[15] This was highly statistically significant in a large replication set and showed good discrimination from both type 1 and type 2 diabetes (C-statistic ≥0.90).[15] Glycan profile is less affected by inflammation than CRP but has the disadvantage that currently there is no high-throughput assay for glycans. This limits immediate translation as a screening test to identify patients at high risk of MODY.

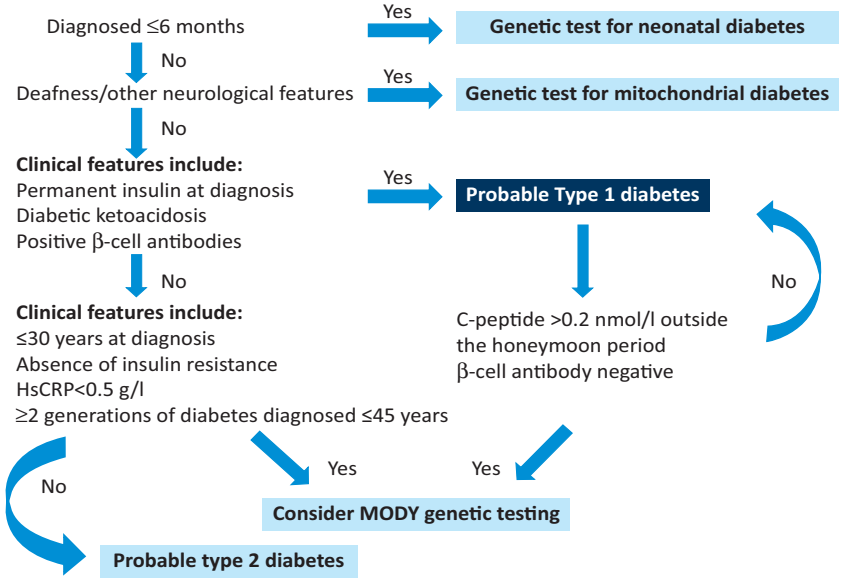

Fig 1. Investigation of diabetes diagnosed in patients aged <45 years. hsCRP = highly sensitive C-reactive protein; MODY = maturity-onset diabetes of the young.

Lessons from GWAS

The role of HNF1A in regulation of CRP and glycosylation is of interest outside the narrow area of diagnostics in monogenic diabetes. HNF1A regulates the expression of a large number of liver proteins involved with inflammatory pathways (including complement components, fibrinogen, plasminogen and α-1 antitrypsin).[16] Meanwhile, aberrant glycosylation is recognised to be of increasing importance in many disease states, including cancer, immune function and inflammation. The study of carriers of *HNF1A* mutations may reveal new insights into these essential physiological processes. Genomewide association studies thus have the capacity to inform us about not just the genetic aetiology of disease but also the underlying pathophysiology and potential therapeutic targets.

Conclusion

Systematic use of widened clinical criteria and specific biomarkers for subtypes of MODY (such as hsCRP) can help improve the current low rate of diagnosis of monogenic diabetes. Fig 1 suggests a diagnostic algorithm that could be used to identify which young adults with diabetes should be referred for MODY testing. The MODY testing centre in the UK is also piloting an online probability model,[17] which can be used to calculate the chance of an individual patient having MODY, with the centre's large database of known cases forming the 'gold standard'. Monogenic diabetes is one of the few areas of medicine in which personalised therapeutics is currently an option, so all children and young adults diagnosed with diabetes should have the opportunity to benefit.

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