What are the practical implications of developments in genetics?

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ABSTRACT In recent years there has been a rapid increase in our understanding of the genetics of diabetes and the implication of genetics for clinical care. The major clinical breakthrough has been in identifying monogenic forms of diabetes. It has now been established that knowing the genetic aetiology alters treatment. This is seen for maturity onset diabetes of the young (MODY) caused by mutations in the *HNF1*α and HNF4α genes (extreme sensitivity to sulphonylureas); mutations in the *glucokinase* gene (no treatment required); and for mutations in the potassium channel genes encoding Kir6.2 and SUR1, where patients with neonatal diabetes can be transferred off insulin with high-dose sulphonylurea treatment. For the more common types of diabetes the clinical impact of genetics has yet to be realised, yet with the dramatic improvements in genotyping/sequencing technologies available it is probably just a matter of time before genetic profiling will be used to predict diabetes risk, or to tailor therapy for the individual.

DECLARATION OF INTERESTS No conflict of interests declared.

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For many years the classical division of diabetes mellitus into type 1 and type 2 diabetes has served clinicians well. Those, usually young people, with an acute presentation had type 1 diabetes and required insulin. Those, usually older obese people, that did not have type 1 diabetes had type 2 diabetes. There were a few rare forms of diabetes known about, but it made no difference to treatment so these were rarely looked for.

The recent developments in the genetics of diabetes have provided considerable insight into the heterogeneity of diabetes, as well as the biology of β-cell dysfunction and insulin action. Initial advances were seen in 'monogenic diabetes', in which a single genetic defect causes diabetes, and it is these forms where there has been direct impact on clinical care and establishing the need for genetic testing in the diabetes clinic. More recently, there has been exponential progress in teasing out the genetics of complex type 1 and 2 diabetes. While this has revealed previously unconsidered pathways associated with diabetes, the potential clinical use of these findings has yet to be established. Therefore this review will focus primarily on monogenic diabetes, with just a brief overview of the developments in common types of diabetes.

NEONATAL DIABETES

Diabetes that presents before the age of six months is unlikely to be type 1 diabetes.[1–2] Infants with neonatal diabetes mellitus (NDM) have low birth weight due to low fetal insulin secretion, and usually develop insulin requiring diabetes in the first three months of life. Presentation is often with severe hyperglycaemia or diabetic ketoacidosis. Clinically, neonatal diabetes can be divided into whether the diabetes persists (permanent, PNDM, 45%) or resolves (transient, TNDM, 45%).[3] This clinical classification has been dissected by recent genetic developments. Transient NDM can now be almost entirely explained either by imprinting anomalies on chromosomal region 6q24, with the remainder due to K_{ATP} gene mutations.[4] Approximately 60% of PNDM can be explained, primarily by mutations in the K_{ATP} genes, but also by mutations in the insulin gene and homozygous mutations in glucokinase.[5–7] This review will focus on the K_{ATP} channel. (For a review of all NDM please refer to Shield.[8])

K_{ATP} channel mutations

The pancreatic β-cell secretes insulin in response to a variety of nutrient and other stimuli. The ATP-sensitive potassium (K_{ATP}) channel is a key step in this process of stimulus-secretion coupling, and consists of four Kir6.2 subunits (encoded by the *KCNJ11* gene) and four SUR1 subunits (encoded by the *ABCC8* gene). Inactivating mutations cause the channel to be closed and thus the β-cells to over-secrete insulin, causing hyperinsulinaemic hypoglycaemia.[9] Activating mutations cause the β-cell to be unresponsive to glucose and therefore are a cause of NDM.[6,10–11]

Activating K_{ATP} channel mutations cause PNDM, TNDM and neurological features

The phenotype seen with mutations in SUR1 and Kir6.2 is very similar. Some mutations cause TNDM and the diabetes remits at 35 weeks (range 2–208 weeks) and then relapses at five years of age (range 1–16 years).[4] Different mutations cause PNDM. About 20% of Kir6.2 mutations are associated with neurological features due to the expression of the K_{ATP} channel in the brain, peripheral nerves and muscle. Approximately 5% have a neurological syndrome that is characterised by severe Developmental delay, Epilepsy and Neonatal Diabetes (DEND). About 15% have an intermediate phenotype

(intermediate DEND or iDEND) with milder developmental delay and without epilepsy.[6] Due to the severity of presentation, often with diabetic ketoacidosis (DKA), and the fact that the majority have no detectable endogenous insulin secretion,[6,12] all patients with NDM, until recently, were initiated on insulin and those with PNDM remained insulin treated for life.

Insulin can be replaced by oral sulphonylureas in patients with NDM

The activating mutations in the K_{ATP} channel genes alter ATP sensitivity or gating of the channel, resulting in a lack of channel closure with a glucose stimulus.[6] Sulphonylureas are a drug class that has been used for more than 50 years to treat type 2 diabetes. These drugs act on the SUR1 subunit of the K_{ATP} channel to bring about channel closure. When three patients with Kir6.2 mutations were challenged with the intravenous sulphonylurea tolbutamide they produced measurable insulin secretion.[6] This paved the way to conversion off insulin to oral sulphonylurea therapy, first in one case[13] and then in a large series.[12] In this series, 90% of patients were able to transition from insulin to sulphonylurea, and every individual who did so improved their glycaemic control (Figure 1) (evidence level 2++).

Over and above the ability to transfer off insulin, what is striking about the glucose control in patients with PNDM treated with sulphonylureas is their near normoglycaemia and minimal hypoglycaemia. This reflects prandial regulation of insulin secretion, an effect that is mediated by sulphonylureas enabling the β-cell to respond to incretins.[12] For many patients who have successfully transferred to sulphonylureas, they have normal glycosylated haemoglobin (HbA_{1c}), can eat what they want, can do what they want and in essence are non-diabetic.

MATURITY ONSET DIABETES OF THE YOUNG

Maturity onset diabetes of the young (MODY) is non-insulin-requiring diabetes that presents in children or young adults and accounts for about 1–2% of diabetes. It has an autosomal dominant inheritance and hence there is often a strong family history of diabetes. Once again, genetics has divided a clinically defined condition into a number of aetiologically distinct subtypes. The most clinically relevant of these will be discussed. These are MODY due to mutations in the transcription factors hepatocyte nuclear factor (HNF) 1α, HNF4α and HNF1β; and the glycolytic enzyme glucokinase (reviewed in Stride and Hattersley[14]). The diabetes phenotype due to the transcription factor gene mutations is similar, with onset usually in adolescence or early adulthood, gradual progression requiring increasing treatment and ultimately insulin, and association with micro- and macrovascular complications. Glucokinase mutations, as will be discussed, cause stable, mild, non-progressive hyperglycaemia and are therefore quite distinct from the other MODY
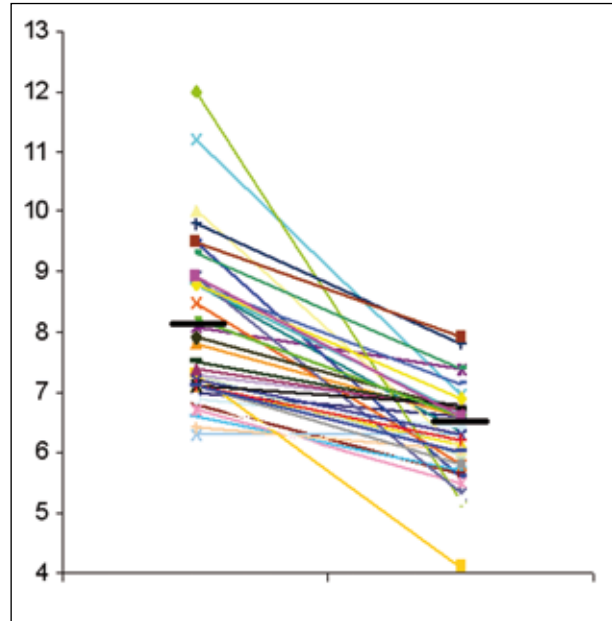
FIGURE 1 HbA_{1c} prior to and after successful transfer off insulin onto sulphonylureas in patients with neonatal diabetes due to Kir6.2 mutations.[12] (With kind permission of *The New England Journal of Medicine*.)

subtypes. Table 1 outlines the key features of the different MODY subtypes.

MODY due to HNF1α and HNF4α mutations are sensitive to sulphonylureas and can transfer off insulin

A series of case reports[15–17] prompted a randomised open-label crossover trial of metformin and sulphonylureas in patients with diabetes due to an HNF1α mutation and patients with type 2 diabetes matched for age and body mass index (BMI).[18] Treatment consisted of gliclazide for six weeks, followed by metformin for six weeks (or vice versa). The primary outcome was fasting glucose reduction from baseline. The results of this study showed dramatically greater response to sulphonylureas in patients with HNF1α mutations compared to their response to metformin and compared to response to gliclazide in patients with type 2 diabetes (Figure 2) (evidence level 1+). The mechanism for this sensitivity reflects the fact that sulphonylureas act downstream of the major defects seen with HNF1α mutations so a patient with an HNF1α mutation is essentially blind to glucose but sensitive to sulphonylureas. Although there are no such robust data for patients with HNF4α similar sulphonylurea sensitivity has been described[19] (evidence level 3).

Because of the marked sensitivity to sulphonylureas in HNF1α diabetes, patients who had been misdiagnosed as having type 1 diabetes and were insulin treated, but who subsequently were found to have an HNF1α mutation were trialled with sulphonylureas.[20] All 34 patients successfully transferred off insulin, some with

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TABLE 1 Features of the MODY subtypes (adapted from Hattersley A et al.[39])			
	Age of onset (range)	Diabetes characteristics	Other features
HNF1α (MODY 3)	14 (4–18)	• Progressive • Sulphonylurea sensitivity	• Low renal threshold for glucose • Normal birthweight
HNF4α (MODY 4)	17 (5–18)	• Progressive (like HNF1α) • Sulphonylurea sensitivity	• Hyperinsulinaemic hypoglycaemia • Macrosomia and increased birth weight
HNF1β (MODY 5)	26 (10–61)	• Diabetes only present in 50% of mutation carriers • Rapidly progresses to insulin • Insulin resistance as well as β-cell dysfunction	• Low birth weight • Renal cysts • Genital tract malformation • Hyperuricaemia • Elevated alanine aminotransferase • Pancreatic exocrine dysfunction • Absent pancreatic body and tail
GCK (MODY 2)	When tested (present from birth)	Stable non-progressive fasting hyperglycaemia	• Fasting glucose 5.5–8 mmol/l • Small increment at oral glucose tolerance test (2 hours increment <3.5 mmol/l)

over 30 years of being 'insulin-dependent' (evidence level 2+). However, 10 patients subsequently had insulin reintroduced. The 24 remaining off insulin remained off for between 17 and 90 months; of these 80% achieved an HbA$_{1c}$ of <7.5% or a >1% reduction.[20]

The decision to be made clinically when treating non-type 1 diabetes is what oral agent to prescribe. In type 2 diabetes, metformin and sulphonylureas have similar efficacy,[21] but metformin is usually favoured because of its macrovascular benefit and weight neutrality. However, if a patient is known to have an HNF1α mutation, even if they are phenotypically indistinct from someone with type 2 diabetes, the randomised trial[18] would support the use of sulphonylureas as first-line agent in this patient group (A grade), as 62% responded at least two-fold better to gliclazide than they did to metformin. This contrasts with just 16% in those with type 2 diabetes. In addition, knowing someone has an HNF1α mutation should prompt an attempt at transfer off insulin to sulphonylureas in patients who have not previously failed on a sulphonylurea (C grade). This is the first robust evidence for genetics impacting on the clinical therapeutic management of diabetes.

HNF4α mutations cause macrosomia and neonatal hyperinsulinaemic hypoglycaemia

Although the diabetes phenotype due to HNF4α mutations is similar to that due to HNF1α mutations, HNF4α mutations cause hyperinsulinaemia in utero, and in some this also manifests as hyperinsulinaemic hypoglycaemia in the neonatal period[22] (evidence level 2+). In the UK series, the affected offspring birth weight was 790 g heavier than the unaffected (no HNF4α mutation) offspring, and as a result there was greater extreme macrosomia (>5 kg birth weight) and two cases of Erb's palsy or shoulder dystocia.[22] Transient hypoglycaemia was reported in 8/54 infants with heterozygous HNF4α mutations, but was reported in none of 54 non-mutation carriers (p<0.003). There was

documented hyperinsulinaemia in three cases. The hypoglycaemia was diazoxide responsive and resolved in all cases by one year of age. The mechanism for this paradoxical oversecretion in utero, and subsequent hypoinsulinaemia causing diabetes in later life has still to be explained. However, clinically, birth weight can be used to guide genetic testing (a birth weight >4.4 kg is 80% specific for an HNF4α mutation compared with an HNF1α mutation[23]) and neonates with hyperinsulinaemia and a family history of diabetes should be tested for an HNF4α mutation (D grade).

Glucokinase mutations are a common cause of incidental hyperglycaemia, and do not require treatment

Glucokinase catalyses the first step in glycolysis and is rate limiting. The kinetics of this enzyme make it able to alter flux through glycolysis according to the glucose
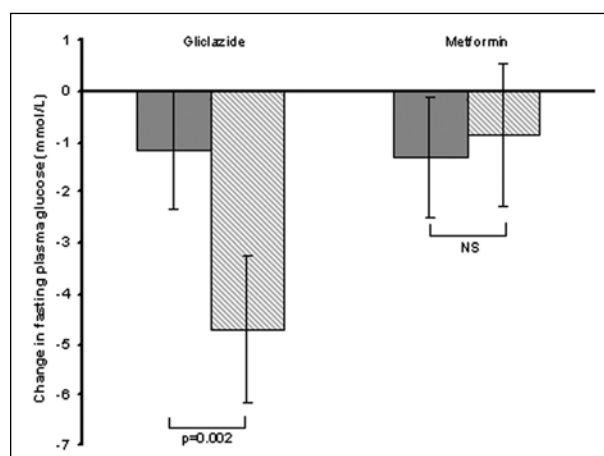
FIGURE 2 Fasting glucose reduction achieved by gliclazide and metformin in patients with HNF1α mutations and patients with type 2 diabetes.[18] Diagonal shaded bars represent HNF1α patients; solid bars are type 2 diabetic patients. (With kind permission of *The Lancet*.)

concentration, and thus it acts as the pancreatic glucose sensor. Heterozygous inactivating mutations in glucokinase cause a shift in the glucose response curve, resulting in patients having a raised fasting glucose. However, given sufficient stimulus, the β-cell will maximally secrete insulin, and thus the post-prandial glucose rise is small. So classically patients with MODY due to a glucokinase mutation will have a high fasting glucose (>5.5 mmol/l) but a small increment at 2 hours after a 75 g oral glucose challenge (<3.5 mmol/l) (evidence level 2+).[24] As a result of this ability to respond to meals, the HbA_{1c} in patients with glucokinase mutations is usually normal or only slightly elevated, and hence the risk of microvascular disease is low.[25] Treatment is not indicated, and in fact the use of insulin or oral agents has little impact on glycaemia.

In a Czech study of 60 paediatric patients referred to secondary care with asymptomatic hyperglycaemia who had persistent hyperglycaemia on retesting, 35 (68%) were found to have glucokinase mutations, two had an HNF1α mutation, one had an HNF4α mutation and one a Kir6.2 mutation. Eleven had type 1 diabetes[26] (evidence level 2+). This study contrasts with a study in an older population, aged 30–70 years, with an increased diabetes risk and with fasting hyperglycaemia (5.5–7.7 mmol/l), where the prevalence of glucokinase mutations was just 5/658 patients.[27] Thus in this paediatric population, where the prior probability of finding a monogenic cause is high, there is a higher prevalence of glucokinase mutations, whereas in the older population where type 2 diabetes is much more likely, fasting hyperglycaemia or a small increment at oral glucose tolerance testing are not discriminatory.

TYPE 1 AND TYPE 2 DIABETES

After many years with little progress, the acceleration in affordable genotyping technology and advances in our understanding of genetic architecture have prompted a flurry of exciting publications. These report in excess of 20 robustly replicated loci for type 2 diabetes[28] (evidence level 1++) and 18 for type 1 diabetes[29] (evidence level 1++). The main output of these discoveries has been that novel pathways have been identified for diabetes, and that there is a considerable overlap between different diseases (e.g. type 2 diabetes and prostate cancer,[30] type 1 diabetes, coeliac disease and rheumatoid arthritis[31]. A full discussion of this is beyond the scope of this review).

Considerable effort is under way to translate these genetic findings into an understanding of the biological mechanism. It is likely that the greatest clinical benefit of genetics of common diabetes will be in the development of novel therapies. However, what remains uncertain is the direct clinical utility of genetics of type 1 and 2 diabetes. While a number of groups have shown that combining all the known type 2 diabetes genes can be

used to predict disease, this is of little added value to traditional risk factors such as BMI, age and family history. To assess predictive utility a receiving operating curve (ROC) is usually plotted, where the area under the ROC (AUROC) is a measure of predictive ability (50% being random, 80–85% the level thought to be clinical useful to predict disease). In a study of two populations (the Botnia study and the Malmö Preventive Project) where longitudinal data was available on the development of diabetes, the AUROC for phenotype alone was 0.74, and this was only increased to 0.75 (p=1*10^{-4}) by the inclusion of genotype at 16 polymorphisms.[32] Even in type 1 diabetes where the sibling relative risk (λ_s) is much greater than in type 2 diabetes (15 vs 3), the utility of genetic testing has still to be established.[33] One problem is that all the loci identified only add up to a small fraction of the heritability of type 1 or type 2 diabetes. The current hope is that this 'missing heritability' consists of multiple rare variants with large effect, and identifying these may enhance prediction.

Another area of hope where genetics might deliver is in pharmacogenetics of type 2 diabetes, a field that also is accelerating fast.[34] However, once again, although the discoveries help unravel drug mechanism, they lack clinical utility due to small effect sizes. In a recent study, 6% of the population who carry two loss-of-function polymorphisms in *CYP2C9*, encoding the key enzyme that metabolises sulphonylureas, were 3.4 times less likely to achieve a treatment HbA_{1c} of <7%.[35] This is the largest pharmacogenetic effect described to date for diabetes drugs, yet the added predictive value of genotype was minimal, and there remains considerable unexplained variation in individual response to diabetes drugs – an area where rare variant discovery might contribute. The field where pharmacogenetics is impacting on clinical medicine is in its utility to predict very rare but extremely severe adverse drug reactions, with the paradigm being that of HLA-B*5701 and abacavir hypersensitivity.[36] In diabetes there are not such severe adverse reactions, but the ability to predict metformin intolerance, thiazolidinedione-induced oedema or hypoglycaemia with sulphonylureas is an area where genetics may begin to play a role in the clinic.

PRACTICAL IMPLICATIONS
Neonatal diabetes

As 60% of permanent neonatal diabetes and 26% of transient neonatal diabetes are due to K_{ATP} channel mutations and 90% of these are able to be treated with sulphonylureas with near normoglycaemia, then:

- All infants, children or adults who develop(ed) diabetes before six months of age should have genetic testing (grade B);
- All infants who have a K_{ATP} channel gene mutation should be trialled with high-dose sulphonylurea (grade B).

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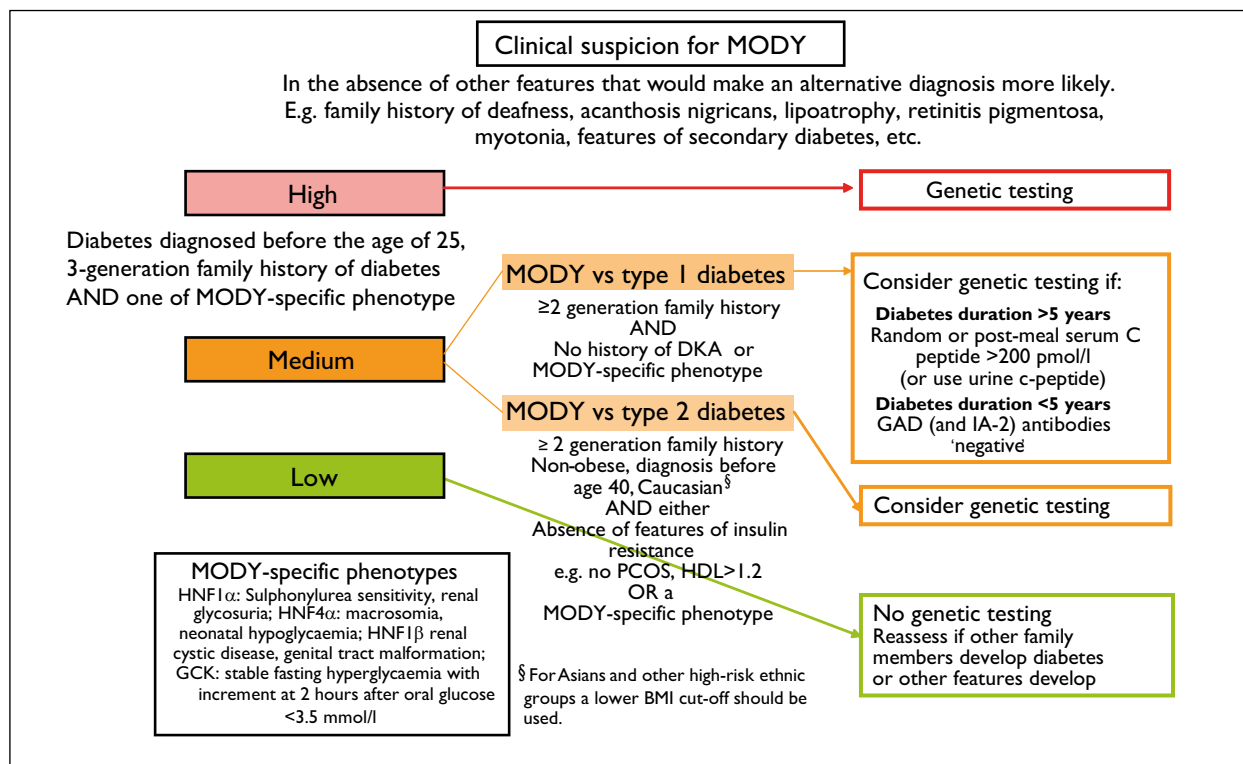
FIGURE 3 A flow chart depicting an approach to deciding whether to screen for MODY, either by direct genetic testing or by testing C-peptide and pancreatic autoantibodies.

Genetic testing should be carried out before considering a trial of sulphonylureas as high doses of sulphonylurea are often required (doses up to five times the maximal adult dose/kg have been used), and knowing that an individual has a K_{ATP} channel mutation will provide confidence in progressing up to such doses before seeing an insulin reduction. Genetic testing for neonatal diabetes is currently free of charge (www.diabetesgenes.org.uk).

Since awareness of neonatal diabetes as a distinct entity has increased, the estimated prevalence has risen and is now estimated at one in 200,000 to 260,000 live births,[37] suggesting there should be about 15–20 cases in Scotland, and about 200–250 in the UK as a whole. A relative lack of adults currently diagnosed suggests that the adult population is a considerable source of undiagnosed neonatal diabetes, and as adults with K_{ATP} channel mutations can still successfully transfer off insulin, efforts should be made by adult diabetologists to identify these people within the 'type 1' patients in secondary care (grade D).

There is increasing, albeit anecdotal, evidence[38] that treating early with glibenclamide can reverse or even prevent the neurological features of iDEND, and therefore all children with a confirmed K_{ATP} channel mutation should be started on a sulphonylurea as soon as possible, and genetic testing should not be delayed (grade D).

Identifying MODY

The classical defining MODY criteria are of a three-generation family history, an age of onset in one family member before 25 and non-insulin-requiring diabetes. Sequencing MODY genes should be considered in families who fulfil these criteria, with specific features being used to guide the gene to be sequenced (see Table 1). For example:

- Increased birth weight – HNF4α
- Renal glycosuria – HNF1α
- Renal cysts – HNF1β

For further information, please refer to the 'Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young'[23] or the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines for diagnosis and management of monogenic diabetes.[39]

Recommendation: Sequence MODY genes in families that fulfil classic MODY criteria (grade D).

Additional clinical criteria may also suggest MODY in patients who do not fulfil the above criteria. Testing pancreatic autoantibodies and C-peptide can be helpful. A suggested approach to identify MODY in the diabetes clinic is shown in Figure 3.

Pancreatic autoantibodies

Type 1 diabetes is defined by the presence of autoimmune destruction of the β-cells, and this is characterised by

detectable pancreatic autoantibodies at diagnosis. A number of autoantibodies can be measured, and if four antibodies are measured (to glutamate decarboxylase, [GAD-65], islet antigen 2 [IA-2], insulin and ZnT8A), only 2–4% of patients are autoantibody-negative.[40] Currently both IA-2 and GAD antibody testing is sensitive and specific, although only GAD antibody testing is routinely available.[41] Where possible, both IA-2 and GAD antibodies should be tested in all individuals presenting with presumed type 1 diabetes, if only to confirm clinical diagnosis. If these antibodies are present in low titre (negative), an alternative diagnosis such as MODY should be considered, although, particularly if only GAD antibodies were tested, type 1 diabetes cannot be excluded. It should be emphasised that whatever the ultimate diagnosis, a clinical presentation with ketosis and severe hyperglycaemia requires insulin treatment to reverse the metabolic abnormalities.

Recommendations:
- Reconsider a diagnosis of type 1 diabetes if GAD (and IA-2) antibodies are negative (grade D);
- If in doubt, treat with insulin initially as this is safe and effective. Insulin treatment can be withdrawn once a diagnosis is made (grade D).

C-peptide

The C-peptide is cleaved in proinsulin processing, and is co-secreted with insulin. It is thus a marker of endogenous insulin secretion and is particularly useful when a patient is insulin treated, as the serum insulin assay cannot be used. Five years after diagnosis of type 1 diabetes in the Diabetes Control and Complications Trial, 0% of adolescents and only 11% of adults had measurable C-peptide (evidence level 2+).[42] So if MODY is queried more than five years after diagnosis of 'type 1 diabetes', the persistence of C-peptide makes a diagnosis of type 1 diabetes unlikely and other causes should be considered. C-peptide needs to reach the laboratory within one hour and thus is not suitable for primary care; however, urinary C-peptide is stable and the urinary C-peptide–creatinine ratio may prove to be useful[43] but is not yet routinely available.

Recommendation: In patients where non-type 1 diabetes is suspected over five years from diagnosis, measure C-peptide (when glucose >8 mmol/l). Persistence of C-peptide after five years of diabetes makes type 1 diabetes unlikely (grade D).

Incidental hyperglycaemia

As highlighted above, 68% of children selected purely on persistent asymptomatic hyperglycaemia had a glucokinase mutation, yet family history of diabetes was not an inclusion criterion for glucokinase sequencing.

Recommendation: Asymptomatic hyperglycaemia that does not progress in children and young adults should prompt sequencing for glucokinase mutations (grade C).

Treatment choice

Recommendations:
- Patients with an HNF1α mutation should be treated with a low-dose sulphonylurea first line (grade A);
- Patients with and HNF4α mutation should be treated with a low-dose sulphonylurea first line (grade D);
- Patients with a glucokinase mutation do not require treatment (grade D).

Prevalence of MODY and availability of testing

There is considerable variation in the number of MODY patients by region in the UK. All data that follow are provided by the Exeter referral lab (Shields, Hattersley, Ellard; personal communication). The highest number of MODY patients is seen in the southwest of England, with a prevalence of 49 per million population. This is likely to reflect the strong research and clinical interest in MODY based in Exeter. Beyond this there is a clear difference in number by country, with Scotland having the highest number of MODY patients per population (27 per million). England has 20 per million, Wales 13 per million and Northern Ireland 5 per million (evidence level 3). There are two probable explanations for this. Firstly, in an initiative set up by the Exeter team, 18 diabetes specialist nurses received additional training on genetic forms of diabetes with an aim to increase and update the knowledge of the local diabetes teams. These Genetic Diabetes Nurses were originally funded within England and Scotland but not in Wales or Northern Ireland. Another barrier to referral for genetic testing is cost and specifically which budget is used to meet these costs. Some primary care trusts have not agreed to meet these costs in England. In Scotland, with the highest referral rate and highest number of MODY patients identified, the cost for genetic testing is met centrally.

Recommendation: Increased awareness and reduced barriers to genetic testing increase detection of MODY. All patients should have similar access to genetic testing irrespective of their geographical location in the UK (grade D).

SUMMARY

In the past five years there have been considerable developments in diabetes genetics. In type 1 and type 2 diabetes the exciting new discoveries are shedding new light on biological mechanisms of disease, but have yet to impact directly on clinical care. In monogenic diabetes the progress has been in translating the genetic discoveries into clinical care, and establishing that knowing the genetic aetiology of diabetes determines the treatment choice. With this established, the critical next step is to fully incorporate genetic testing into routine care.

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