Molecular genetics goes to the diabetes clinic

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ABSTRACT – Diabetes has historically been thought of as a medical specialty which primarily deals with treatment rather than diagnosis. Molecular genetic testing can now be used to make a diagnosis of the 1–2% of all diabetic patients with monogenic diabetes. Making a diagnosis of monogenic diabetes is important as it can have a dramatic effect on the treatment a patient should receive: glucokinase MODY patients need no treatment; HNF1α MODY patients are very sensitive to low dose sulphonylureas; and patients with neonatal diabetes due to Kir6.2 mutations, despite being insulin dependent, can discontinue insulin and be well controlled on high dose sulphonylurea tablets. The challenge for diabetologists is to use clinical skills to detect these monogenic patients whose care will be greatly helped by the treatment changes that follow molecular genetic testing.

KEY WORDS: diabetes, GCK, genetic testing, HNF1α, Kir6.2, MODY, pharmacogenetics

Diagnostic dilemmas in the young adult with diabetes

Diabetes has historically been thought of as a medical specialty which primarily deals with treatment rather than diagnosis. A diagnosis of diabetes is simply made by assessing if the patient has glycaemia levels above agreed standards defined by association with diabetes-specific complications in epidemiological studies. Subsequent division into the two largest diagnostic categories, type 1 and type 2, is usually made on the basis of simple clinical criteria such as age of onset, whether the patient is obese and the presence of ketoacidosis. Into which of these two categories a young adult is classified will make a fundamental difference to their management as they will either be treated with diet and exercise and then go on to metformin or they will immediately start on insulin. Despite the clear different therapeutic outcomes, there are limited clinical guidelines on how to diagnose these two major subgroups or how to recognise patients who have neither type 1 nor type 2 diabetes but have a defined genetic aetiology.

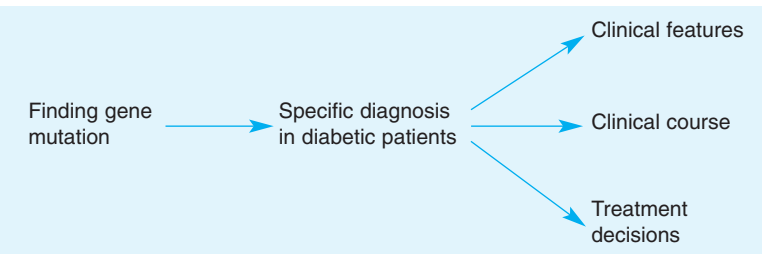

Fig 1. Finding a gene mutation can make a diagnosis of monogenic diabetes, with implications for clinical features, prognosis and treatment.

Molecular genetics as a diagnostic test in diabetes

Is there any role for molecular genetics in the difficult decisions about classification and treatment of children and young adults diagnosed with diabetes? Considerable advances have been made in our understanding of the genetics of type 1 and type 2 diabetes, but gene variants only *predispose* to these polygenic conditions and cannot be used in diagnostic testing. In contrast, in monogenic diabetes a mutation in a single gene *causes* diabetes, so genetic testing can potentially have an important role in the diagnosis. In these conditions a single base change often results in the diabetes phenotype, so it is possible to make a specific diagnosis by sequencing. This becomes important if it helps explain associated clinical features, anticipate the patient's prognosis and alter treatment decisions (Fig 1). The emphasis in this article will be on how a molecular genetic diagnosis can influence treatment decisions in maturity-onset diabetes of the young (MODY) and neonatal diabetes.

Maturity-onset diabetes of the young: moving from clinical diagnosis to molecular classification

With the advances in the last two decades in molecular genetic methodology it has been possible to define the underlying gene or genes in most clinically recognised subgroups of monogenic diabetes. In many cases this has altered the understanding of a clinically defined condition. One of the clearest and commonest examples is MODY, first recognised by Tattersall in 1974 as autosomal, dominantly inherited beta cell dysfunction resulting in early-onset, non-insulin dependent diabetes, typically before the age of 25.[1] Six different genes have now been identified:

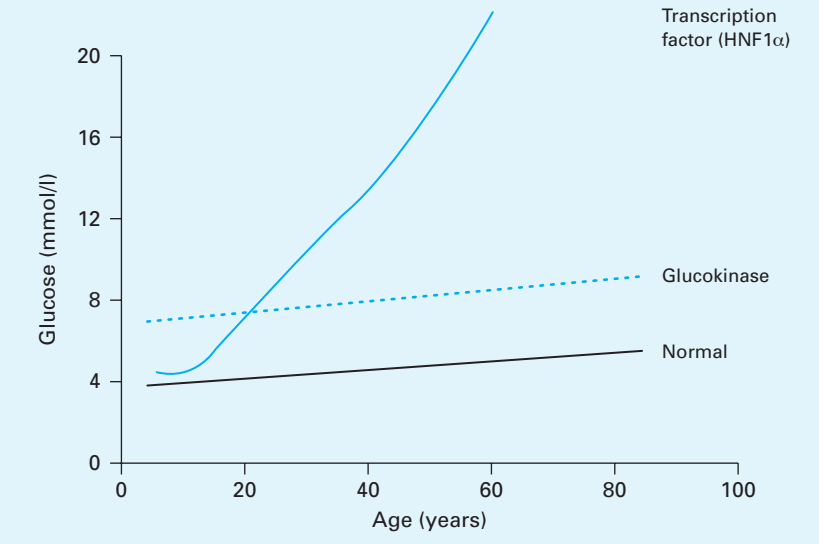

Fig 2. Diagram showing the variation of fasting glucose with age in normals and patients with glucokinase and transcription factor (usually hepatic nuclear factor (HNF)1α mutations.

- glycolytic enzyme glucokinase, and
- beta cell transcription factors: hepatic nuclear factor (HNF)1α, HNF4α, HNF1β, insulin promoter factor 1, and NeuroD1.[2,3]

There are marked differences between the diabetes caused by the different genes, but it was not recognised that there were discrete clinical entities until the genes were defined.[2,4] Patients with glucokinase mutations have mild fasting hyperglycaemia from birth; this deteriorates very little with age, pharmacological treatment is rarely required and it is only occasionally associated with microvascular complications (Fig 2).[4]

In contrast, patients with transcription factor mutations (most commonly HNF1α) are born normoglycaemic. There is then progressive hyperglycaemia – which results in diabetes being diagnosed in adolescence or young adult life – continuing thereafter to become more markedly severe (Fig 2).[4] Almost all patients will require pharmacological treatment. If hyperglycaemia is not adequately treated, complications are frequent.

The features of the two main subgroups of MODY are as marked as the differences between type 1 and type 2 diabetes (Fig 3).

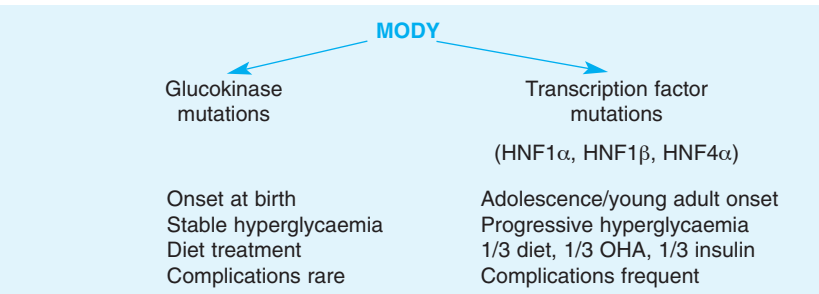

Fig 3. Clinical features of the two major subgroups of maturity onset diabetes of the young (MODY) (OHA = oral hypoglycaemic agent).

Making a molecular genetic diagnosis of a glucokinase mutation can allow treatment to be discontinued

The stable, mild fasting hyperglycaemia in patients with glucokinase mutations rarely results in osmotic symptoms. These patients typically present when their blood glucose is tested incidentally or during screening (eg in pregnancy, hospital admissions or routine medicals).[5] The clinical diagnosis frequently depends on the age at diagnosis:

- children who are slim are often thought to be in the early stages of type 1 diabetes
- patients diagnosed when pregnant are classified as gestational diabetes, and
- older patients are diagnosed as having type 2 diabetes.

The different diagnostic labels given to a disease with a single aetiology and prognosis often impacts on the patient's management. Those diagnosed with type 1 diabetes are treated with insulin. Making a molecular genetic diagnosis of a glucokinase mutation can result in the physician being confident that the child can stop insulin treatment immediately without any significant change in their glycaemic control, resulting in a dramatic reduction in hospital appointments and glucose monitoring.

Evidence of pharmacogenetics in MODY

HNF1α MODY is the most common form of monogenic diabetes and one of the commonest monogenic disorders, with over 20,000 people affected in the UK. Depending on the age of diagnosis, the subjects are frequently misdiagnosed as type 1 or type 2 diabetes. This is because it is uncommon to perform confirmatory tests for type 1 or type 2 diabetes (beta cell autoantibodies and C-peptide measurement) and the significance of the strong autosomal dominant family history is often not appreciated. It could be argued that if the patient is appropriately treated, the failure to make a diagnosis is only of academic interest. This clearly would be true if the patients responded to oral therapy in a similar way to type 2 patients and if glycaemic control achieved on insulin was better or similar to that achieved on tablets. Recent work has questioned both these assumptions.

Sulphonylureas in HNF1a MODY

HNF1α MODY patients can be extremely sensitive to the hypoglycaemic effects of sulphonylureas given in standard doses.[6] Their glycaemic control can markedly deteriorate when they are switched from a sulphonylurea to an equivalent dose of metformin, only to improve again on reintroducing the sulphonylurea (Fig 4).[6] This is

in marked contrast to type 2 diabetes where meta-analysis showed sulphonylureas and metformin have a similar hypoglycaemic effect.

In a recent randomised crossover study, HNF1α patients had a four-fold greater response to the sulphonylurea gliclazide than body mass index and glycaemia matched type 2 diabetes patients (Fig 5).[7] Physiological tests showed that this pharmacogenetic response was not due to altered drug metabolism but to greater insulin secretion in response to the drug, this effect being amplified by increased insulin sensitivity.[7] Animal and cellular models with reduced HNF1α levels suggest the defects in the beta cell are early in glucose metabolism before the sulphonylurea receptor in the K_{ATP} channel, explaining why the insulin secretion to sulphonylureas is relatively well preserved.[7]

This result has clear practical implications. Metformin has become the first-line pharmacological treatment for type 2 diabetes. Making a diagnosis of HNF1α will therefore change the recommended initial drug. Many patients with both HNF1α and HNF4α mutations have been well maintained on sulphonylureas for decades. The most dramatic response has been in patients with an HNF1α mutation misdiagnosed as type 1 diabetes, and hence treated with insulin from diagnosis.[8] These patients can transfer to sulphonylureas with no deterioration in glycaemic control. At first sight this might appear to give little benefit as one effective treatment has been changed for another. However, patients report a dramatic change, with an enormous impact on their quality of life.[9] Many patients have been successfully treated with sulphonylureas for over 25 years; not only is good glycaemic control maintained but there are also reduced risks of hypoglycaemia compared with insulin treatment.

Mutations in the gene encoding Kir6.2 cause neonatal diabetes

There have been recent advances in the genetics and treatment of neonatal diabetes which is usually diagnosed in the first three months of life. There are two forms of neonatal diabetes:

- transient neonatal diabetes mellitus (TNDM) which resolves, usually within three months, and

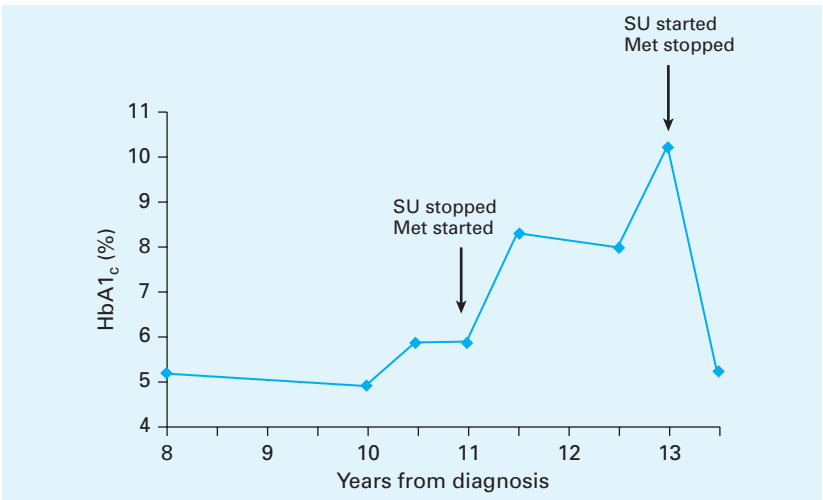

Fig 4. Sulphonylurea (SU) sensitivity in a patient with an hepatic nuclear factor (HNF)1α mutation shown by deterioration in glycosylated haemoglobin ($HbA1_c$) on stopping SUs and starting metformin (Met) and improvement of $HbA1_c$ on restarting SUs and discontinuing Met (adapted from Ref 6).

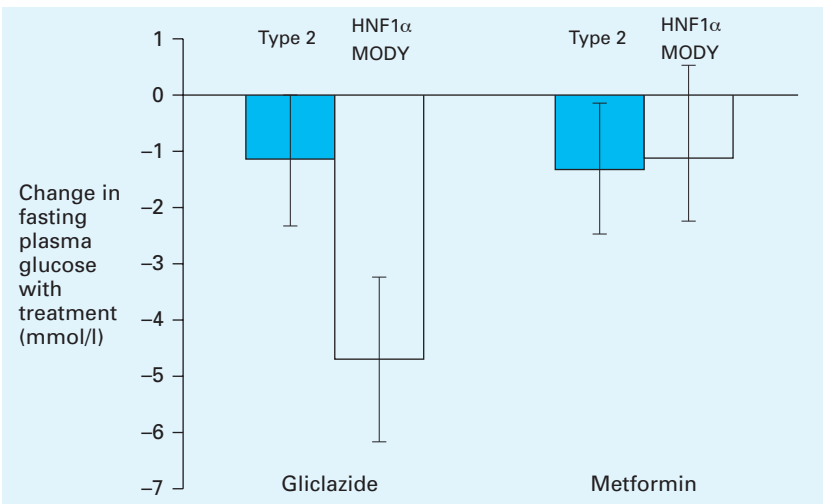

Fig 5. In a randomised crossover trial, hepatic nuclear factor (HNF)1α MODY patients had a four-fold greater fall in fasting glucose on gliclazide than body mass index and glycaemia matched type 2 patients (shown on the left), while the response to metformin was similar (shown on the right) (MODY = maturity-onset diabetes of the young) (adapted from Ref 7).

- permanent neonatal diabetes mellitus (PNDM) which may require lifelong insulin.

A few rare causes of PNDM were described before 2004 but a genetic cause was not defined in over 90% of patients. A candidate gene approach led to mutations in the gene encoding the Kir6.2 subunit of the beta cell K_{ATP} channel being identified in 31–64% of patients with PNDM.[10,11,13] These mutations are heterozygous, activating mutations which result in the K_{ATP} channel failing to close in the presence of ATP. This results in a large influx of potassium which holds the cell membrane of the beta cell in a hyperpolarised state, preventing insulin secretion. Most of these mutations are spontaneous, which explains why an autosomal dominant family history was seen in only a minority of cases.

Clinical features associated with mutations in Kir6.2

Some interesting clinical features are associated with these mutations.[10,13] Low birth weight is a common feature, with 61% below the 3rd centile, reflecting the very low insulin secretion *in utero* by the fetus which results in reduced insulin-mediated growth. The median age of diagnosis of diabetes is seven weeks (range birth to six months). Most patients are markedly hyperglycaemic, some with ketoacidosis. Investigations and the insulin dose required are in keeping with no or minimal endogenous insulin secretion. These patients differ from type 1 diabetes in that pancreatic autoantibodies are not detectable and they are diagnosed before six months of age.

DEND syndrome

Although most patients with Kir6.2 mutations have isolated diabetes, some patients have multisystem disease, termed DEND syndrome (**d**evelopmental delay, **e**pilepsy and **n**eonatal **d**iabetes).[10,13] The developmental delay can be extremely severe, with patients unable to stand independently or speak even in early adulthood. Severe cases have generalised epilepsy diagnosed under the age of 12 months. These neurological features make the management of their diabetes with insulin extremely difficult. The pattern of disease is in keeping with the distribution of the Kir6.2 subunit of the K_{ATP} channel: as well as being present in the beta cells it is also in the brain, peripheral axons and skeletal muscle. Altered action at these sites is likely to be responsible for the neurological features.

Fortunately, the full DEND syndrome is relatively rare, but there is a more common, intermediate subgroup of patients with less severe developmental delay and neonatal diabetes but who do not have epilepsy.

Other mutations

Activating mutations do not always cause permanent neonatal diabetes. A minority of patients with mutations in the Kir6.2 gene have transient neonatal diabetes which resolves around the age of two years. Finally, a common polymorphism, E23K, present in approximately 40% of the population, predisposes to type 2 diabetes, increasing the risk by approximately 20%.

From genotype to functional abnormality to phenotype in neonatal diabetes

A striking feature is a strong genotype/phenotype relationship in Kir6.2: the vast majority of patients with mutations at R201 have isolated neonatal diabetes, whilst most patients with mutations at V59 have neurological features. Structural models show mutations at the R201 residue directly interfere with ATP binding to the Kir6.2 channel. The mutations that result in a more severe phenotype are in regions of the molecule involved in changing the conformation of the channel: the slide helix and the pore of the K_{ATP} channel. Functional studies show that the severity of the clinical phenotype is reflected in the functional changes seen in the mutated channel.[12] Therefore, the current remaining in the presence of ATP is reduced in all the disease associated mutations, but the severity of the reduction is less in TNDM than in PNDM which, in turn, is less than in DEND syndrome.[12,13] The clinical severity associated with most patients in Kir6.2 therefore directly reflects the functional severity of these mutations (Fig 6).

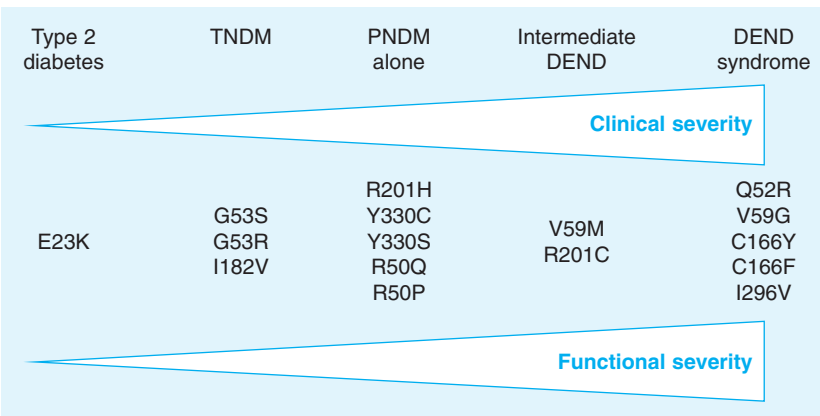

Fig 6. Clinical spectrum associated with specific Kir6.2 mutations and the polymorphisms (E23K) reflects the functional severity of the mutations *in vitro* (DEND syndrome = **d**evelopmental delay, **e**pilepsy and **n**eonatal **d**iabetes; PNDM = permanent neonatal diabetes mellitus; TNDM = transient neonatal diabetes mellitus).

From gene to treatment in neonatal diabetes

Defining the genetic aetiology has the greatest impact if it improves treatment for patients. As soon as activating Kir6.2 mutations were found to cause PNDM, knowledge of the K_{ATP} channel suggested that sulphonylurea tablets might offer an alternative to insulin injections.

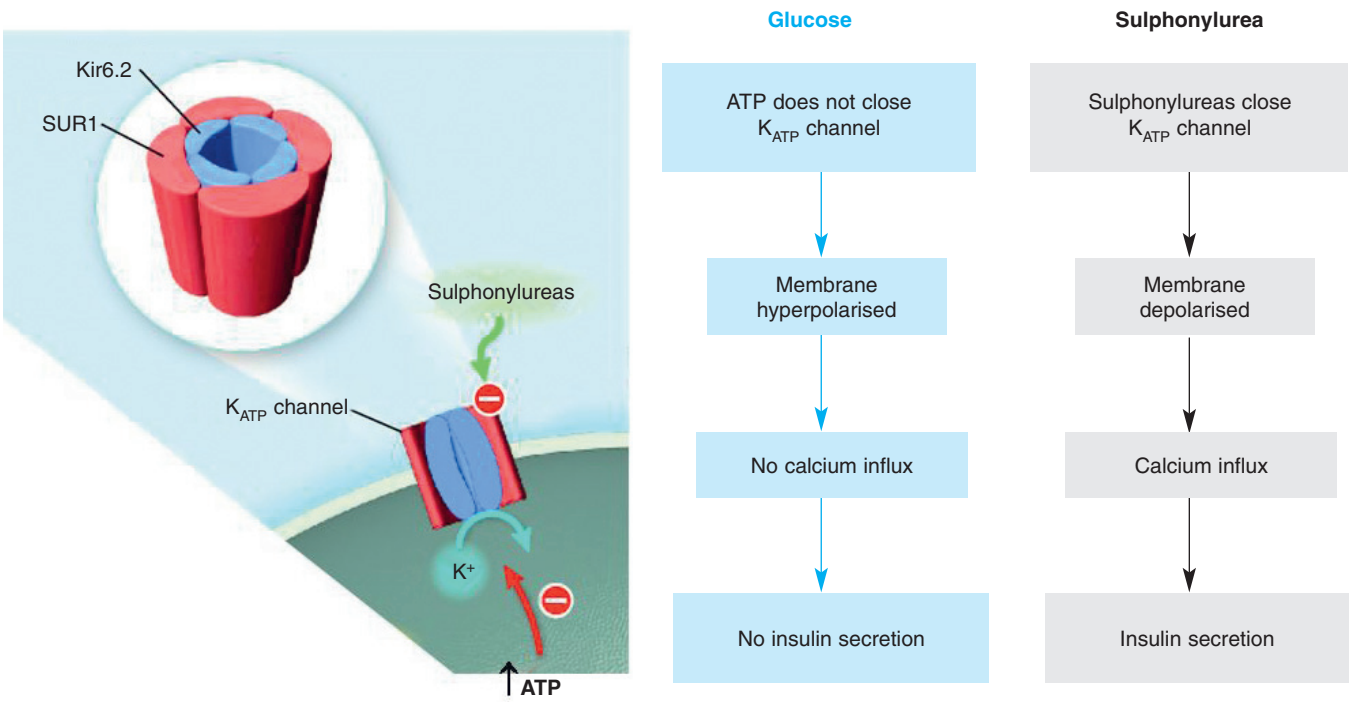

Fig 7. Diagram showing that patients with a mutation in Kir6.2 cannot close the K_{ATP} channel and hence secrete insulin in the presence of increased ATP, but can secrete insulin when the channel is closed independently of ATP by sulphonylureas (adapted from Ref 10).

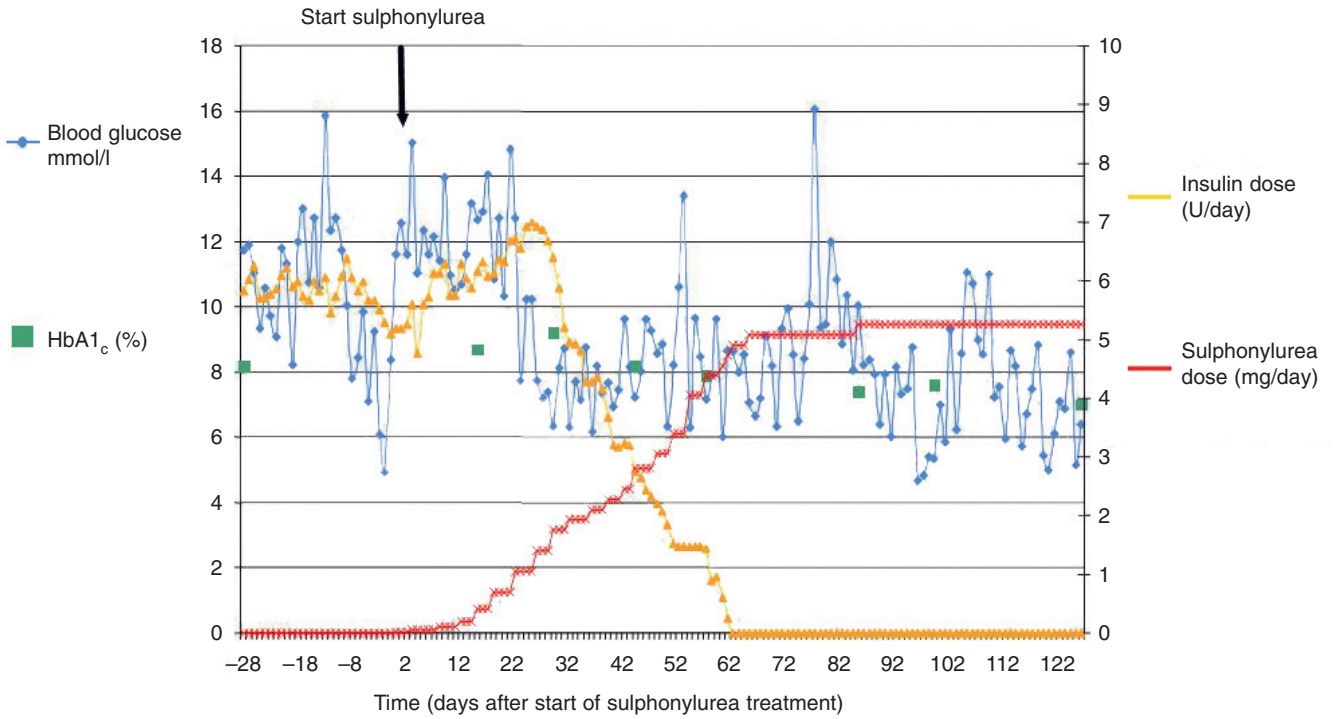

Fig 8. Sulphonylurea treatment in an apparently insulin-dependent infant with permanent neonatal diabetes mellitus (PNDM) caused by a mutation in Kir6.2, showing measurements of capillary blood glucose (mean of 4–6 daily measurements) (blue), glycosylated haemoglobin (HbA_{1c}) (green), administered dose of sulphonylurea (red) and insulin requirement (orange). The arrow indicates initiation of oral sulphonylurea (glibenclamide). The dose was increased every three days, with a parallel reduction in the insulin dose according to capillary glucose measurements made at home. As revealed by HbA_{1c} measurements, the metabolic control did not deteriorate after insulin was discontinued (from Ref 11).

Table 1. Making a diagnosis of genetic diabetes alters treatment in diabetes: the initial pharmacological treatment in the different subgroups.

Diabetes aetiology	Treatment
Type 1	Insulin
Type 2	Metformin
MODY GCK	None
MODY HNF1α	Low-dose silphonylurea
Neonatal Kir6.2	High-dose sulphonylurea

GCK = glucokinase; HNF = hepatic nuclear factor; SU = sulphonylurea.

Although mutations in the Kir6.2 subunit prevent an increase in ATP closing the K_{ATP} channel, sulphonylureas are known to be able to bind to the sulphonylurea receptor 1 (SUR1) subunit and close the K_{ATP} channel through an ATP independent route (Fig 7). Although patients with Kir6.2 mutations secrete no insulin in response to intravenous (iv) glucose, physiological tests show that they have good insulin secretion in response to iv tolbutamide.[10] This led to testing whether patients could transfer from insulin to sulphonylurea tablets. High doses of glibenclamide or other sulphonylureas enabled most patients to discontinue insulin completely and to show improved glycaemic control (Fig 8).[11,13] This is the first time that insulin-dependent patients have been able to be effectively treated with tablets.

Do the neurological features resulting from K_{ATP} channel activation also improve with sulphonylureas? Glibenclamide binds to both SUR1 and SUR2, so it should act in nerve, muscle and brain, but to act in most of the brain it would need to cross the blood–brain barrier which is uncertain in man. *In vitro* tests suggest that mutations associated with DEND syndrome respond poorly to sulphonylureas compared with mutations associated with isolated diabetes.[12] In keeping with this, three patients with the full set of DEND syndrome features were unable to discontinue insulin. However, most patients with mild developmental delay and diabetes associated with the V59M mutation not only discontinued insulin with glibenclamide but also showed improved muscle strength, concentration and speech. In some cases the change was dramatic: a two-year old patient, who was unable to stand unaided, was able to walk without help within days of starting sulphonylurea treatment. It is hard to prove causality in these cases, especially as the children and their parents were reluctant to return to insulin, but the timing certainly suggests that neurological features improve with sulphonylureas.

Conclusions

We have shown that making a diagnosis of monogenic diabetes is important as it can have a dramatic effect on the treatment a patient should receive (Table 1). These patients are seen not infrequently: although they represent only 1–2% of all diabetic patients, 20–40,000 people have monogenic diabetes in the UK. The recognition of these patients will remain a clinical skill, with molecular genetic testing confirming the diagnosis. As technological advances continue in molecular genetics, the price of testing is likely to fall and it will become more widely used. It is important that, while numbers of patients are limited, we learn the appropriate use of molecular genetics in the diabetic clinic.

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