Multiple facets of diabetes in young people*

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Genetic influences are important in the aetiology of diabetes. The majority of the major advances in our understanding have been in the rare monogenic subgroups like maturity-onset diabetes of the young (MODY). In MODY the description of the aetiological genes has lead to new understanding in many diverse areas including clinical management, pancreatic beta-cell function and foetal growth and development. Major advances in defining predisposing genes for Type 2 diabetes are likely in the next decade.

Diabetes in young people

The challenges

Diabetes in young people is a challenge for both the patient and the doctor. For both of them there is the prospect of many decades of diabetes and so the correct diagnosis and treatment is a priority. In this article I will try to highlight how recent advances in the genetics of diabetes have resulted in the crucial understanding of novel discrete types of diabetes that are diagnosed in young people. I will then develop the thesis that the study of these genetic subgroups has in turn offered new insights into diabetes, diabetes treatment, foetal growth, foetal development, identification of new clinical syndromes and new basic scientific understanding. These advances have only come through the collaboration of clinicians and scientists, and continuing collaboration is crucial.

Many diseases

As recently as 1970, diabetes was often considered a single disease of different severities. Now it is clearly established that Type 1 and Type 2 diabetes have very different aetiologies and require different treatment. It has really only recently been appreciated that the variation in the diabetes seen in the young adult is greater than just the dichotomy of Type 1 and Type 2. Monogenic diabetes is most likely to be present in young adults. There are at least five different types of maturity-onset diabetes of the young (MODY) (see below), maternally inherited diabetes and deafness (MIDD), genetic syndromes associated with obesity, e.g. Alstrom's syndrome and genetic syndromes associated with neurological conditions, e.g. Wolfram syndrome.

Crucial role of genetic predisposition

In all types of diabetes there is a balance between genetic and environmental factors, but as in other diseases, genes play a greater role in patients with a younger-onset for their diabetes. This is clearly seen in Type 2 diabetes[1]. There is considerable evidence that genes, as well as major environmental influences like obesity, are important in the aetiology of Type 2 diabetes. Children and siblings of patients with Type 2 diabetes, who share 50% of their genes, are 3–4 times more likely to have Type 2 diabetes than subjects without diabetic relatives. Identical twins of patients with Type 2 diabetes, who share 100% of their genes, have a $>90\%$ chance of developing diabetes themselves. The variation in prevalence among ethnic groups also provides evidence for the role of genes. Migration studies show that the genetic predisposition varies markedly among races that live in a similar environment. The important role of genetics in young-onset Type 2 patients is shown by the higher rate of parental diabetes than patients diagnosed in middle and old age.

Defining genes in diabetes

While Type 1 and Type 2 diabetes both cluster within families, they do not show the clear inheritance patterns seen with diseases that are caused by a single gene (monogenic). This is because the common form of Type 2 diabetes is predisposed to by multiple genes (polygenic) and whether any individual within a family develops diabetes depends on precisely which combination of genetic variation he/she may have inherited as well as non-genetic factors such as their age, physical activity and obesity.

The definition of the genes involved in diabetes was described by O'Rahilly as 'a genetic nightmare'[2] as the late age of onset and diabetes-related mortality make it difficult to collect large multi-generation pedigrees and the large role of the environment dilutes the role of genes.

The pattern is quite different in monogenic diabetes where a clear pattern of inheritance is seen as the result of a mutation in a single gene and the environment only

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has a minor modifying role (Figure 1). The best example of this is MODY, where there is an autosomal dominant inheritance of early-onset (frequently diagnosed before 25 years), non insulin-dependent diabetes. In this case diabetes will be passed from one generation to the next, with on average 50% of the children of an affected individual developing diabetes. The simplicity of analysis has meant most of the major advances in the genetics of diabetes have all come in the monogenic diabetes. In this article I will concentrate on MODY, where the majority of my personal work has been over the last decade.

Molecular basis of MODY

The monogenic nature of MODY and the availability of large multigenerational pedigrees greatly assisted definition of the underlying molecular genetics. It could only be elucidated once large number of families were collected, where it could be established that there was a single gene disease and not just an early diagnosis due to a high level of polygenic susceptibility. To date, five genes have been shown to cause MODY[3] (Figure 2). The glycolytic enzyme glucokinase was defined by a candidate gene approach in 1992 (refs 4 and 5). The transcription factor, hepatocyte nuclear factor HNF-1α, was identified by positional cloning of the MODY3 region on 12q (ref. 6). This rapidly led to the identification of mutations in HNF-4α (ref. 7) and HNF-1β (ref. 8) in a minority of MODY families. The last gene to be identified (to date) was another transcription factor, the insulin promoter factor IPF-1 (ref. 9). In European Caucasians with strictly defined MODY, defects in these genes account for approximately 90% of the cases.

MODY: Different genes, different diseases

Though mutations in all these genes result in MODY, there are subtle but important differences in the spectrum of the disease (see Table 1)[3]. The pathophysiology and phenotype of MODY caused by mutations in the glucokinase gene (glucokinase-MODY) are quite distinct from those caused by the transcription factor mutations (transcription factor-MODY) or non-MODY T2D, and imply quite different management strategies. The prognostic and management information associated with a precise molecular diagnosis explains why MODY has become the first area in diabetes in which molecular genetics has played a clear clinical role.

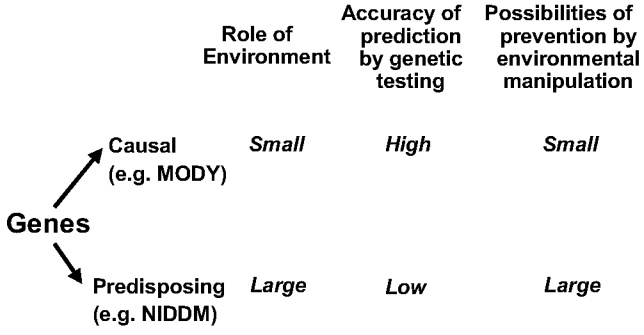

Figure 1. Differences between genes that cause or predispose to diabetes.

Glucokinase (MODY2)

Patients with glucokinase mutations have a unique phenotype: modest fasting hyperglycaemia is present from birth, but there is very little decline in glycaemia with age[3]. Consequently, patients rarely have symptomatic diabetes, pharmaceutical treatment is unusual and micro-vascular complications are rare. In contrast to patients with T2D or transcription factor mutations, increasing body weight makes little difference to the level of glycaemia in individuals with glucokinase mutations. This is because glucokinase deficiency leads to an abnormality in glucose sensing rather than a failure of insulin secretion *per se*; therefore, as in the normal subject, obesity-related insulin resistance is still capable of eliciting a compensatory hyperinsulinaemia, with glucose levels remaining fixed at the same (albeit elevated) setpoint.

Transcription factor MODY (MODY1, MODY3, MODY4 and MODY5)

In contrast to glucokinase-deficient MODY, patients with transcription factor mutations (HNF-1α, HNF-4α, HNF-1β and IPF-1) have a normal fasting glucose level in childhood, but develop progressive hyperglycaemia in adolescence and early adulthood[3]. They are usually present with symptomatic diabetes, have increasing treatment requirements with age and frequently develop microvascular complications (retinopathy, nephropathy), if good glycaemic control is not achieved. As in glucokinase, the primary pathophysiology is a beta-cell defect, so the associated features of the metabolic syndrome, typical of T2D, are unusual in both groups.

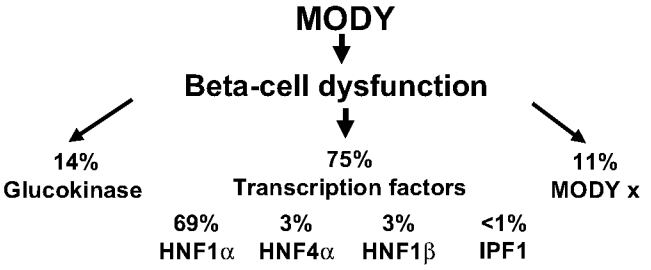

Figure 2. The genetic causes of MODY (adapted from Frayling *et al.*[25]).

Table 1. Characteristics of different subtypes of MODY

	HNF-4α (MODY1)	Glucokinase (MODY2)	HNF-1α (MODY3)	IPF-1 (MODY4)	HNF-1β (MODY5)	MODY-X
Frequency in a large UK series	2%	20%	64%	< 1%	2%	12%
Penetrance of mutations at 40 years of age	> 80%	45% (> 90% with FPG > 6 mmol/l)	> 95%	> 80%	> 95%	Not known
Onset of hyperglycemia	Adolescence; early adulthood	Early childhood (from birth)	Adolescence; early adulthood	Early adulthood	Similar to HNF-1α	Uncertain
Severity of hyperglycemia	Progressive; may become severe	Mild with little deterioration with age	Progressive; may be severe	Similar to HNF-4α	Similar to HNF-1α	Variable
Microvascular complications	Frequent	Rare	Frequent	Few data	Frequent	Variable
Pathophysiology	β-cell dysfunction (glucose sensing)	β-cell dysfunction	β-cell dysfunction	β-cell dysfunction	β-cell dysfunction	β-cell dysfunction
Other features		Reduced birth weight	Low renal threshold; sensitivity to sulphonylureas	Pancreatic agenesis in homozygotes	Renal cysts; proteinuria; renal failure. Uterine and genital abnormalities	

Despite these shared features, there are subtle differences between the patterns of disease associated with different forms of transcription factor MODY (see Table 1). Extra-pancreatic features differentiate subtypes of transcription factor-MODY. HNF-1α is characterized by a low renal threshold for glucose and consequent glycosuria[10]. This is thought to be due to reduced expression of the high-capacity/low-affinity sodium/glucose transporter-2 in the proximal tubule[11]. In HNF-4α MODY, the main extra-pancreatic manifestations result from reduced transcription of HNF-4α hepatic target genes. The levels of apolipoproteins apoAII and apoCIII and of triglycerides are all reduced[12].

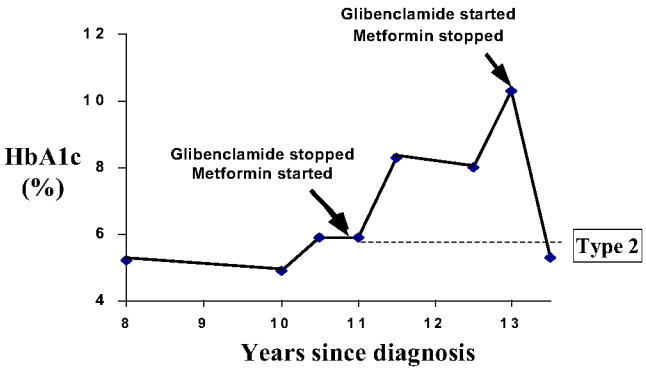

Figure 3. Sensitivity of patients with HNF-1α mutations to sulphonylureas (adapted from Pearson *et al.*[13]).

Different genes, different treatment

One of the most interesting features is the difference in treatment of the different major subgroups of MODY[3]. Glucokinase patients with their stable mild hyperglycaemia rarely require any pharmocological treatment and indeed frequently benefit from the discontinuation of insulin or oral agents that were prescribed before the diagnosis was made. A very marked increased sensitivity to the hypoglycaemic action of sulphonylureas is seen in HNF-1α patients[13]. This is particularly noticeable at diagnosis and during the first 10 years of the disease and can result in symptomatic hypoglycaemia when initiating therapy, even on conventional dosing recommendations. Although meta-analysis shows metformin and the sulphonylureas are equally effective in reducing hyperglycaemia in patients with T2D, this is clearly not so in patients with HNF-1α mutations[13]. Case reports have noted a marked deterioration in glycaemic control on transferring from sulphonylureas to metformin, with an improvement in HbA1c level of 4–5% on returning to sulphonylureas (Figure 3). This is probably the first example of pharmacogenetics in the field of diabetes and provides a clear rationale for establishing the precise molecular diagnosis in young-onset, non-insulin-dependent diabetes.

Diagnostic molecular genetic testing in MODY

The clear genetically-defined subtypes and important differences in prognosis and treatment requirements would appear to make MODY ideal for diagnostic molecular genetic testing. This testing has recently been introduced in the UK as a routine diagnostic investigation for clinicians. Testing involves the full direct sequencing of the genes which requires expensive equipment and considerable laboratory time, making it an expensive investigation.

The technical and financial considerations mean that there needs to be careful selection of which diabetic patients have diagnostic testing and which gene is tested for initially. The clinical assessment needs to estimate firstly, if the patient (and his/her family) has the clinical criteria for MODY and secondly, to define which gene is likely to be involved. The most important role in patient management for diagnostic testing is in children, where it is often assumed that slim patients have Type 1 diabetes and so will need treatment with insulin. Defining the underlying gene will establish that they have MODY and therefore are not insulin-dependent and insulin treatment is not necessary. The subgroup definition also helps to define the best treatment for them: most glucokinase patients require no treatment apart from a healthy living diet, while HNF-1α patients can be successfully controlled with very small doses of sulphonylureas.

Different mutations in the same gene, different diabetes

In the commonest types of MODY a clear association between the severity of the mutation and that of the diabetes has been difficult to establish, but a clear relationship has been established in the insulin promoter factor 1 (*IPF-1*) gene. The key role of *IPF-1* in the development of the pancreas was shown by the pancreatic agenesis found in transgenic mice in which there was a homozygous knockout of *IPF-1*. Stoffers and colleagues[14] studied a child with pancreatic agenesis and found a severe homozygous frameshift mutation which had dominant negative characteristics *in vitro*. They noticed that there were multiple generations of diabetes on both sides of the family and went on to show that the heterozygous mutation caused MODY[9].

Although mutations have only been found in a single family which conforms to clinical criteria for MODY, other milder missense *IPF-1* mutations were shown to predispose to young-onset Type 2 diabetes in both the UK and French collections[15,16]. Therefore there is a clear severity of genotype/phenotype relationship with a homozygous severe mutation causing pancreatic agenesis, a heterozygous severe mutation causing MODY, and a heterozygous mild mutation predisposing to Type 2 diabetes.

Diabetes genes alter more than glucose levels

Foetal growth: Lessons from glucokinase

Patients with glucokinase mutations are frequently diagnosed during screening in pregnancy. In the European Caucasian series, the prevalence of glucokinase mutations varies between 0 and 6% (ref. 17). The recognition of glucokinase subjects is favoured by the clinical characteristics of persistent fasting hyperglycaemia (> 5.5 mmol/l), a small increment on the oral glucose tolerance test (less than 3 mmol/l), and a family history of mild hyperglycaemia[17].

Foetal growth has been shown to vary with whether the foetus has inherited a glucokinase mutation[18]. Foetal insulin secretion is a key determinant of foetal growth acting mainly during the third trimester when the weight of the foetus increases markedly and much of growth is mediated by foetal insulin secretion. Macrosomic children born to mothers with diabetes in pregnancy have increased foetal insulin secretion in response to foetal pancreatic sensing of maternal hyperglycaemia. Factors which alter foetal insulin secretion will therefore alter intra-uterine growth by altering foetal insulin-mediated growth. A glucokinase mutation in a pregnant mother will result in maternal hyperglycaemia increasing foetal insulin secretion, whilst the inheritance of a mutation by the foetus will result in reduced sensing of the maternal glucose by the foetal pancreas and hence reduce foetal insulin secretion and intra-uterine growth. The two contradictory effects are shown in Figure 4. The effects of inheriting a glucokinase mutation by either the mother or the foetus are marked. Inheritance of the mutation by the mother resulted in the foetus being 601 g heavier, whilst inheritance of a glucokinase mutation by the foetus resulted in a reduction in birth weight of 521 g. Figure 4 shows the effects of foetal and maternal mutations on mean birth weight centiles; the two effects are additive. In both, the mother and foetus who had the glucokinase mutation, two opposing effects cancelled out as the baby was of normal weight.

These observations have important theoretical implications. They establish that glucokinase plays a key role in the sensing of glucose by the foetus *in utero*. It also led to a more wide-ranging hypothesis described as the 'Foetal Insulin Hypothesis'[19] (Figure 5). This proposes that genetic defects that alter either foetal insulin secretion or foetal insulin action could, by reducing insulin-mediated growth, reduce birth weight. This therefore

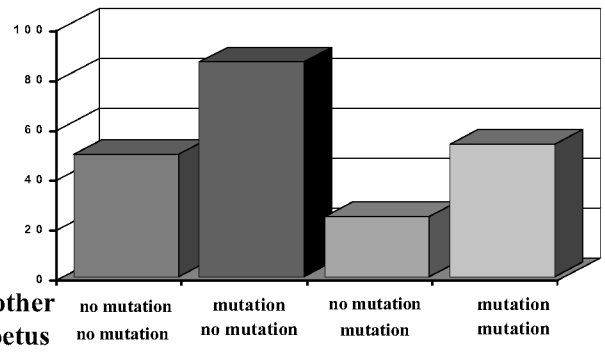

Figure 4. Centile birth weight according to maternal and foetal genotype (adapted from Hattersley *et al.*[18]).

provides an explanation of the association seen between low birth weight and diabetes in adult life. This association had previously been attributed to intra-uterine programming as the result of intra-uterine malnutrition. The relative importance of these two factors will vary among populations and requires further study. It is likely that both the intra-uterine environment and foetal genetics are important in explaining the association.

Treatment of glucokinase pregnancy

The observations on foetal growth are important for the management of pregnancy of mothers who have glucokinase mutations. These subjects will have fasting hyperglycaemia which will not respond to dietary measures. Most modern recommendations would suggest that patients should be treated with insulin to achieve normoglycaemia and many glucokinase subjects are treated with insulin during pregnancy. However recent observations have suggested that the foetal genotype is a far greater determinant of pregnancy outcome than treatment of the mother with insulin. In addition it is debatable whether to achieve maternal euglycaemia is desirable if the baby has a mutation, as this may result in a small baby. A small baby is seen when the foetus inherits a mutation from the father and is born in a normal mother[18,20]. Treatment decisions in glucokinase gestational diabetes therefore should be related to foetal growth as shown by scans, rather than purely be related to maternal glycaemia levels. Foetal hypoglycaemia is not seen when the foetus has inherited the mutation and it is clear that within a few days the newborn child will have mild fasting hyperglycaemia, which is unlikely to progress. The realization that a patient has a glucokinase mutation will also be important for the subsequent management of the mother. It is likely that even if she has been on very large doses of insulin, no pharmacological therapy will be required post-pregnancy. In contrast to subjects with non-glucokinase gestational diabetes, deterioration to Type 2 diabetes over the following 10–20 years is unlikely.

Foetal development: Lessons from HNF-1β

One of the exciting developments has been that new syndromes have been recognized as a result of the definition of the underlying genetics. Probably the commonest is MIDD, resulting from a mutation in the mitochondrial DNA at position 3243 (ref. 21). The crucial features are diabetes inherited from an affected mother but not an affected diabetes, usually diagnosed in middle age often requiring insulin, associated with neural deafness and other neurological and systemic features. This may account for 0.2–3% of diabetes with a higher prevalence in Asia[21]. Recently a new syndrome associated with HNF-1β mutations has been described where like HNF-1α patients have MODY, but also have cystic renal disease with different histologies and degrees of renal failure[22]. In HNF-1β MODY, the associated renal disease which precedes diabetes, typically results in presentation via the renal rather than diabetic clinic. Non-diabetic cystic renal disease is a feature of most HNF-1β mutations and this has led to the description of the previously-unrecognized clinical syndrome of renal cysts and diabetes (RCAD). Three discrete histologies have been described: oligomeganephronia, cystic dysplasia and familial hypoplastic glomerulocystic kidney disease. The renal manifestations are the result of abnormal

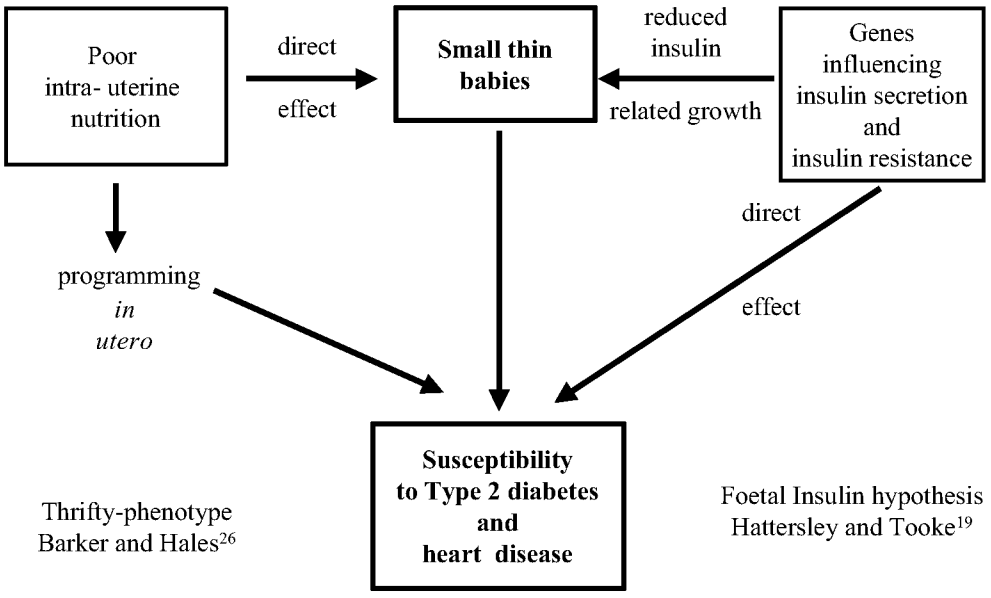

Figure 5. Intra-uterine environment or predisposing genes.

nephron development, and the aberrant renal morphology may be observed on scans as early as 17 weeks of pregnancy. Approximately 40% of subjects will develop non-diabetic end-stage renal failure before the age of 45. Other recently described features of HNF-1β mutation[3] are uterine and genital anomalies and elevated urate levels.

Defining genes in Type 2 diabetes

Defining the predisposing genes in Type 2 diabetes is considerably more difficult than defining causal genes in rare monogenic subgroups[1]. Recent studies have shown that unlike HLA in Type 1 diabetes, there is no single major predisposing Type 2 diabetes gene and no gene that is important in all populations. Future analysis will be considerably helped by very large collaborative collections of DNA from patients and modern high throughput genetic analysis methods.

To date over 250 candidate genes have been investigated in Type 2 diabetes, but none have been shown to be major predisposing genes.

Recently evidence has accumulated that a variant in the *PPARγ* gene is protective for the development of Type 2 diabetes[23]. This variant only results in a very slightly altered relative risk of diabetes and the definitive study for its role reported results from over 3000 patients and controls. This is an indication of the size required for future studies.

Another important recent study reported that variation in a novel gene, the protease Calpain 10, was associated with susceptibility to Type 2 diabetes in Mexican Americans. This is the first time that an unknown gene has been identified using the positional cloning approach. This approach does not require the pathophysiology to be known, as it involves searching the whole genome for a genetic region (in this case the short arm of chromosome 2) that is inherited more frequently in diabetic family members. The region is then further investigated to determine which of the genes in this region are important.

Conclusion

Genetic influences are important in the aetiology of diabetes. To date the majority of advances in our understanding have been in the rare monogenic subgroups like MODY. In MODY, description of the genes has lead to new clinical insights and diagnostic testing. The major predisposing genes for Type 2 diabetes will be much more difficult to define, but in the next few years large studies should lead to considerable progress in this area.

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