

# Learning From Molecular Genetics

## Novel Insights Arising From the Definition of Genes for Monogenic and Type 2 Diabetes

Mark I. McCarthy<sup>1,2</sup> and Andrew T. Hattersley<sup>3</sup>

**G**enetic factors for many decades have been known to play a critical role in the etiology of diabetes, but it has been only recently that the specific genes have been identified. The identification of the underlying molecular genetics opens the possibility for understanding the genetic architecture of clinically defined categories of diabetes, new biological insights, new clinical insights, and new clinical applications. This article examines the new insights that have arisen from defining the etiological genes in monogenic diabetes and the predisposing polymorphisms in type 2 diabetes.

### MONOGENIC DIABETES

**Defining monogenic diabetes genes by candidate gene and positional cloning approaches.** There has been rapid progress in defining the etiological genes for monogenic diabetes reflecting the relative simplicity of gene discovery in single gene disorders. The candidate gene approach has been remarkably successful in defining monogenic genes; this reflects that key rate-limiting steps in insulin secretion and action are known, and severe mutations affecting these proteins will result in  $\beta$ -cell dysfunction or insulin resistance. Examples of this approach include the genes encoding insulin (1), glucokinase (2,3), the two ATP-sensitive  $K^+$  channel ( $K_{ATP}$  channel) subunits Kir6.2 (4) and SUR1 (5,6), peroxisome proliferator-activated receptor (PPAR) $\gamma$  (7), and the insulin receptor (8). Finding human subjects with mutations in these candidate genes has allowed confirmation of a critical role in humans of the encoded protein, helped define structure and function of the protein, and allowed confirmation of the associated pathophysiology (e.g., abnormal glucose sensing in glucokinase mutations) (9), but it has not led to the identification of novel pathways in glucose homeostasis.

Completely unexpected critical pathways for insulin secretion and action have resulted from the positional cloning of novel monogenic diabetes genes. The most

striking example was the identification of *HNF1A*, encoding the transcription factor hepatic nuclear factor (HNF)-1 $\alpha$ , as the maturity-onset diabetes of the young (MODY) gene linked to 12q (10). Before this finding it was not known that *HNF1A* was expressed in the  $\beta$ -cell, and diabetes had not been noticed in the *hnf1a*-knockout mouse (11), although it was noticed subsequently (12). This result rapidly led to mutations in other hepatic transcription factor genes, *HNF4A* (13) and *HNF1B* (14), shown to cause MODY. These findings have led to a whole new area of  $\beta$ -cell biology seeking to explain why haploinsufficiency of these genes resulted in progressive  $\beta$ -cell dysfunction (15,16). Mutations in *CEL*, which encodes the lipolytic enzyme carboxyl-ester lipase, responsible for the hydrolysis of cholesterol esters, was also an unexpected MODY gene identified through positional cloning (17). *CEL* is only expressed in the pancreatic acinar cell, so it was unexpected that there was  $\beta$ -cell dysfunction. Further studies of the mechanism will lead to new understanding of the close relationship between the exocrine and endocrine pancreas. Familial partial lipodystrophy was shown, following linkage to 1q21, to arise from mutations in *LMNA*, encoding Lamin A/C (18). Mutations in *LMNA* can also result in myopathy, dilated cardiomyopathy, or atypical progeria (19), and the biology of how these mutations alter fat distribution is still incompletely understood. Therefore, positional cloning has led to exciting novel pathways of glucose homeostasis.

**Most monogenic diabetes genes are  $\beta$ -cell genes.** A key result has been that the vast majority of genes where mutations cause early-onset diabetes have reduced  $\beta$ -cell function rather than increased insulin resistance. Heterozygous haploinsufficiency results in dominant early-onset diabetes for many  $\beta$ -cell genes, including *GCK*, *HNF1A*, *HNF4A*, and *HNF1B*, but this is not seen in insulin resistance genes. This shows that even when faced with severe insulin resistance, a healthy  $\beta$ -cell is usually able to compensate, but there is no compensation possible when faced with marked insulin deficiency. There are many mechanisms of  $\beta$ -cell dysfunction seen in monogenic diabetes, including reduced  $\beta$ -cell development, failure of glucose sensing, and increased destruction of the  $\beta$ -cell (Table 1).

**Gene discovery can also lead to recognition of novel phenotypes.** For many genetic syndromes, such as Wolcott Rallison, and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndromes, a discrete cluster of clinical features including diabetes was initially recognized as a clinical syndrome, and subsequently the gene responsible was identified (20,21). In these cases, the gene discovery gave new biological insights but only limited insights into the phenotype.

From the <sup>1</sup>Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, U.K.; the <sup>2</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K.; and <sup>3</sup>Diabetes Genetics, Peninsula Medical School, Exeter, U.K.

Corresponding authors: Andrew T. Hattersley, [andrew.hattersley@pms.ac.uk](mailto:andrew.hattersley@pms.ac.uk), and Mark I. McCarthy, [mark.mccarthy@dr1.ox.ac.uk](mailto:mark.mccarthy@dr1.ox.ac.uk).

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See accompanying commentary, p. 2918.

TABLE 1  
Examples of some mechanisms of  $\beta$ -cell dysfunction seen in monogenic diabetes

Mechanism of $\beta$ -cell dysfunction	Gene/mutation
Reduced $\beta$ -cell number	
Pancreatic aplasia	<i>IPF1</i> homozygous
Reduced $\beta$ -cell development	<i>PTF1A</i> <i>HNF1B</i>
Reduced metabolism	
Reduced glucose sensing	<i>GCK</i>
Reduced metabolism	Mitochondrial mutations <i>HNF1A</i> <i>HNF1B</i> <i>HNF4A</i> <i>IPF1</i> heterozygous
Failure to depolarise membrane	
Failure to close $K_{ATP}$ channel	<i>KCNJ11</i> <i>ABCC8</i>
Increased destruction of $\beta$ -cells	
Immune-mediated destruction	<i>FOXP3</i> <i>INS</i>
Endoplasmic reticulum stress	<i>EIF2AK3</i> <i>WFS1</i>
Increased apoptosis cause uncertain	<i>HNF1A</i> <i>HNF4A</i> Mitochondrial mutations

However, in other clinically defined categories, the identification of the etiological genes helped the recognition of novel clinical subgroups. MODY was clinically defined as autosomal dominantly inherited, non-insulin-dependent, early-onset diabetes, but now there are at least eight genetic subgroups of MODY, most of which have a discrete phenotype (Fig. 1) (22,23). Similarly, diabetes diagnosed in the first few months of life was defined clinically as permanent neonatal diabetes mellitus (PNDM) or transient neonatal diabetes mellitus (TNDM), depending on whether the diabetes resolved (22,23). Molecular genetic advances have identified three genetic subgroups of TNDM, four genetic subgroups of PNDM, and five genetic syndromes that include neonatal diabetes (Fig. 2); many of these genetically defined subgroups can, at least in part, be separated by their clinical features (22,23).

Many entirely novel multisystem clinical syndromes were only recognized clinically after the molecular genetics were defined. The most common is maternally inherited diabetes and deafness, resulting from a heteroplasmic mitochondrial gene mutation at position 3243, which was recognized when the gene was identified in a single large

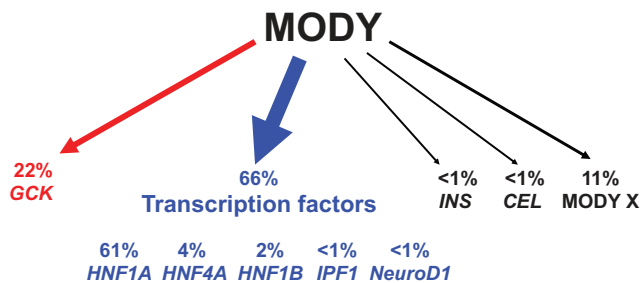


FIG. 1. The genetic heterogeneity of clinically defined MODY. The percentages given are based on over 1,000 referrals to the Exeter lab for genetic testing in MODY. (S. Ellard and A.T.H., unpublished data). MODY X represents patients with a clear clinical diagnosis of MODY who do not have a mutation in any of the known genes.

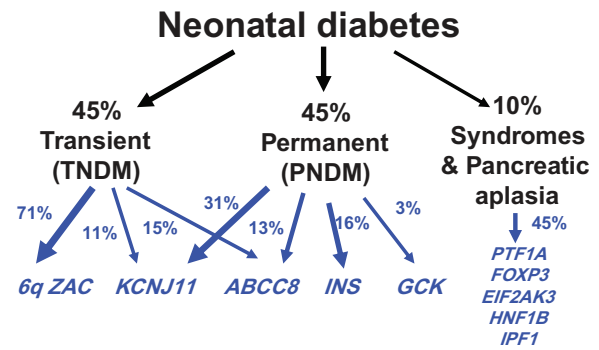


FIG. 2. The genetic heterogeneity of the clinically defined subdivisions of neonatal diabetes. The percentages given are based on over 400 referrals to the Exeter and Salisbury labs for genetic testing for neonatal diabetes (S. Ellard, A.T.H., D. Mackay, and I.K. Temple, unpublished data). *KCNJ11*, *ABCC8*, *INS*, and *HNF1B* mutations are dominantly acting; *GCK*, *PTF1A*, *EIF2AK3*, *IPF1*, and some *INS* and *ABCC8* mutations are recessively acting; *FOXP3* is sex linked; and the 6q *ZAC* region for TNDM results from altered imprinting at this locus.

Dutch family (24). Patients with this mitochondrial mutation not only have diabetes typically diagnosed in early adult life and sensorineural deafness, but also other features resulting from the associated mitochondrial dysfunction including myopathy, pigmented retinopathy, cardiomyopathy, and focal glomerulosclerosis (25,26). Prevalence studies have suggested that 3243 accounts for ~1–2% of diabetes in Japanese and 0.2–0.5% in European series (26). Mutations in the transcription factor *HNF1B* mutations were first described as a subgroup of MODY (14), but it was quickly realized that the most consistent feature was renal cysts (27–29); therefore, the novel syndrome renal cysts and diabetes was proposed (30). Further studies have shown that heterozygous mutations or whole gene deletions (31) of this ubiquitous transcription factor result in a wide range of developmental kidney disease and other features including uterine and genital abnormalities, gout, hyperuricemia, exocrine pancreatic dysfunction, abnormal liver function tests, and insulin resistance (32).

Clinical features reflect the function of the encoded protein outside glucose regulation. One striking example of how extra pancreatic features give new insights into the role of the encoded proteins is the way in which birth weight is affected in the different genetic subtypes of MODY (Fig. 3). In utero fetal insulin is a major growth factor, and therefore altered fetal insulin secretion will alter birth weight. Increased birth weight resulting from increased fetal insulin secretion in response to maternal hyperglycemia may be seen in all types of diabetes including monogenic diabetes. A key additional feature of monogenic diabetes is that if the genes alter fetal insulin secretion or fetal insulin action in utero, birth weight is also altered (33).

Because patients with glucokinase mutations have moderate stable hyperglycemia in the neonatal period (34), it is expected that the glucose sensing abnormality was also present in utero, resulting in reduced fetal insulin secretion and reduced birth weight by 550 g (35). Conversely, in *HNF1A* mutations, where glucose tolerance is normal at birth and diabetes rarely develops before adolescence,  $\beta$ -cell function was likely to have been normal in utero, hence fetal *HNF1A* mutations would have no impact on birth weight (38). The marked reduction in birth weight (900 g) with fetal *HNF1B* mutations (36) suggests that fetal insulin secretion is disrupted even though diabetes

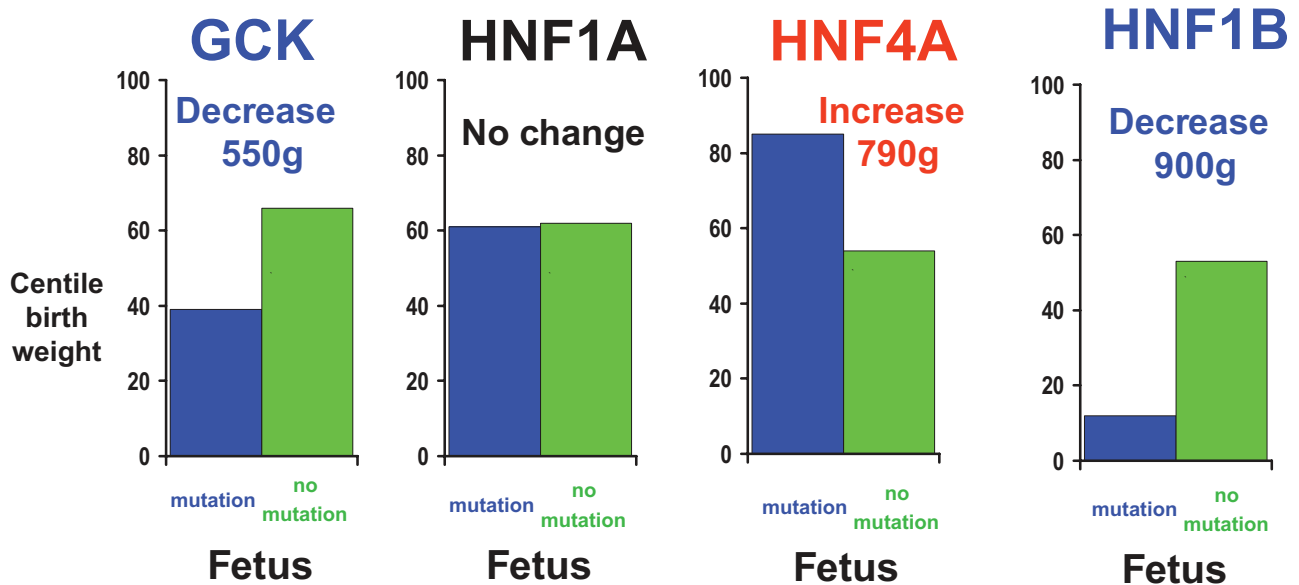


FIG. 3. The impact on birth weight of a fetus inheriting the four most common MODY gene mutations. Birth weight is presented as centile birth weight with the fetus inheriting the mutation in blue and in comparison a fetus without the mutation in green. Data are from refs. 35, 36, and 38.

typically presents in early adulthood. This is in keeping with the role of the fetal  $\beta$ -cell. HNF1- $\beta$  is a critical transcription factor in the maturation of the pancreatic stem cell before differentiation into the exocrine and endocrine cells (37). A marked increase in birth weight (790 g) seen with a fetal *HNF4A* mutation, even when inherited from the father, is an unexpected finding (38). Macrosomia reflects increased insulin secretion (38), and some neonates with *HNF4A* mutations have transient (38) or prolonged hypoglycemia (39). This means that increased insulin secretion in the fetus progresses to reduced insulin secretion in adolescence or early adulthood. This pattern is also seen in some dominant *SUR1* mutations (40).

**Defining genetic etiology can alter treatment.** Geneticists have long argued that defining the etiology of subgroups of diabetes should help the development of appropriate treatment, and in monogenic diabetes there are now clear examples of this (41).

The best example of pharmacogenetics has been in the treatment of patients with PNDM resulting from mutations in the Kir6.2 and *SUR1* subunits of the  $K_{ATP}$  channel. These patients frequently present with ketoacidosis and no detectable endogenous insulin secretion, and therefore insulin injections are the only treatment option. Insulin treatment is difficult in a young child, and outstanding glycemic control is rarely achieved. Finding that one-third of the patients with PNDM had mutations in the Kir6.2 channel that reduced channel closure in response to ATP led to the possibility of treating these patients with sulfonylureas that close the channel by an ATP-independent route (4,42). It was then possible to replace insulin injections with high-dose oral sulfonylureas in 90% of patients and also to achieve improved glycemic control without an increase in hypoglycemia (43,44). Insulin secretion is regulated despite the  $\beta$ -cell having a limited response to ATP; this is predominantly mediated through nonclassical pathways for insulin secretion, particularly GLP1 (43). Excellent glycemic control is also seen in the majority of patients with *SUR1* mutations treated with sulfonylureas (45). Therefore, ~50% of patients diagnosed before 6

months with permanent diabetes can benefit greatly from a molecular diagnosis. To date, patients with  $K_{ATP}$  channel mutations have maintained near normoglycemia for over 4 years (A.T.H., unpublished data). Doses tend to reduce over time, suggesting that the effectiveness of this treatment will be long lasting.

There is also clear evidence of pharmacogenetics within the genetic subcategories of MODY. The fall in fasting glucose with sulfonylurea therapy is fourfold greater in *HNF1A* MODY patients compared with glycemia- and BMI-matched type 2 diabetic subjects (46). This sensitivity to sulfonylureas frequently means that patients who have been misdiagnosed as type 1 diabetic and treated with insulin from diagnosis can successfully transfer to sulfonylureas without deterioration in glycemic control (47). This improved hypoglycemic response to sulfonylureas compared with that in type 2 diabetes represents, in part, greater insulin secretion (46). Animal and cellular models of HNF-1 $\alpha$  deficiency have suggested that the major defect in the  $\beta$ -cell is in glucose metabolism, which may explain why these subjects are very responsive to a drug that binds to the  $K_{ATP}$  channel, which is downstream of glucose metabolism. Pharmacogenetics may result from a specific genetic subgroup having reduced instead of increased response to a drug. In *HNF1B* MODY there is reduced pancreatic size (48), reflecting reduced development of the endocrine and exocrine pancreas (37). In these patients, treatment with sulfonylureas is relatively unsuccessful and insulin treatment is rapidly required (49). In *GCK* MODY patients, glucose is regulated to remain at a higher fasting level, hence oral hypoglycemic agents or low/moderate-dose insulin does not alter glycemia. In 24 *GCK* MODY patients on insulin or oral hypoglycemic agents, A1C was 6.3% on treatment and 6.3% after 3 months off treatment (O. Gill-Carey and A.T.H., unpublished data).

**Introduction of monogenic diabetes testing into clinical practice.** Guiding treatment decisions is not the only clinical role for genetic information in monogenic diabetes; it can also be used to make a definitive diagnosis, explain the cluster of clinical features, and predict the clinical course (26). The clear clinical utility of this has led



TABLE 2  
Summary details of the first 17 loci with a proven role in type 2 diabetes susceptibility

Signal	Chromosome	Representative SNP	Risk allele frequency	Effect size	How found	Hypothecated biology
<i>PPARG</i>	3	rs1801282	0.85	1.23	Candidate	Adipocyte differentiation and function
<i>KCNJ11</i>	11	rs5219	0.40	1.15	Candidate	$\beta$ -Cell $K_{ATP}$ channel
<i>TCF7L2</i>	10	rs7901695	0.40	1.37	Large-scale association	Incretin signaling in the islet
<i>HHEX</i>	10	rs5015480	0.63	1.13	GWA	Pancreatic development Zn transport in $\beta$ -cell insulin granules
<i>SLC30A8</i>	8	rs13266634	0.72	1.12	GWA	Hypothalamic effect on weight regulation
<i>FTO</i>	16	rs8050136	0.45	1.23	GWA	$\beta$ -Cell function and mass
<i>CDKAL1</i>	6	rs10946398	0.36	1.16	GWA	Cell cycle regulation in the $\beta$ -cell
<i>CDKN2A/B</i>	9	rs10811661	0.86	1.19	GWA	mRNA processing in the $\beta$ -cell
<i>IGF2BP2</i>	3	rs4402960	0.35	1.11	GWA	Endoplasmic reticulum stress
<i>WFS1</i>	4	rs10010131	0.60	1.11	Large-scale association	$\beta$ -Cell development and function
<i>TCF2/HNF1B</i>	17	rs757210	0.43	1.08	Large-scale association	Transcriptional repression in the islet
<i>JAZF1</i>	7	rs864745	0.50	1.10	GWA	Cell cycle regulation ( <i>CDC123</i> )
<i>CDC123/CAMK1D</i>	10	rs12779790	0.18	1.09	GWA	Cell surface glycoprotein
<i>TSPAN8</i>	12	rs7961581	0.27	1.09	GWA	Apoptosis
<i>THADA</i>	2	rs7578597	0.90	1.12	GWA	Metalloprotease
<i>ADAMTS9</i>	3	rs4607103	0.76	1.06	GWA	Pancreatic development
<i>NOTCH2</i>	1	rs10923931	0.11	1.11	GWA	

All loci have been shown to attain significance levels consistent with genome-wide significance in European populations. Note that in most cases the single nucleotide polymorphisms (SNPs) denoted are unlikely to be causal. Effect size is given as the estimated OR per copy of the risk allele. The biological processes listed are based on best available knowledge, but empirical data confirming these are not yet available for most. The data populating this table are derived mostly from refs. 55–67 and based on populations of European descent only.

to the very rapid adoption of diagnostic genetic testing in clinical practice with noncommercial and commercial diagnostic services for monogenic diabetes (50). Guidelines for laboratories offering this testing have now been produced (51). As this is a new area of diabetes practice, guidelines are also needed for both patients and physicians (22), and websites such as [www.diabetesgenes.org](http://www.diabetesgenes.org) offer both educational material and guidelines about who should be tested. As the costs of genetic testing fall and more advantages are established, genetic testing to detect monogenic diabetes will increase in clinical practice.

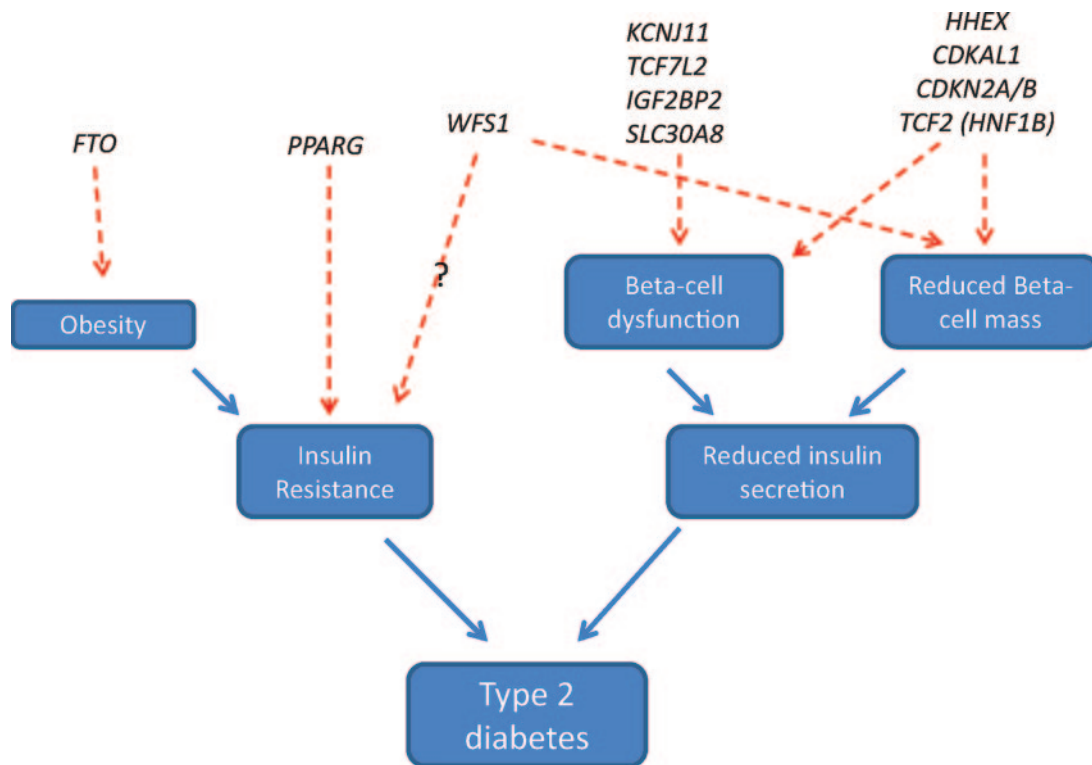
#### LESSONS LEARNED FOR MULTIFACTORIAL DISEASE

Monogenic and syndromic forms account for only a small, though highly informative, proportion of cases of nonautoimmune diabetes. The challenge for medical science lies in bringing equivalent mechanistic insights and translational benefits to the hundreds of millions of people already affected by, or at risk of, more common, typical forms of diabetes. For type 2 diabetes, there is abundant evidence that individual susceptibility is influenced by both the combination of genetic variation at multiple sites and a series of environmental exposures encountered during life (52). Tracking down the specific genetic variants involved has been tougher than for monogenic forms of disease, since the correlations between genotype and phenotype are far weaker (53,54). However, recent efforts have now identified at least 17 confirmed type 2 diabetes-susceptibility variants (Table 2) (55–67), a count certain to increase further in the months ahead. Though effective

type 2 diabetes gene discovery remains very much in its infancy, several important lessons are emerging.

**Inherited susceptibility to common forms of type 2 diabetes derives from multiple genes of modest effect.** The linkage approaches used in monogenic diabetes are successful precisely because linkage analysis is intrinsically adept at finding highly penetrant variants, irrespective of allele frequency. Efforts to use similar linkage approaches to identify type 2 diabetes-susceptibility genes have met with only limited success, yielding few, if any, consistently replicating signals (68). The lesson is clear: common variants of large effect (what might once have been called “major” genes) do not make an important contribution to type 2 diabetes susceptibility.

Association-based approaches are far better suited to identification of signals of modest effect (69), and development and exploitation of this methodology has had the greatest impact on susceptibility gene discovery. Even so, many of these discoveries have been hard-won. One reason for this is that the “candidate” gene-based approach has proved, with notable exceptions (55,56), to be an inefficient route to susceptibility gene discovery; it is only with the advent of functionally agnostic genome-wide approaches that the floodgates have opened (70). Another reason is that detection of the variants of modest effect that appear to be responsible for much of type 2 diabetes susceptibility (per-allele odds ratios [ORs] 1.10–1.40, for risk-allele frequencies 10–90%) has required association studies conducted in extremely large sample sizes (thousands of individuals) (54). Variants within *TCF7L2* have



**FIG. 4.** Simplified schematic of the processes involved in genetic predisposition to type 2 diabetes. Assignments of loci to particular processes are based on current knowledge of the presumed function of the best candidates within each signal and human physiological studies. These assignments should be considered provisional until the causal variants have been identified and the molecular mechanisms through which they act are established. Current evidence shows, however, that the majority of genes implicated in diabetes susceptibility act through effects on  $\beta$ -cell function and/or mass. This figure does not include the six type 2 diabetes–susceptibility loci reported most recently.

the largest effects seen so far, with a per-allele OR of 1.4 (57): the 15% of Europeans carrying two copies of the risk allele are at approximately twice the lifetime risk of type 2 diabetes as the 40% who have none.

It is important to remember that for many of the newly discovered susceptibility loci (57–67), all we have at present is an initial association signal derived from an incomplete survey of genome-wide common variation. Deeper inspection of these association signals, using resequencing to derive more complete inventories of local genomic variation, and fine mapping to explore the relationships between these variants and disease susceptibility may reveal that the variants of current interest are merely weak surrogates for other stronger effects nearby. It is also possible that future discovery efforts—targeting a wider range of types of genome sequence variation than the subset of common single nucleotide polymorphisms captured by current genotyping platforms—will reveal additional type 2 diabetes–susceptibility variants with more impressive effect sizes (see below).

Nevertheless, it seems likely that many of the undiscovered type 2 diabetes–susceptibility variants will have effects similar to, or smaller than, those found thus far; there may well be scores (even hundreds) of these (71). Very large sample sets (requiring collaboration between multiple groups) will be required to detect them, to confirm that they are truly associated, and to identify the causal variants. Researchers planning to examine the consequences of these variants on whole-body physiology, or on molecular events *in vitro*, can expect that such low-penetrance variants will often have equally subtle effects on intermediary metabolism and cellular function.

From a translational point of view, low-penetrance variants may have limited value for individual prognostication (72) (see below). Nevertheless, they are already providing valuable insights into the biology of type 2 diabetes (Table 2 and Fig. 4). Demonstrating that a particular variant has a genuine effect on type 2 diabetes susceptibility generates the most direct evidence available about pathways critical to the maintenance of normal glucose homeostasis in humans. These pathways might, with luck, be amenable to therapeutic or preventative manipulation. From this perspective, the effect size of the associated variant is irrelevant: more is likely to be learned from discovering a weak (but genuine) genetic effect in an entirely novel pathway than from a much larger effect that highlights once again a process with an established role in pathogenesis.

**Most type 2 diabetes–susceptibility variants impact on  $\beta$ -cell function and/or mass.** Individuals with type 2 diabetes typically display concomitant defects in both insulin secretion and action. While it is axiomatic that hyperglycemia implies some degree of relative or absolute failure of  $\beta$ -cell function, there has been a long-standing debate about the relative importance (even “primacy”) of the two processes in the pathogenesis of type 2 diabetes (73). Notwithstanding the efforts of epidemiologists and physiologists, this may be one debate where genetics (precisely because of its focus on inherited rather than acquired phenomena) may provide the answers.

The relative prevalence of mutations causal for monogenic forms of diabetes suggests that mutations in  $\beta$ -cell-related processes are a more frequent cause of severe early-onset diabetes than those influencing insulin action

(see above). Studies of the relative heritabilities of indexes of  $\beta$ -cell function and insulin action in the general population also hint at a preponderance of  $\beta$ -cell effects (52).

Recent gene discovery efforts have provided further evidence to support such assertions. Though, at this point, the identity of some of the genes mechanistically responsible for the association signals uncovered remains uncertain, it remains possible to determine, through studies of healthy populations, whether the type 2 diabetes–susceptibility variants themselves are mediating their effects through disruption of  $\beta$ -cell function or insulin action. With the exception of *FTO* (known to influence type 2 diabetes risk through a primary effect on adiposity) and *PPARG* (long implicated in insulin action), all confirmed susceptibility alleles appear to exert their predominant effect on diabetes pathogenesis through abrogation of  $\beta$ -cell function (or mass) (62,74–77). It would be wrong to extrapolate too far: the known variants account for only a small proportion of overall genetic risk, and the focus on lean type 2 diabetes cases, which has characterized several of the genome-wide association (GWA) studies (58,59), may have generated a bias toward detection of variants detrimental to  $\beta$ -cell performance. Nonetheless, the picture that emerges is one where alterations of  $\beta$ -cell function seem to be playing the predominant role with respect to the inherited component of disease predisposition.

When it comes to further insights—to the identification of specific pathways responsible, for example—caution is warranted. Colocalization of an association signal to the same interval as a particular gene does not prove a causal connection. In some instances, causal variants may be influencing type 2 diabetes pathogenesis through remote regulatory effects on genes whose coding sequences lie some distance away. Indeed, several of the recently identified type 2 diabetes signals map to “gene deserts,” and others (such as the association within the *HHEX/KIF11/IDE* region on chromosome 10) map to regions containing several good candidates (58–61). Until the causal variants have been defined, and unequivocal connections made to the genes whose function and/or expression is altered, any presumptions about the mechanisms involved must remain provisional. Having said that, certain themes are emerging (Table 2 and Fig. 4). Perhaps the most exciting involve the roles played by variants within the islet Zn transporter (58) and cell-cycle regulators (59–62,67) in type 2 diabetes predisposition. The latter discovery, in particular, may help to resolve the controversy over the part played by reduced  $\beta$ -cell mass in the development of diabetes (78).

**Unexpected connections between diabetes and other diseases.** One of the most unexpected outcomes of large-scale association approaches has been the identification of variants, and loci, that influence predisposition to multiple diseases. These “pleiotropic” effects often cross conventional nosological boundaries. In the case of type 2 diabetes, the most compelling is the overlap with prostate cancer. It has emerged that several of the genes implicated in type 2 diabetes susceptibility (particularly *TCF2/HNF1B* and *JAZF1*) are also involved in predisposition to prostate cancer, a finding that hints at hitherto unsuspected common pathways influencing these two disease processes (64,65,67). Since the predisposition effects seem to lie in opposing directions, this finding also highlights the risk that efforts at pharmaceutical modulation of this

pathway designed to benefit diabetes may have adverse consequences for cancer risk.

A further example concerns the region of type 2 diabetes susceptibility mapped to chromosome 9, in the vicinity of the *CDKN2A/2B* genes (59–61). Not only does this region harbor at least two distinct type 2 diabetes–susceptibility signals, it also contains a third, statistically independent, signal with a profound effect on coronary artery disease risk (79–81) and aneurysm formation (82). Surprisingly, the diabetes and vascular association signals involve quite distinct sets of variants, though these may act through modulation of similar molecular pathways. In the case of type 2 diabetes, this most plausibly involves an effect on  $\beta$ -cell regeneration mediated by *CDKN2A* overexpression (83). Since loss of *CDKN2A* expression is a common feature of many cancers, this may represent another instance of molecular events with reciprocal effects on cancer and diabetes risk.

**The common variants so far uncovered have limited capacity to provide individual prediction.** In analysis of large subject groups, it can be shown that the known type 2 diabetes–susceptibility variants influence clinically relevant phenotypes such as disease progression (84), risk of complications, and therapeutic response (85). However, it does not follow that those differences will be sufficient to provide clinically relevant information where individual patients are concerned. Indeed, the modest effect sizes of the variants identified to date mean that their individual impact is likely to be limited.

This is best illustrated by considering variants in *TCF7L2* (57). GWA studies have demonstrated that variants in this gene have the strongest effect on diabetes risk currently known, and a genetic test is commercially available. Assuming an average lifetime risk of type 2 diabetes of ~10%, someone with no copies of the risk allele would (all else being equal) find that figure falling to ~7.5%, whereas the lifetime risk for an individual with two copies increases to 14.5%. It is not yet clear that personal information of this kind (particularly when other pertinent factors such as an individual’s age, ethnicity, family history, and BMI are not explicitly taken into account) will lead individuals toward beneficial changes in health-related behaviors (86) or alterations in their clinical management. Indeed, if such information were to be poorly presented, there is a danger that overestimation of the deterministic qualities of genetic information could motivate individuals toward counterproductive changes to their lifestyle (through unwarranted fatalism or feelings of personal immunity).

Of course, individual small effects can amount to more when considered collectively, and it is true that genetic testing (for the 17 known genes, for example) can identify subsets of individuals who have inherited particularly high or low numbers of risk alleles and therefore have marked differences in individual risk (87). However, the numbers of individuals in these “extreme” high- and low-risk groups are comparatively small, and for many, their risk will already be obvious through conventional factors (family history, BMI, and previous gestational diabetes, for example). When the information from the known type 2 diabetes–susceptibility variants is examined using approaches such as receiver-operating curve analysis, which are better suited for evaluating the performance of diagnostic tests at the population level, the results look far less spectacular (72,87).



Progress toward wider use of genetic testing in the prediction of type 2 diabetes and its complications will require three developments. The first involves identification of a growing number of risk variants that, collectively, deliver greater predictive and discriminative performance than the subset thus far known. The second involves understanding how genetic information can be combined with other conventional risk factors (and possibly with non-DNA-based biomarkers, as these emerge) to provide a more accurate assessment of individual risk. It should be kept in mind that susceptibility genotype information will not be orthogonal to those traditional factors, since several of them (such as ethnicity, family history, and BMI) capture overlapping genetic information. The third development will be evidence that imparting such information results in clinically meaningful differences in individual behavior or provides a more rational basis for therapeutic or preventative interventions.

#### A GLIMPSE INTO THE FUTURE

**Getting from the extremes to a comprehensive view of diabetes genetics.** As described above, success in the identification of genes impacting on individual risk of diabetes has come from two distinct approaches to gene discovery. The first, linkage mapping within monogenic and syndromic families, has delivered causal variants that are rare but highly penetrant. The second, large-scale association mapping, is now yielding growing numbers of common variants: these have, at best, modest effect sizes and low penetrance. Several genes are featured in the lists generated by both approaches. For example, mutations in *KCNJ11*, *PPARG*, *WFS1*, and *TCF2* (*HNF1B*) are causal for syndromic and/or monogenic forms of diabetes, while common variants in these same genes influence predisposition to typical type 2 diabetes (55,56,64–66). While common variants in *GCK* (another gene causal for MODY) do not influence type 2 diabetes risk per se, they have a clear impact on fasting glucose levels within the population (88).

Of course, none of this should come as a surprise. Once a gene has been shown to harbor one variant associated with, or causal for, a diabetes-like phenotype, it becomes far more likely that other nearby variants (provided they exert some effect on the expression and/or function of the gene) will also have a detectable phenotypic effect. By the same token, the genotype-phenotype relationships revealed by these gene discovery efforts highlight the pathways involved as prime candidates for beneficial therapeutic or preventative manipulation, a view reinforced by the fact that at least two of the genes involved in both monogenic and multifactorial forms of diabetes (*PPARG*, *KCNJ11*) encode the targets of proven diabetes drugs.

However, it should be obvious that these two “flavors” of polymorphism—rare and highly penetrant on the one hand and low penetrant on the other—are not the only options when it comes to the variants that might influence disease susceptibility. It seems probable, even likely, that between these extremes lies a class of medium frequency, medium penetrance variants that have until now escaped the gaze of the gene mappers.

Such variants would have penetrances too low to generate Mendelian patterns of segregation and frequencies too low to be covered by current GWA approaches. Despite this, such variants have particularly attractive

translational properties. For example, a variant where the risk allele has a frequency of 1% and produces in a per-allele OR of  $\sim 3$  would provide greater predictive power than the known variants in *TCF7L2*. Variants with such characteristics are increasingly being reported in other disease states (breast cancer and hyperlipidemia) (89,90) and have even been reported in type 2 diabetes (91). In principle, just 30 such variants across the genome could explain the observed familial aggregation of type 2 diabetes in a way that the current set of common, low-penetrance variants cannot. Such a pool of variants would also provide an excellent tool for individual diabetes-risk prediction, generating a discriminative accuracy on receiver-operating characteristic analysis close to 80%. The advent of new high-throughput sequencing technologies, allied to large-scale association analysis, brings variants in this class within the range of genetic discovery and should allow researchers to evaluate the contribution to disease susceptibility attributable to variants that lie between the extremes where previous attention has been focused.

There are many other challenges to be faced and opportunities to be realized in the years ahead. The first of these lies in extending the range of variants that are accessible to scrutiny, beyond the low-frequency variants referred to in the previous paragraph, to a systematic evaluation of structural polymorphisms (insertions, deletions, and duplications) and variants that influence methylation status (92). Another lies in characterizing the association signals that have been found: large-scale resequencing and fine-mapping strategies will be required to recover the full allelic spectrum of causal variants and thereby obtain the most precise quantification of the genetic effects attributable to each locus. The part played by nonadditive interactions between different genetic loci and between susceptibility variants and environmental exposures needs to be charted, and discovery and replication studies need to be extended beyond the European populations that have been the focus of much of the current research.

Moving beyond genetics, there is work to be done to understand the novel (molecular, cellular, and physiological) biology revealed by these discoveries. If, as seems probable, many of the causal variants lie in noncoding regions, often some distance from the nearest coding sequence, they will often have subtle, spatially and/or temporally restricted effects. In such circumstances, gathering experimental evidence of their functional impact will be seriously difficult.

The final challenge lies in placing gene discovery into translational context. The clinical utility and validity of genetic diagnostics are already established in monogenic diabetes, where such testing can influence clinical practice and treatment. However, diagnostic genetic testing, still underutilized by most diabetologists, and further research, development, and education are required. It is a major challenge to establish how to use knowledge from the identification of predisposing polymorphisms in type 2 diabetes to improve the care of the diabetic patient. Definition of the underlying polymorphisms and genes is but a first step on this road.

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This article is dedicated to the memory of Robert Turner.

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