12 The Genetics of Type 2 Diabetes: From Candidate Gene Biology to Genome-wide Studies

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Keypoints

- Type 2 diabetes (T2DM) is a multifactorial heterogeneous disease with a complex interplay of genetic and environmental factors influencing intermediate traits such as β-cell mass and development, insulin secretion and action, and fat distribution.
- Three general approaches to identifying genetic susceptibility factors are used: the study of candidate genes selected as having a plausible role in glucose homeostasis; a genome-wide scan to detect chromosomal regions with linkage in nuclear families; a genome-wide scan for association with common variants (single nucleotide polymorphisms and copy number variant); and the study of spontaneous, bred or transgenic animal models of T2DM.
- Common T2DM shows familial clustering, but does not segregate in a classic Mendelian fashion. It is thought to be polygenic and probably multigenic (many different gene combinations among people with diabetes). Only a small fraction of the genetic risk for common adult T2DM is known.
- Common nucleotide variants within or near the genes implicated in monogenic forms of diabetes (e.g. *GCK, HNF4A* or *HNF1B/TCF2* in maturity-onset diabetes of the young [MODY], or *WFS1* in Wolfram syndrome) may contribute to T2DM with modest risk effects.
- Candidate genes identified as having an association with common polygenic T2DM include the insulin promoter, class III alleles of the variable region upstream of the insulin gene, the peroxisome proliferator-activated receptor γ (*PPARG*), *KCNJ11* encoding the pore forming Kir6.2 subunit of the β-cell inwardly rectifying K_{ATP} channel. Minor susceptibility might operate in some populations from other genes, including insulin receptor substrate 1 (*IRS-1*), adiponectin (*ACDC*) or ectonucleotide pyrophosphatase/phosphodiesterase 1 enzyme (*ENPP1*) in a context of obesity or diabesity.
- In genome scans of diabetic families, loci for T2DM have been found at several sites, including chromosomes 1q, 2q (*NIDDM1*), 2p, 3q, 12q, 11q, 10q and 20. *NIDDM1* has been identified as coding for calpain 10, a non-lysosomal cysteine protease with actions at the mitochondria and plasma membrane, and also in pancreatic β-cell apoptosis.
- In 2007, five large genome-wide association studies in European descent populations have identified new potential T2DM genes, including the Wnt signaling related transcription factors *TCF7L2* and *HHEX*, the zinc transporter ZnT8 (*SLC30A8*), the CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) and a regulatory protein for IGF2 (*IGF2BP2*). A consensus of close to 20 confirmed T2DM-susceptibility loci to date provided novel insights into the biology of T2DM and glucose homeostasis, but individually with a relatively small genetic effect. Importantly, these genes implicate several pathways involved in β-cell development and function.
- Compared with clinical risk factors alone, the inclusion of common genetic variants (at least those identified to date) associated with the risk of T2DM has a small effect on the ability to predict future development of T2DM. At the individual level, however, a combined genotype score based on 15 risk alleles confers a 5–8 fold increased risk of developing T2DM. Identifying the subgroups of individuals at higher risk is important to target these subjects with more effective preventative measures.

Genetic architecture of type 2 diabetes: a complex interaction of genetic susceptibility and environmental exposures

Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disease resulting from defects of both insulin secretion and action

[1,2]. The prevalence of diabetes has been estimated as 12.3% of the population of the USA, and worldwide to affect 285 million people with a projection to increase to 435 million by 2030 [3]; the vast majority of these individuals have T2DM. The disease affects various groups differently; minority racial groups including Hispanics, African-Americans, Native Americans or people living in the Middle East are affected at a higher rate than white individuals.

The etiology of T2DM is multifactorial, including genetic as well as prenatal and postnatal factors that influence several

different defects of glucose homeostasis, primarily in β-cell function and also in muscle and liver. It is generally accepted that T2DM results from a complex interplay of genetic and environmental factors influencing a number of intermediate traits of relevance to the diabetic phenotype (β-cell mass, insulin secretion, insulin action, fat distribution and obesity) [3]. Quantitative phenotypes related to glucose homeostasis are also known to be heritable, with a greater relative impact of genetic components on *in vivo* insulin secretion [4].

Although several monogenic forms of diabetes have been identified (see Chapter 15), such as maturity-onset diabetes of the young (MODY) and maternally inherited diabetes and deafness (MIDD) [5,6], diabetes in adulthood seems to be a polygenic disorder in the majority of cases. T2DM shows a clear familial aggregation, with a risk for people with familial diabetes that is increased by a factor of 2–6 compared with those without familial diabetes; it does not segregate in a classical Mendelian fashion, and appears to result from several combined gene defects, or from the simultaneous action of several susceptibility alleles, or else from combinations of frequent variants at several loci that may have deleterious effects when predisposing environmental factors are present [3,7]. T2DM is probably also multigenic, meaning that many different combinations of gene defects may exist among subgroups of people with diabetes. Whereas the current worldwide epidemic of T2DM is greatly driven by lifestyle and dietary changes, a combination of these environmental factors and susceptible genetic determinants contribute to the development of T2DM [7].

The primary biochemical events leading to common diabetes in adulthood are still largely unknown in most cases, even if new genetic and biologic insights into T2DM etiology have recently arisen from the completion of genome-wide association (GWA) studies in several European descent populations [3,7]. Genetic and environmental factors may affect both insulin secretion and insulin action [1], and a variety of environmental factors can be implicated in the clinical expression of T2DM (see Chapter 4), such as the degree and type of obesity, sedentary lifestyle, malnutrition in fetal and perinatal periods, lifestyle and different kinds of drugs such as steroids, diuretics and antihypertensive agents (see Chapter 16). It is noteworthy that obesity, which is one of the so-called risk factors of T2DM, is also clearly under genetic control. Both disorders are frequently associated and share many metabolic abnormalities, which suggests that they might also share susceptibility genes [8,9]. Moreover, retrospective studies have shown that low birth weight is associated with insulin resistance and T2DM in adulthood [10,11]. It has been proposed that this association results from a metabolic adaptation to poor fetal nutrition [12]. The identification of gene variants that contribute both to variation in fetal growth and to the susceptibility to T2DM, however, suggests that this metabolic "programming" could also be partly genetically determined [13].

These complex interactions between genes and environment complicate the task of identifying any single genetic susceptibility factor for T2DM. Three general approaches have been adopted

Table 12.1 Genetic and genomic approaches used for the study of type 2 diabetes genes.

Family-based studies
Linkage studies (genome-wide scan with microsatellite markers)
Transmission disequilibrium test (in sibships or nuclear families)
Study of monogenic diabetes (extreme phenotypes, syndromic diseases)

Population-based studies
Survey of candidate genes and targeted genomic regions (~100 SNPs)
Genome-wide association study (HapMap-based SNPs choice, 500K–1M SNPs)
Other types of genetic markers (rare variants, CNVs, insertion/deletion)
Study of general populations and prospective cohorts (epidemiology-based data)

Functional genomic approaches
Gene expression profiling, transcriptomics (microarray gene expression data in human and animal models)
Molecular and cellular analyses (*in vitro* and *in vivo* model systems)
Expressed quantitative trait linkage (in human and animal models)
Epigenetics (in humans, and animal models)

CNV, copy number variant; SNP, single nucleotide polymorphism.

to search for genes underlying complex traits such as T2DM [6], as described in Table 12.1.

The first approach was to focus on candidate genes, that is, genes selected as having a plausible role in the control of glucose homeostasis and/or insulin secretion, on the basis of their known or presumed biologic functions. Although this approach has led to the identification of several susceptibility genes with small effects (see below), no genes with a moderate or major effect on the polygenic forms of diabetes have been found. Possible explanations for this failure to identify genes with a major effect include the possibility that they do not exist, or our incomplete knowledge of the pathophysiologic mechanisms of T2DM and the genes that control them, has obscured the choice of candidates.

The second approach is to perform genome-wide scans for linkage in collections of nuclear families or sib-pairs with T2DM [14], or for GWA in large case–control samples, as was performed in recent years [15]. These "hypothesis-free" approaches require no presumptions as to the function of the susceptibility loci. Although a large number of genomic regions with presumed linkage have been mapped [11–14], identification of the susceptibility genes and causal variants within these regions has proceeded at a very slow pace. In contrast, the recently completed GWA studies from several independent European case–control cohorts have released at least a dozen of confirmed at-risk loci, with unexpected susceptibility genes for T2DM and a number of at-risk variants having been replicated in most of the European populations investigated [6,15,16].

A third approach has used microarray gene expression analysis in attempt to define genetic alterations in T2DM. Through such analysis, a defect in skeletal muscle of people with diabetes was

discovered and characterized by a coordinated decrease in the expression of nuclear-encoded genes involved in mitochondrial oxidative phosphorylation [17]. This defect appears to be secondary to reduced expression of the transcriptional coactivators PGC-1α and PGC-1β. Similar changes in expression have been observed in some cohorts of first-degree relatives of individuals with diabetes, suggesting that these may be heritable traits [18]. A second potential gene is the transcription factor ARNT/HIF1β, which was identified using islets isolated from individuals with T2DM compared with normal glucose-tolerant controls. Reduced ARNT levels in human diabetic islets were associated with altered β-cell expression of other genes involved in glucose sensing, insulin signaling and transcriptional control [19], suggesting an important role for ARNT in the impaired islet function of human T2DM.

Another complementary approach to identifying diabetes genes is to study spontaneous (such as the Zucker diabetic fatty rats or fat/fat mice, [20]), bred (like the GK rats, [21]) or transgenic (using β-cell-specific gene inactivation, [22–24]) animal models of T2DM. The genes responsible for diabetes in these models may not necessarily be major players in typical T2DM in humans, but such studies provide the most direct way of improving the overall understanding of the molecular circuitry that maintains glucose homeostasis. Nevertheless, despite the evidence of a strong genetic background in T2DM, very little is yet known about the genetic risk factors for T2DM. By far the most striking results in defining etiologic genes have been obtained by studying the highly familial MODY form of young-onset diabetes or other rare forms of monogenic diabetes.

Genes involved in monogenic diabetes and their relevance to adult-onset T2DM

Some of the most compelling evidence that inherited gene defects can cause glycemic dysregulation comes from the clinical and genetic description of monogenic forms of diabetes, including MODY characterized by an autosomal dominant inheritance of young-onset diabetes, neonatal or early infancy diabetes, or diabetes with extrapancreatic features (see Chapter 15) [4,6]. Although rare, these monogenic forms of diabetes provide a paradigm for understanding and investigating some of the genetic components of more complex forms of diabetes in adults.

The well-defined mode of inheritance of MODY, with a high penetrance and early-onset diabetes, allows the collection of multigenerational pedigrees, making MODY an attractive model for genetic studies. MODY usually develops in thin young adults (usually before 25 years of age; in childhood, adolescence or young adulthood), and is associated with primary insulin-secretion defects [4,5]. The prevalence of MODY is estimated to be less than 1–2% of patients with T2DM, although it could represent as many as 5% of European cases of diabetes [4,25]. MODY is not a single entity, but involves genetic, metabolic and clinical heterogeneity. So far, heterozygous mutations or chromosome rearrangements in seven genes have been identified as responsible for the disease (Table 12.2). These genes encode the enzyme glucokinase (*GCK*, MODY type 2) [26–28], the transcription factors hepatocyte nuclear factor 4α (HNF-4α//*HNF4A*, MODY type 1) [29], hepatocyte nuclear factor 1α (HNF-1α/*HNF1A*,

Table 12.2 The different subtypes of maturity-onset diabetes of the young (MODY).

MODY type	Gene locus	Gene name	Year of discovery	Distribution	Onset of diabetes	Primary defect	Severity of diabetes	Complications	OMIM
MODY1	20q	*HNF4A* (*TCF14*)	1996	Rare (2–3%)	Adolescence/ early adulthood	Pancreas/other	Severe	Frequent	600281
MODY2	7p	*GCK*	1992	20–60%*	Early childhood/ life-long	Pancreas/liver	Mild IFG	Rare	138079
MODY3	12q	*TCF1* (*HNF1A*)	1996	15–60%*	Adolescence/ early adulthood	Pancreas/kidney other	Severe	Frequent	142410
MODY4	13q	*IPF-1*	1997	Rare (<1%)	Early adulthood	Pancreas/other	Severe	Unknown; pancreas agenesis and NDM in homozygote (rare)	600733
MODY5	17q	*TCF2* (*HNF1B*)	1997	Rare (2%)	Early adulthood	Kidney/pancreas	Severe	Kidney disease (RCAD); pancreas agenesis and NDM in homozygote (rare)	189907
MODY6	2q32	*NEUROD1*	1999	Rare (<1%)	Early adulthood	Pancreas	Severe	Unknown; NDM in homozygote (rare)	601724
MODY7	11p15.5	*INS*	2008	Rare (<1%)	Early childhood/ early adulthood	Pancreas	Mild to severe	Rare	176730

IFG, impaired fasting glycemia; NDM, neonatal diabetes mellitus; RCAD, renal cysts and diabetes syndrome.
*Different distributions in different populations.

MODY type 3) [30,31], insulin promoter factor 1 (IPF-1, MODY type 4) [32,33], hepatocyte nuclear factor 1β (HNF-1β/*TCF2,* MODY type 5) [34] and NEUROD1/β2 (MODY type 6) [35], and the preproinsulin (*INS*) (MODY type 7) [6]. Moreover, additional unknown genes (MODY-X) related to the MODY phenotype remain to be discovered from families in which early-onset diabetes cosegregates with genetic markers outside the known *MODY* loci [36–38].

The relative prevalence of the different subtypes of MODY has been shown to vary greatly in studies of British, French, German and Spanish family cohorts [39–42]. These contrasting results may be caused by differences in the genetic background of these populations, or else may reflect, at least partly, ascertainment bias in the recruitment of families. Altogether, mutations in *GCK* and *HNF1A* are the cause of the two most prevalent MODY2 and MODY3 subtypes, accounting for around 50–60% of all MODY cases. Mutations in *HNF4A* and *TCF2*/Hnf-1β were identified in many dozens of families and the other defects caused by mutations in *PDX1* and *NEUROD1* are rarer disorders [43,44].

Recent challenging studies have tried to identify a connection between monogenic diabetes genes and common T2DM, that is to test whether common but less severe variants might have a role in the pathogenesis of common multifactorial forms of the disease. Indeed, if major mutations (i.e. causing a substantial functional defect and normally rare or absent in the general population) lead to a highly penetrant form of diabetes, it seems plausible that more subtle genetic changes affecting the structure or expression of the gene product might have a role in determining (minor) susceptibility to T2DM. Our current understanding of genetic variants influencing T2DM supports this hypothesis [3,4,6], but common variants (also referred as single nucleotide polymorphisms [SNPs]) in the known MODY genes seem to contribute very modestly to the common forms of T2DM, as recently assessed in a staged case–control study from multiple clinical samples [45]. In this study, the strongest effects were found for an intronic variant of *TCF2*/HNF-1β and for the −30 G/A variant of *GCK*. The −30 G/A polymorphism in the β-cell specific promoter of glucokinase was found to modulate diabetes risk, with the (−30) A-allele being associated with an increased risk) [45]. A similar genetic association was observed with a stronger effect in a French prospective study of a middle-aged general population, along with a significant impact on the modulation of fasting glycemia and insulin secretion HOMA-B index [46]. Furthermore, a meta-analysis of previously reported association results for *GCK* (−30A) with T2DM showed a modest overall effect on disease risk of 1.08 (*P* = 0.004) in European populations [45].

Mutations in *HNF1α* were identified in African-Americans and Japanese subjects with atypical non-autoimmune diabetes with acute onset [47,48]. In the Oji-Cree native Canadian population the G319S mutation in *HNF1A*, found in approximately 40% of patients with diabetes, accelerates the onset of T2DM by 7 years [49]. Such findings show that *HNF1A* mutations can be associated with typical adult-onset insulin-resistant obesity-related diabetes in addition to MODY.

A population-based study in Swedish and Finnish cohorts using both *in vitro* and *in vivo* experiments has shown that common variants in and upstream of the *HNF1A* gene influence transcriptional activity and insulin secretion *in vivo* [50]. Some of these variants are associated with a modestly higher risk of diabetes in subsets of elderly overweight individuals. Mutations in *HNF4A* and *IPF1* genes were also identified in a number of families with late-onset T2DM [51]. IPF-1/Pdx-1 has a dosage-dependent regulatory effect on the expression of β-cell-specific genes and therefore assists in the maintenance of euglycemia. As a consequence, frequent variants in the regulatory sequences controlling IPF-1/Pdx-1 expression in the β-cell, or in genes coding for transcription factors known to regulate IPF-1, could contribute to common T2DM susceptibility. Two independent studies, from the Ashkenazim [52] and Finnish [53] populations, have reported significant associations between common variants adjacent to the *HNF4A* P2 promoter and T2DM. Interestingly, some of the diabetes-associated variants account for most of the evidence of linkage to chromosome 20q13 reported in these two populations. Consistent with these results, genetic variation near the P2 region of *HNF4A* is associated with T2DM in other Danish and UK populations, but not in French and other Caucasian populations [54] which argues for genetic heterogeneity in *HNF4A* variants susceptibility [55].

Recent studies have evaluated the genotype–phenotype correlation between the MODY genes and the common form of T2DM and have reported that several common variants (MAF >0.05) in *TCF2/HNF1β* may contribute to T2DM risk but with modest effects (allelic odds ratio [OR] <1.25); the strongest effect was found for an intronic variant with corrected *P* values <0.01 and OR of 1.13 [45]. Independently, a GWA scan performed to search for sequence variants conferring risk of prostate cancer demonstrated with replication from eight case–control groups that two intronic variants located in the first and second intron of *TCF2* gene confer protection against T2DM (OR 0.91, $P \sim 10^{-7}$) in individuals of European, African and Asian descent [56]. Several epidemiologic studies have reported an inverse relationship between T2DM and the risk of prostate cancer, and a recent meta-analysis estimated the relative risk of prostate cancer to be 0.84 (95% CI 0.71–0.92) among patients with diabetes [57]. Previous explanations of this inverse relationship between T2DM and prostate cancer have centered on the impact of the metabolic and hormonal environment in men. The protective effect of the *TCF2* SNPs against T2DM is too modest to explain their impact on prostate cancer risk by a consequence of an effect on diabetes. The primary functional impact of *TCF2* variants may lie within one or more metabolic or hormonal pathway, and incidentally may modulate the risk of developing prostate cancer and T2DM throughout life.

Genetic variation in the *WFS1* gene, which encodes a 890-amino-acid polypeptide named wolframin, a transmembrane protein located in the endoplasmic reticulum (ER), which serves as an calcium channel, not only results in the rare Wolfram

syndrome (known as DIDMOAD and characterized by early-onset non-autoimmune diabetes mellitus, diabetes insipidus, optic atrophy and deafness) [58,59] but is also associated with susceptibility to adult T2DM [60]. In a pooled case–control analysis comprising >20 000 individuals, several SNPs in *WFS1* (including a non-synonymous SNP, R611H) were shown to modulate diabetes risk (OR ~0.92 for a minor allele frequency of ~40% [60]), with a population attributable fraction of 9%, which could explain 0.3% of the excess familial risk. This study provides further evidence that wolframin has an essential role in the ER stress response in insulin-producing pancreatic β-cells, and contributes to the risk of common T2DM.

Search for T2DM genes in the pre-GWA era

Before the completion of GWA studies in early 2007, numerous focused candidate gene studies, driven by biologic or functional hypotheses, and the hypothesis-free linkage approach, have delivered a small number of validated T2DM susceptibility genes which effects the disease process (both insulin secretion and insulin sensitivity), although contributing at the individual level with a modest risk effect.

Candidate gene approach

The molecular screening of candidate genes to search for genetic variants (either rare when the allele frequency is <0.01, or common in the population tested) potentially associated with diabetes status (i.e. more frequent in individuals with T2DM) has so far been the most frequently used approach to tackle the genetic determinants of T2DM [61]. There are many reasons why specific genes may be candidates:

• A gene may have a known or presumed biologic function in glucose homeostasis or energy balance in humans.
• It may be implicated in subtypes of diabetes, such as MODY or neonatal diabetes.
• It may be associated with diabetes or associated traits in animal models.
• It may be responsible for an inherited disease that includes diabetes (e.g. mitochondrial cytopathies, Wolfram syndrome).
• The gene products may be differentially expressed in diabetic and normal tissues.

For obvious reasons, the insulin gene was among the first genes to be studied. Apart the more recent identification of rare mutations in the sequence of the preproinsulin gene responsible for neonatal and non-autoimmune early infancy diabetes [5,6], mutations in the coding regions of the insulin gene have been reported to be associated with familial hyperinsulinemia in less than 10 families, but are not consistently associated with T2DM [62]. Mutations in the promoter region, however, could affect the regulation of the insulin gene, leading to absolute or relative hypoinsulinemia. A variant allele of the promoter has been observed in about 5% of African-Americans with T2DM, and shown to be associated with decreased transcriptional activity

[63]. An association between T2DM and paternally transmitted class III alleles of the variable number tandem repeat (VNTR) region upstream of the insulin gene (*INS-VNTR*) was observed in British families [64]. Interestingly, class III alleles (one of the two main classes of *INS-VNTR* allele length, with 141–209 repeats) were also found to be associated with increased length and weight at birth [65] and with a dominant protection against type 1 diabetes [66] compared with type I alleles.

Key components of the insulin signaling pathways have also been tested. They were at first thought to be important players in the context of the insulin resistance of T2DM. Several of these genes are also expressed in pancreatic β-cells, and several studies from knockout animals have demonstrated that they may also have an important role in the mechanisms of insulin secretion [23,24]. More than 50 different mutations have been found in the coding regions of the insulin receptor gene on chromosome 19p (see Chapter 15) [67]; patients with these mutations seldom present with the common form of T2DM [68], but rather with a syndrome of severe insulin resistance associated with leprechaunism, or with acanthosis nigricans, hirsutism and major hyperinsulinemia [69]. Missense variants in the gene encoding the first substrate for the insulin receptor kinase (*IRS1*) on chromosome 2q have been detected in several populations [70–73] but an association of these variants with diabetes was not observed in all studies [74,75].

Similarly, an association between polymorphisms of the muscle glycogen synthase gene (*GYS1*) on chromosome 19q and T2DM has been observed in Finnish [76] and in Japanese [77] subjects, but not in French subjects [78]. Taken together, these results suggest that the *IRS1* and *GYS1* genes may act in some populations as minor susceptibility genes, which are neither necessary nor sufficient for disease expression, but may nevertheless modulate the phenotype in the patients. This is important in the light of more recent data obtained from combined and meta-analyses conducted by GWA follow-up studies, which report a significant effect of common variants at the *IRS1* locus on T2DM risk in European descent populations (unpublished results).

Functionally significant polymorphisms have also been identified in other proteins involved in insulin action, such as the phosphatidylinositol 3 kinase [79] and the ectonucleotide pyrophosphatase/phosphodiesterase 1 enzyme (ENPP1 or PC-1), a transmembrane glycoprotein that downregulates insulin signaling by inhibiting insulin-receptor tyrosine kinase activity [80]. ENPP1 is expressed in multiple tissues including three targets of insulin action: adipose tissue, muscle and liver. A missense variation in its gene *ENPP1*, in which lysine 121 is replaced by glutamine (K121Q, rs1044498), results in a gain-of-function mutation leading to greater inhibition of the insulin receptor and clinical insulin resistance [81]. Pizutti *et al.* [82] first identified the association while studying healthy non-obese non-diabetic Sicilian subjects. *ENPP1* K121Q is also associated with an earlier onset of T2DM in Europeans and obese children, suggesting that the variant may accelerate disease onset in predisposing individu-

als. Not all studies have replicated these results, and some studies have reported that high body mass index (BMI) exacerbates, or in some cases is a prerequisite for, the deleterious role of *ENPP1* K121Q on insulin resistance-related phenotypes and T2DM [83,84]. Meyre *et al.* [85] reported an effect of the Q121 variant on the risk of both morbid obesity and hyperglycemia in large samples of French children and adults, as observed in children from Germany [86] and British adults [87]. Given the major role of obesity in deteriorating glucose homeostasis, the different effect of the Q121 variant on BMI in different samples may be, at least partly, responsible for the non-homogeneous results so far reported on the risk of across different studies. A recent large meta-analysis performed on published case–control studies has shown that, although results are not homogeneous across all samples, individuals carrying the variant have an approximately 20% increased risk of T2DM [88].

Another biologic candidate gene that was extensively studied is the peroxisome proliferator-activated receptor γ gene (*PPARG*), where mutations that severely decrease the transactivation potential were found to cosegregate with extreme insulin resistance, diabetes and hypertension in two families, with autosomal-dominant inheritance [89]. A common amino-acid polymorphism (Pro12Ala) in *PPARG* has been associated with T2DM; homozygous carriers of the Pro12 allele are more insulin resistant than those having one Ala12 allele and have a 1.25-fold increased risk of developing diabetes [90]. This common polymorphism has a modest, yet extensively replicated effect on the risk of T2DM. There is also evidence for interaction between this polymorphism and the insulin secretion in response to fatty acids [91], and BMI [92]; the protective effect of the alanine allele was lost in subjects with a BMI greater than 35 kg/m². A widespread Gly482Ser polymorphism of PGC1-α (known as *PPARGC1*), a transcriptional coactivator of a series of nuclear receptors including *PPARG*, has been associated with a 1.34 genotype relative risk of T2DM [93]. In this study, a test for interaction with the Pro12Ala variant in *PPARG* gave no indication for additive effects on diabetes status.

Other genes have been shown to be implicated in the genetic susceptibility to insulin resistance. Although they do not appear to be directly linked or associated with T2DM, they may also modulate the disease expression. A common and widespread polymorphism at codon 905 in the gene encoding the glycogen associated regulatory subunit of protein phosphatase 1 (PP1), which is expressed in skeletal muscle and plays a crucial part in muscle tissue glycogen synthesis and breakdown, has been shown to be associated with insulin resistance and hypersecretion of insulin in Danish subjects with T2DM [94]. A missense mutation in the intestinal fatty acid binding protein 2 (*FABP2*) gene on chromosome 4q has been found to be associated with increased fatty acid binding, increased fat oxidation and insulin resistance in the Pima Indians of Arizona [95], an ethnic group with the highest reported prevalence of T2DM and insulin resistance in the world. A rare P387L variant in protein tyrosine phosphatase-1B (PTP-1B), a negative regulator for insulin and leptin signaling, has been described to be associated with a 3.7 genotype-relative risk of T2DM in a Danish Caucasian population, resulting in impaired *in vitro* serine phosphorylation of the PTP-1B protein [96]. In an extensive analysis of the *PTPN1* gene locus, Bento *et al.* [97] found convincing associations between multiple SNPs and T2DM in two independent Caucasian American case–control samples. A modest effect of *PTPN1* gene variants on disease risk was observed in the French population, whereas consistent associations with metabolic variables reflecting insulin resistance and dyslipidemia were found for two intronic SNPs, thereby influencing susceptibility to metabolic diseases [98].

Mutations in transcription factors have also been reported to contribute to the genetic risk for T2DM through various mechanisms: dysregulation of target genes involved in glucose or lipid metabolism (HNFs, *PPARG*, *IPF-1*, *IB1*, *TIEG2/KLF11*), impaired β-cell development and differentiation (*IPF-1*, *NEUROD1*/β2, *TIEG2/KLF11*), and increased β-cell apoptosis (*IB1/MAPK8IP1*). Deleterious mutations that significantly impair the transactivation activity of these transcription factors can be responsible in some families for monogenic-like forms of diabetes with late age of onset, which may represent an intermediary phenotype between MODY and the most common forms of T2DM. This is the case for the *TIEG2/KLF11* gene encoding the Krüppel-like factor 11 (KLF11), an SP1-like pancreas expressed transcription factor that is induced by the transforming growth factor β (TGF-β) and regulates cell growth in the exocrine pancreas. A common polymorphism (Q62R) in *KLF11* was reported to be associated with polygenic T2DM developing in adulthood and to affect the function of KLF11 *in vitro* [99]. Insulin levels were found to be lower in carriers of the minor allele at Q62R [99] but attempts of replication in other populations only found a minor, or no detectable effect of the Q62R common variant on diabetes risk [100]. Sequencing of *KLF11* gene in families enriched for early-onset T2DM uncovered two missense mutations which segregated with diabetes in three pedigrees [99], but proof of their causality was only based on *in vitro* experiments. These findings suggest a role for the TGF-β signaling pathway in pancreatic diseases affecting endocrine islets (diabetes) or exocrine cells (cancer) [101].

Another example is the identification of a mutation in islet brain 1 (*IB1*, *MAPK8IP1*) found to be associated with diabetes in one family [102]. *IB1* is a homologue of the *c-jun* amino-terminal kinase interacting protein 1 (JIP-1), which has a role in the modulation of apoptosis [103]. *IB1* is also a transactivator of the islet glucose transporter GLUT-2. The mutant *IB1* was found to be unable to prevent apoptosis *in vitro* [102]. It is thus possible that the abnormal function of this mutant *IB1* may render the β-cells more susceptible to apoptotic stimuli, thus decreasing β-cell mass. As glucotoxicity and lipotoxicity are known to induce both apoptosis and transcription factor down regulation in pancreatic β-cells, inherited or acquired defects in *IB1* activity could have deleterious effects in β-cell function.

Other genes encoding key components of insulin secretion pathways have also been tested as potential candidates for a role in the genetic susceptibility of T2DM. The pancreatic β-cell ATP-sensitive potassium channel (IKATP) has a central role in glucose-induced insulin secretion by linking signals derived from glucose metabolism to cell-membrane depolarization and insulin exocytosis (see Chapter 6) [104]. IKATP is composed of two distinct subunits: an inwardly rectifying ion channel forming the pore (Kir6.2), and a regulatory subunit, which is a sulfonylurea receptor (SUR1) belonging to the ATP-binding cassette (ABC) superfamily [105]; both subunits are expressed in neuroendocrine cells. The *KCNJ11* and *ABCC8* genes encoding these two subunits are located 4.5 kb apart on human chromosome 11p15.1. Inactivating mutations in each of these genes may result in familial persistent hyperinsulinemic hypoglycemia in infancy, and gain-of-function mutations are responsible of the opposite phenotype of neonatal diabetes (either permanent or transient forms of the disease with distinct distributions of mutations in each gene), demonstrating their role in the regulation of insulin secretion (see Chapter 15) [106]. Studies in various populations with different ethnic backgrounds have provided evidence for associations of SNPs in these genes with T2DM [107–112]. In particular, a common variant, E23K, of *KCNJ11*/K_{IR}6.2 has now been convincingly associated with an increased risk of T2DM and decreased insulin secretion in glucose-tolerant subjects [113,114]. Large-scale studies and meta-analyses have consistently associated the lysine variant with T2DM, with an OR of 1.15 [115].

Increasing evidence from more recent studies also suggested that inflammatory processes may have a pivotal role in metabolic diseases: prospective studies have shown that high plasma interleukin 6 (IL-6) levels increased T2DM risk [116], but conflicting associations were found between a promoter polymorphism (G-174C) in *IL6* and T2DM [117,118]. In a large joint analysis of 21 case–control studies, representing >20 000 participants in one of the largest association studies addressing the role of a candidate gene in T2DM susceptibility, the *IL6* promoter variant was found to be associated with a lower risk (OR 0.91, $P = 0.037$) [119]. In addition, association between T2DM and *IL6R*-D358A was reported in Danish white people [120], and with *TNF* G-308A promoter SNP in the Finnish Diabetes Prevention Study [118]. The effects of both *IL6* and *IL6R* variants on developing T2DM risk in interaction with age have been reported in a prospective study of a general French population [46].

Hypothesis-free genome-wide approach and positional cloning of T2DM genes

The second approach used in the years 1995–2005 for identifying genes underlying common polygenic T2DM, the so-called genome-wide familial linkage (GWL) studies, was based on genome-wide scans to detect chromosomal regions showing linkage with diabetes in large collections of nuclear families or sib-pairs. This strategy required no assumptions regarding the function of genes at the susceptibility loci, because it attempted to map genes purely by position. Genotyping of approximately

400 multiallelic markers (short tandem repeats or microsatellites, with a density of ~1 marker/10 cmol) allows identification of polymorphic markers showing strong allele identity by descent in diabetic family members (i.e. allele sharing in sibships is significantly higher than 50%). Once identified, such susceptibility genes for diabetes may then be positionally cloned in the intervals of linkage.

This total genome approach has been used for some time in other multifactorial diseases such as type 1 diabetes [121] and obesity [5]. More than 20 genome scans for T2DM have been completed, involving thousands of pedigrees from different populations and ethnic groups. One of the limitations of the genome-scan approach is the relatively low power of the method, which is unable to detect a weak linkage signal because of the low relative risk for diabetes in siblings (about a three- to fivefold increase in comparison with the general population). Working on very large family collections (>500 sib-pairs), in more homogeneous ethnic groups (e.g. island populations), or in large pedigrees using quantitative intermediary traits instead of the dichotomous diabetes status, has improved the effectiveness of this approach in detecting linkage. Indeed, intermediary phenotypes were shown to have greater heritability than the dichotomous endpoint. To avoid bias brought about by the effect of chronic hyperglycemia on many traits, other strategies have been proposed to analyze the general population [122] or to collect sibships of the offspring of subjects with diabetes who are not yet diabetic but are at high risk of developing the disease [123]. False-positive results are likely to occur. Thus, stringent criteria for linkage (in the magnitude of $P < 10^{-5}$) need to be used to minimize bias brought about by multiple testing. Indeed, a level of statistical significance at the genome-wide level (i.e. giving a less than 5% chance that a linked locus is false) implies a multipoint lod score higher than 3.6 at a given chromosomal region. This value is not a rigid one; each case is different, and sophisticated simulations are needed to provide an empirical *P* value, and therefore to assess the robustness of the data obtained. Indeed, the validation (or the absence of validation) of a linked locus has strong implications for further research, and careful examination is necessary. For this reason, another criterion for a true locus has been the replication of the findings by others. Although less stringent *P* values are required (10^{-3}), replication is difficult, because genetic heterogeneity, ascertainment biases and phenotypic differences across studies may mask a confirmatory linkage. Furthermore, genotyping errors have a substantial effect on linkage analyses.

Since 1996, the results of several genome scans in families with diabetes have been published [123]. A summary of the best T2DM linkage replication results is presented in Table 12.3. A locus for T2DM on chromosome 2q (*NIDDM1*) was initially found in Mexican Americans [14], and it was shown that an interaction between this locus and a locus on chromosome 15 further increases the susceptibility to diabetes in this population [124]. A region of replicated linkage between diabetes and

Table 12.3 Genomic locations of best type 2 diabetes linkage replications.

Chromosome location	LOD ≥ 3.0*	LOD ≥ 3.6*	Ethnic contribution†	Phenotypes‡ in the interval	Potential or validated candidate genes
1q21-q25.3	6	4	Af(1)CJ(2)Eu(9)MA(4)	10D6Q	PKLR, USF1, DUSP12, APOA2, RXRG
2q24.1-q31.1	2	1	Eu(4)MA(2)Yc(3)	4D5Q	
2q35-q37.3	3	1	Af(1)CJ(2)Eu(6)MA(1)Yc(3)	8D5Q	CAPN10
3q27-q29	3	1	Eu(4)MA(1)Yc(1)	2D4Q	ACDC, IGF2BP2
6q23.1-q24.1	3	2	CJ(3)Eu(6)MA(2)Yc(1)	8D4Q	ENPP1
8p23.3-p12	3	2	Af(1)CJ(1)Eu(7)MA(4)Yc(2)	9D6Q	
9q21.11-q21.31	2	1	CJ(4)Eu(2)MA(1)	4D3Q	
10q25.1-q26.3	2	0	Af(2)CJ(1)Eu(5)MA(4)	4D8Q	TCF7L2
14q11.2-q21.1	1	0	CJ(2)Eu(7)MA(1)Yc(1)	7D4Q	
17p13.3-q11.2	3	1	Af(1)CJ(1)Eu(7)MA(1)Yc(3)	5D8Q	
18p11.32-p11.21	2	1	Af(1)CJ(1)Eu(7)Yc(1)	6D4Q	
19p13.3-p13.12	1	1	Af(2)Eu(5)MA(3)	4D6Q	
19q13.32-q13.43	2	0	Af(1)Eu(6)MA(1)Yc(1)	1D8Q	
20q11-q13.32	2	1	Af(1)CJ(5)Eu(5)MA(1)	9D3Q	HNF4A, PTPN1
22q11.1-q12.3	2	0	Af(1)CJ(1)Eu(5)MA(3)	5D5Q	

*The data on the number of LOD scores in each interval that met the threshold criteria are adapted from the study by Lillioja & Wilton [206].

†Ethnic origins of the populations showing a genetic linkage in each interval (Af, African; CJ, Chinese/Japanese; Eu, European; MA, Mexican American; Yc, other/combined data) followed in parentheses by the number of such results.

‡Phenotypes in each interval (D, type 2 diabetes; Q, quantitative trait) and the number of each type of result.

chromosome 1q21-25 was reported in different populations of European origin and in Pima Indians [123]. Linkages with diabetes and with the age at onset of diabetes were found on chromosome 3q27 [125–127], and in a region on chromosome 10q in Mexican American families from San Antonio, Texas [128]. Linkage was found at a locus near *TCF1* on chromosome 12q in Finnish T2DM families, characterized by a predominant insulin secretion defect [13]. Evidence for an obesity–diabetes locus on chromosome 11q23-q25 [129] and linkage of several chromosomal regions with prediabetic traits [14] were observed in Pima Indians from Arizona, an ethnic group with a high prevalence of diabetes and obesity. Evidence for the presence of one or more diabetes loci on chromosome 20 was found in different populations [130,131]. In these and other studies, a large number of loci showing only suggestive or weak indication of linkage with diabetes-related traits have also been reported, several of which fall in overlapping regions.

Some concerns have therefore been raised about the heterogeneity and reliability of genetic data in multifactorial diseases in general (e.g. the lack of replication), and in T2DM in particular. A new series of genome scans published in 2000 and later [125–127], however, has surprisingly shown that results obtained from T2DM families may be reproducible, despite differences in ethnicity and in environmental factors. It is likely that progress both in automated genotyping and statistical analyses of larger sample sets have improved the quality of the data. A meta-analysis of the data obtained from all European studies has confirmed findings

on 1q, 2p and 12q, and also revealed a new locus on 17p linked to the metabolic syndrome in the US population [132,133]. These data provide a strong basis for believing that some important genetic contributors to common T2DM exist.

Although genome-wide scans have mapped T2DM loci, each hit represents 10–20 million nucleotides. The challenge was to identify the diabetes-related genes within these intervals, knowing that hundreds of genes may be present. To identify the true etiologic gene variants associated with an enhanced risk for T2DM, one first positional cloning approach was to refine the chromosomal regions of linkage using a dense map of biallelic SNPs as markers for the detection of linkage disequilibrium (LD) mapping [134–136]. DNA polymorphisms located away from the true functional variant may be associated with the disease, or with the variation of a diabetes-associated trait. The strength of LD is quite variable within the genome, ranging from 10 to 300 kb or more. It has been postulated that working in a so-called "isolated" population should significantly enhance results with LD, but even research in Finns or in Icelanders may not resolve the problems [137].

The identification of the *NIDDM1/CAPN10* gene (coding for calpain-10, a non-lysosomal cysteine protease) on 2q37 confirmed that LD mapping may be a successful strategy for unravelling other polygenic diseases [138]. This work, however, also showed the complexity of the search, because in this case an intronic polymorphism (UCSNP-43) was associated with T2DM in Mexican Americans. In fact, three non-coding polymorphisms,

including SNP-43, SNP-44 and SNP-63, have been identified as defining an at-risk haplotype [138]. In other ethnic groups, such as French Caucasians, the rarity of this high-risk haplotype made it difficult to achieve a definite answer concerning the role of calpain-10 in T2DM. Nonetheless, recent pooled and meta-analyses as well as the LD and haplotype diversity studies suggest a role for genetic variation in *CAPN10* affecting risk of T2DM in Europeans [139]. *CAPN10* was thus the first diabetes gene to be identified through a genome-wide scan approach. Many investigators, but not all, have subsequently found associations between *CAPN10* polymorphism(s) and T2DM as well as insulin action, insulin secretion, aspects of adipocyte biology and microvascular functions. This has not always been with the same SNP or haplotype or the same phenotype, suggesting that there might be more than one disease-associated *CAPN10* variant and that these might vary between ethnic groups and the phenotype under study. Indeed, both genetic and functional data indicated that calpain-10 has an important role in insulin resistance and intermediate phenotypes, including those associated with the adipocyte [140]. In this regard, emerging evidence would suggest that calpain-10 facilitates GLUT-4 translocation and acts in the reorganization of the cytoskeleton. Interestingly, calpain-10 is also an important molecule in the β-cell. It is likely to be a determinant of fuel sensing and insulin exocytosis, with actions at the mitochondria and plasma membrane, respectively, and also of pancreatic β-cell apoptosis [141].

Only a few other chromosomal regions have shown highly significant lod scores in several populations. The best example is probably the chromosome 1q21-25 region (in particular the 30 Mb stretch adjacent to the centromere), which ranks alongside the regions on chromosomes 10 and 20 as amongst the strongest in terms of the replicated evidence for GWL to T2DM. Linkage has been reported in samples of European (UK, French, Amish, Utah), East Asian (Chinese, Hong Kong) and Native American (Pima) origin (summarized in [123]). The homologous region has also emerged as a diabetes-susceptibility locus from mapping efforts in several well-characterized rodent models [142]. The human region concerned is gene-rich and contains a disproportionate share of excellent biologic candidates, and genetic variation in a number of them has already been investigated with details in the populations showing 1q21-linkage (e.g. the genes *USF1* encoding the upstream stimulatory factor 1, *LMNA1* encoding Lamin A/C, *PBX1* encoding a TALE-homeodomain transcription factor that has a role in pancreatic development and is an important cofactor of Pdx1, the "master regulator" of pancreatic development and function, *ATF6* encoding activating transcription factor 6, or *DUSP12* encoding the glucokinase-associated, dual-specificity phosphatase 12). These studies have been conducted by an international consortium, which represent a coordinated effort by the groups with the strongest evidence for 1q linkage, by using a high-density, custom LD mapping approach applied to a well-powered set of multi-ethnic samples to identify variants causal for that signal [143]. Detection of low-frequency susceptibility variants also requires new approaches and the

future plans of the 1q consortium include deep resequencing of the 1q region of interest, focusing at least initially on exons and conserved sequence.

Linkage with chromosome 20 has been also found in several ethnic groups, from Finns to Japanese subjects [147], but the linked region is rather large, raising the issue of whether there may be several susceptibility genes. While common variants upstream of *HNF4A* that maps to chromosome 20q13 have been reported to explain the linkage signals seen in Finns and Ashkenazim [144,145], these associations have proved difficult to replicate in many other populations [146].

Loci overlapping between T2DM, obesity and the metabolic syndrome have appeared on chromosome 2p [125,147] and on chromosome 3q27 [125,126,127]. Several candidate genes map to the 3q27 region, including the *APM1/ACDC* gene encoding the differentiated adipocyte-secreted protein, adipocyte complement-related protein 30 (Acrp30/adiponectin), which is an adipokine abundantly present in plasma. The purified C-terminal domain of adiponectin has been reported to protect mice on a high-fat diet from obesity, and to rescue obese or lipoatrophic mice models from severe insulin resistance, by decreasing levels of plasma free fatty acids (FFAs) and enhancing lipid oxidization in muscle [148]. Moreover, plasma levels of adiponectin have been shown to be decreased in obese subjects with diabetes and to correlate with insulin sensitivity [149], which makes *ACDC/ACRP30* an attractive candidate gene for fat-induced metabolic syndrome and T2DM.

Both common and rare variants of *ACDC/ACRP30* were found to be associated with T2DM in numerous obese populations, but do not contribute to the 3q27 linkage in the French families, suggesting that other genes may be involved. Common variants in and upstream of *EIF4A2* (encoding the eukaryotic translation initiation factor 4 α2) have been shown to contribute to both age of onset of diabetes and linkage in the French families studied [150].

One notable exception is the identification of *TCF7L2* as a T2DM gene with the largest risk effect of intronic variants of any diabetes gene identified to date. In early 2006, common intronic variants of the *TCF7L2* gene encoding the transcription factor 7-like 2 (TCF7L2) were identified to be strongly associated with T2DM risk in the Icelandic population by DeCODE Genetics (Reykjavik, Iceland) during a follow-up positional cloning study from a modest linkage signal on chromosome 10q [151]; but it turned out that despite not explaining this linkage signal, multiple intronic SNPs also showed strong association in US and Danish samples [151]. This initial study was rapidly followed by widespread replications in different ethnic backgrounds from white Europeans, West Africans, Mexican Americans, Indian and Japanese populations [152], confirming that *TCF7L2* variants have the largest risk effect of any of the known T2DM susceptibility loci. As shown in a meta-analysis from >20 studies in diverse ethnicities, the estimated risk of rs7903146 T allele (the most T2DM-associated variant) was 1.46 ($P = 5.4 \times 10^{-140}$) [153].

At the individual level, carrying one *TCF7L2* risk allele may increase diabetes risk by 40%–60% [152]. As a marked variation in *TCF7L2* rs7903146 T allele frequency exists between individuals from different ethnic groups, this implies a variable impact in the respective general populations. In European general populations, rs7903146 T allele has been estimated to contribute to 10–25% of all cases of diabetes in lean individuals [152]. In Eastern Asians, the population attributable risk fraction was assessed at 18.7% for other specific risk alleles [154]. The rs7903146 variant has been shown to influence progression to diabetes (with a hazard ratio of 1.81 for the homozygote TT group in the participants from the Diabetes Prevention Program) [155].

In the French and UK populations, rs7903146 T allele was associated with lower BMI in individuals with T2DM and with a higher risk for T2DM in non-obese subjects [152], but it is not a risk factor for obesity in European populations, whereas its effect on T2DM risk was found to be modulated by adiposity [156]. Watanabe *et al.* [157] suggested that an interaction between TCF7L2 and adiposity may be caused by a better β-cell compensation in obese individuals.

TCF7L2 is a member of the T-cell specific high mobility group (HMG) box containing family of transcription factors which, upon binding β-catenin, transduces signals generated by Wnt receptors at the cell surface to modify expression of multiple genes, many of which are associated with the cell cycle. Mutations in *TCF7L2* are also implicated in certain types of cancer [158], but rare *TCF7L2* mutations are not contributing to monogenic forms of diabetes such as MODY and neonatal diabetes [159]. One mechanism whereby *TCF7L2* variants increase diabetes risk might involve an impairment of incretin effects (i.e. GLP-1) on islet functions [160,161]. Indeed, variants of *TCF7L2* specifically impair GLP-1-induced insulin secretion [162], and the stimulation of β-cell proliferation by GLP-1 is TCF7L2 dependent [163]. TCF7L2 is also required to maintain glucose-stimulated insulin secretion and β-cell survival because its deletion in human islets reduces insulin secretion and increased apoptosis [164]. Furthermore, the overexpression of TCF7L2 protects β-cells from glucose and cytokine-induced apoptosis [164]. Clinical studies have demonstrated that the at-risk SNP is associated with defects in β-cell function and insulin release. The study by Lyssenko *et al.* [161] suggested that pancreatic islets from subjects with T2DM bearing the at-risk TT genotype have increased TCF7L2 RNA levels, indicating that excessive TCF7L2 expression may be associated with the reported insulin secretory defects. Expression studies carried out by Elbein *et al.* [165] on adipose tissue and muscle, however, showed that TCF7L2 expression was not altered by genotype and did not correlate with insulin sensitivity or BMI. As the most associated SNP rs7903146 is found in an intronic region, a potential mechanism through which it may confer an increased risk of diabetes is by altering TCF7L2 expression levels, but the functional mechanisms through which TCF7L2 modulates glucose sensing and insulin release in pancreatic β-cells remain to be clarified. A recent study showed that *TCF7L2* silenc-

ing in primary mouse islets as well as in clonal MIN6 and INS-1(832/13) cells decreases both basal and glucose-stimulated insulin secretion [166]. *TCF7L2* silencing also abolished the stimulatory effects of GLP-1 on secretion, whilst having little impact on glucose-induced changes in intracellular ATP or free Ca^{2+} concentrations. The study by da Silva Xavier *et al.* [166] showed that TCF7L2 controls the expression of genes involved in insulin granule fusion at the plasma membrane (notably syntaxin 1A and Munc 18-1 which may be direct targets for regulation by TCF7L2). Thus, defective insulin exocytosis may underlie increased diabetes incidence in carriers of the at-risk *TCF7L2* alleles.

The era of GWA studies improved knowledge of T2DM susceptibility genes

New discoveries in the genetic etiology of T2DM

Important advances in T2DM genetics have been made with the completion of GWA studies based on HapMap-selected common SNPs. This has become reality with the outstanding breakthroughs made in the knowledge and assessment of human genome variations, their mapping and their links with the genetic background of common diseases [167], and in the development and accessibility to very high throughput genotyping techniques based on microarray technology and to biostatistical tools for large cohort data analyses.

Within only few months of 2007, five independent GWA screens for T2DM, based on the Illumina 300K or Affymetrix 500K genotyping arrays both capturing about 80% of common variants present in the human genome of Caucasian individuals, identified dozens of potential candidates; at least 15 new loci emerged as being most consistently associated with risk of T2DM across multiple studies (Table 12.4). These include variants within or near *SLC30A8*, *HHEX*, *CDKAL1*, *CDKN2A/2B*, *IGF2BP2* and *JAZF1* [15]. Three of the associated loci correspond to genes that had already been implicated in T2DM (*TCF7L2*, *KCNJ11*, *PPARG*). These studies also confirmed that *TCF7L2* was the highest genetic risk factor for T2DM in populations of European descent. The next strongest SNP is in a 33-kb LD block within the coding region of *SLC30A8* encoding a zinc membrane transporter (ZnT8) which is highly expressed in pancreatic islets [168]. Interestingly, the overexpression of ZnT8 in the HeLa cell line is associated with an accumulation of zinc in intracellular vesicles [168]. In the INS-1E β-cell line, which overexpresses ZnT8, the intracellular zinc level is increased and the insulin secretion is stimulated [169]. Furthermore, overexpressing ZnT8 does not sensitize the cells to toxic doses of zinc but confers a resistance to apoptosis after zinc depletion [169]. The non-synonymous polymorphism showing an increased risk of T2DM (OR per risk allele: 1.18) corresponds to an arginine to tryptophan substitution at position 325, and the at-risk C-allele is associated with impaired β-cell function *in vivo*. An impaired transporter function may decrease the amount of zinc available for co-crystallization with insulin in the secretory vesicles of

Table 12.4 Validated type 2 diabetes susceptibility genes in the post genome-wide association era.

Chromosome location	Representative SNP	Gene symbol	Predicted disease mechanism	Approximate effect size for T2DM risk	Year of major publication (key references)
3p25	rs1801282	PPARG	Insulin resistance/muscle/adipocyte	1.23	2000 [90]
2q37	rs2975760	CAPN10	Glucose transport/insulin resistance/β-cell dysfunction	1.18	2003 [139]
11p15	rs5219	KCNJ11	β-cell dysfunction (K_{ATP} channel)	1.15	2003 [115]
20q12	Multiple SNPs at P2 site	HNF4A	β-cell dysfunction/other	1.05–1.70*	2004 [52,53,55]
6q23	rs1044498	ENPP1	Insulin resistance/muscle/adipocyte	1.38[†]	2005 [83,85,88]
10q25	rs7903146	TCF7L2	β-cell dysfunction/β-cell mass/incretin effect	1.47	2006 [15,153]
8q24	rs13266634	SLC30A8	β-cell dysfunction/Zn transport	1.15	2007 [16]
10q23-q25	rs1111875	HHEX-IDE	Pancreatic development	1.15	2007 [15,16]
6p22	rs10946398	CDKAL1	β-cell dysfunction/β-cell mass	1.16	2007 [15,172]
9p21	rs10811661	CDKN2A/2B	β-cell dysfunction/cell cycle regulation	1.20	2007 [15]
3q28	rs4402960	IGF2BP2	mRNA processing in the β-cell	1.14	2007 [15]
4p16	rs10010131	WFS1	β-cell dysfunction/ER stress/other	1.11	2007 [15,60]
17q21	rs4430796	TCF2	β-cell dysfunction/development	1.10	2007 [45,56]
1p12	rs10923931	NOTCH2	Pancreatic development	1.13	2008 [174]
3p14	rs4607103	ADAMTS9	Metalloprotease/cell–cell interactions; insulin resistance	1.09	2008 [174]
2p21	rs7578597	THADA	β-cell dysfunction/apoptosis	1.15	2008 [174]
1p12	rs2641348	ADAM30	Metalloprotease/cell–cell interactions	1.10	2008 [174]
12q21	rs7961581	TSPAN8-LGR5	Cell surface glycoprotein	1.09	2008 [174]
10p13-p14	rs12779790	CDC123-CAMK1D	Cell cycle regulation in the β-cell	1.11	2008 [174]
7p15	rs864745	JAZF1	Transcriptional regulation in the β-cell	1.10	2008 [174]
11p15	rs2237892	KCNQ1	β-cell dysfunction (K^+ channel)	1.40	2008 [207,208]
11q21	rs10830963	MTNR1B	β-cell dysfunction (melatonin signaling)/ other	1.09	2009 [181–183]

* Difference in effect size for T2DM risk between populations (the strongest effect was found in the Ashkenazim [55]).
[†] Under a recessive model of inheritance in white populations (according to the meta-analysis by McAteer *et al.* [88]).

β-cells. *SLC30A8*/ZnT-8 has also recently been identified as an autoantigen in human type 1 diabetes [170].

A locus on 10q23 ranked third for association with T2DM in the French GWA study was then independently replicated in the other large studies, but with a combined OR of only 1.13. SNPs at this locus have been found to be associated with measures of insulin secretion in a large multicentric study from Europe and in the Diabetes Prevention Program (DPP) study [171]. The association signal lies in a 295-kb block of LD that includes at least three potential T2DM genes: *HHEX* (a homeobox transcription factor); *KIF11* (a kinesin interacting factor); and *IDE* (an insulin degrading enzyme).

Another well-replicated association signal maps to 6q22 within a 15-kb LD block in the intron 5 of the *CDKAL1* gene coding for CDK5 regulatory subunit-associated protein 1-like 1, with the strongest association observed in the DeCODE study [172]. CDK5 itself has been shown to blunt insulin secretion in response

to glucose and to have a permissive role in the decrease of insulin gene expression that results from glucotoxicity. Thus, it is speculated that reduced expression of *CDKAL1* would result in enhanced activity of CDK5 in β-cells, and lead to decreased insulin secretion; in agreement, variants at this locus were significantly associated with small decreases in insulin response to a glucose load [171,173].

A meta-analysis of three GWA scans followed by a large-scale replication (Diagram consortium including more than 50 000 individuals in total) has identified additional susceptibility loci for T2DM, with OR ranging from 1.09 to 1.15, near six genes: *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9* and *NOTCH2* [174]. Variants at *JAZF1*, *CDC123-CAMK1D* and *TSPAN8-LGR5* are associated with small alterations in insulin secretion, whereas the mechanisms linking the other loci to T2DM remain to be clarified [175]. In each GWA scan, other loci showed significant associations with T2DM, but were not fol-
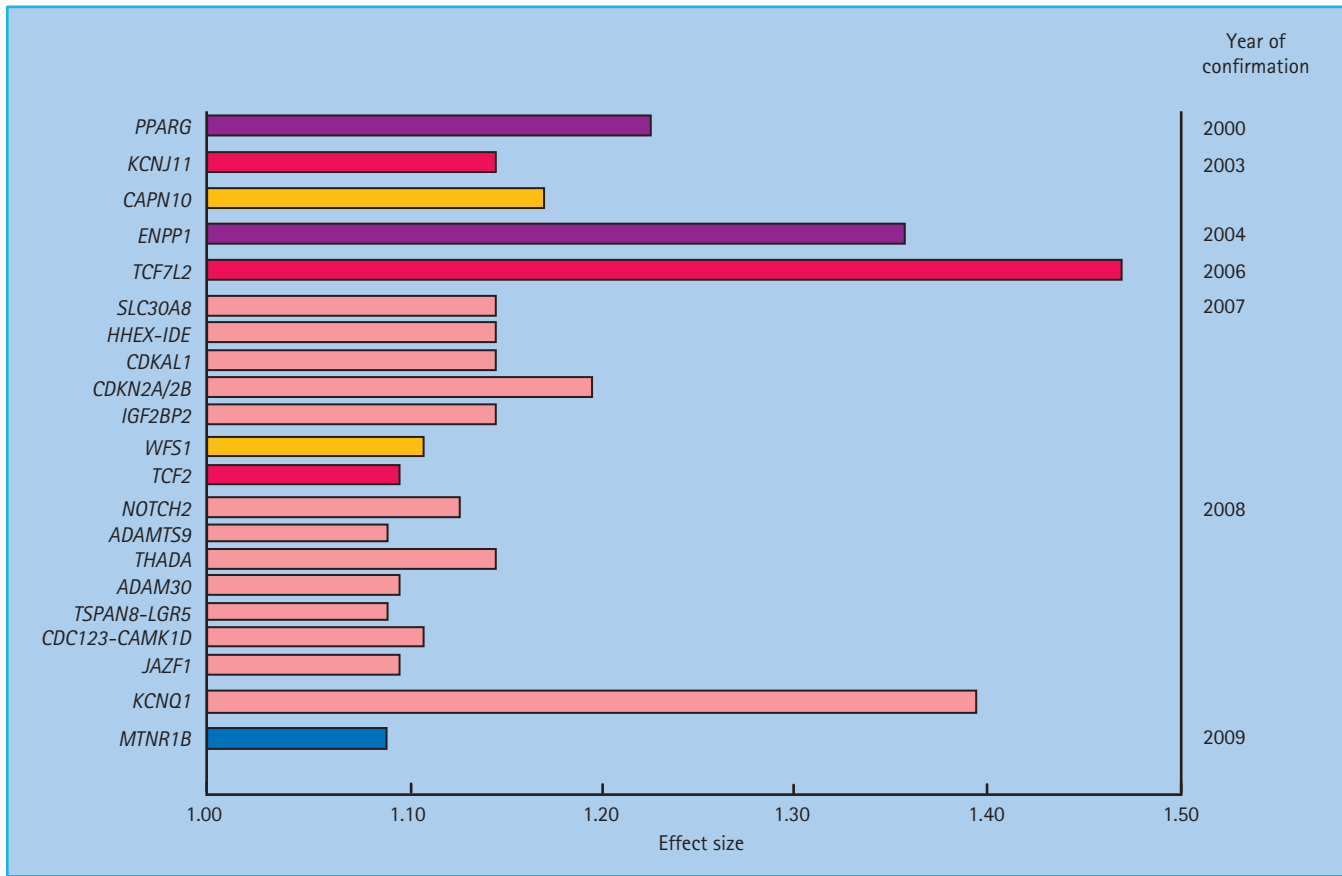
Figure 12.1 Chronology in the discovery of the genetic loci associated with type 2 diabetes. Validated type 2 diabetes-associated genes are plotted by year of publication and approximate effect size. Allelic effect sizes (OR value) are derived from major publications of definitive associations (as indicated in Table 12.4). Color code: purple indicates candidate genes with a biologic effect on insulin sensitivity at different sites; red with an effect on pancreatic islet function and insulin secretion; yellow with effects on both insulin secretion and sensitivity; red with pink lines indicates genes uncovered via genome-wide association studies, for which common variants increase disease risk through impaired β-cell function (for most of them); blue for one gene modulating fasting plasma glucose and type 2 diabetes risk through impaired β-cell function and other mechanism(s).

lowed up because of lack of replication across multiple studies. A larger meta-analysis study including almost all GWA studies for T2DM performed in European populations will extend the list of potentially at-risk alleles and genes (Figure 12.1).

These GWA results suggest that T2DM may be more heterogeneous and more polygenic than previously believed, and thereby represents a large number of different disorders which might also differ at a clinical level. The second finding is the relatively high frequency of each of the risk variants in the population (ranging from 0.26 for *TCF7L2* to 0.90 for *THADA*), together with a small risk of disease conferred by each allele translating into a very large number of individuals who carry several of the susceptibility variants but do not develop T2DM. Such low penetrance is consistent with the mild effect of the risk variants, requiring the presence of other factors, presumably environmental for the development of hyperglycemia, and may also be explained by the fact that most of these variants are in non-coding regions of the gene, where they may produce only subtle differences in regulation of expression. This is in contrast

with Mendelian forms of diabetes, such as MODY, which are caused by rare mutations in the coding sequence resulting in significant amino acid substitutions or truncated proteins, leading to hyperglycemia even in the absence of other diabetogenic exposures.

It is also worthy of note that the loci identified to date appear to explain only a small proportion of the familial clustering of T2DM, and thus are just the tip of the iceberg. Indeed, there is a discrepancy between the linkage and GWA studies, and this may be explained by the presence of multiple, uncommon, mildly deleterious polymorphisms scattered throughout the regulatory and coding regions of genes for T2DM.

The current genetic models of how genes interact with each other and with the environment may be too simplistic, and probably clouded by our underestimation of the role of genetic imprinting and/or epigenetics on gene expression and disease.

These new discoveries lead to the proposal of new etiologic mechanisms for T2DM, and would suggest that the genetic components to T2DM risk act preferentially through β-cell
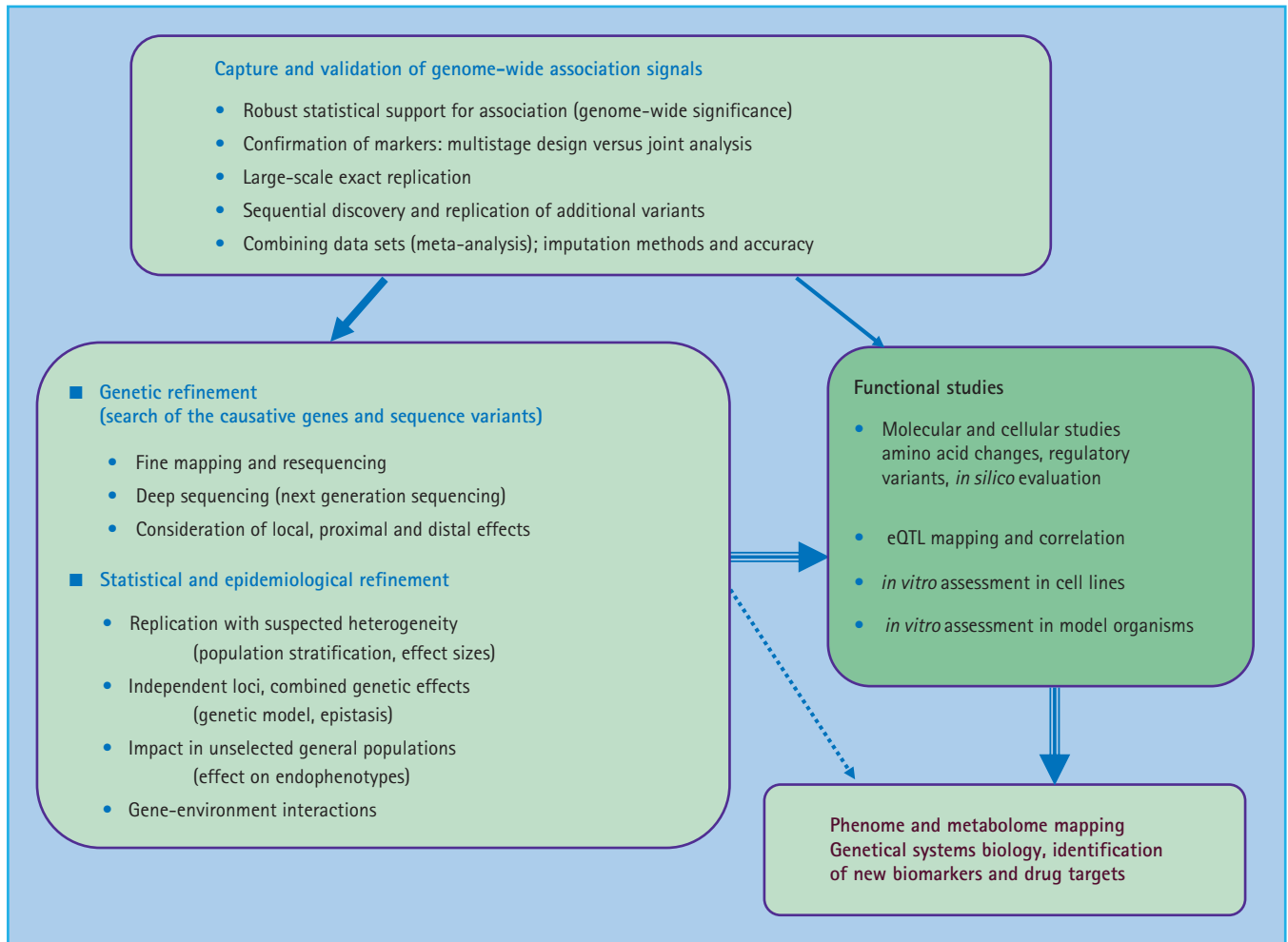
Figure 12.2 From the capture of genomic information to phenome and metabolome mapping. Flow chart of the research approaches and methods required to document, refine and validate genome-wide association signals. These steps have a rationale sequence, but this can also be modulated by the availability of resources and data, and the specific challenges that arise in each field.

defects, given that several of the genes placed in the proximity of GWA signals are expressed in β-cells and risk alleles at many of these loci alter insulin secretion capacity of the β-cells. Variants predisposing to diabetes through effects on insulin sensitivity, however, may be more difficult to track down because of strong environment–gene interactions for insulin resistance with body weight, physical activity, nutrient intake and other factors, compared with the potentially purer genetic control on insulin secretion. More generally, exploring these novel association data and truly causative DNA sequences as well as functional mechanisms requires pursuing other research efforts (Figure 12.2).

A concept emerging from these new discoveries is that there is a genetic overlap between T2DM and prostate cancer. Common variants in the T2DM susceptibility genes *TCF2* and *JAZF1* also influence prostate cancer risk [176]. For *TCF2*, the risk allele for T2DM has a protective effect on prostate cancer susceptibility, whereas two variants in *JAZF1* predispose to the two diseases, which are not correlated with each other [174,177]. The mecha-

nisms by which these genes influence disease are unknown; however, they are thought to be involved in cell-cycle control.

Additional loci associated with quantitative metabolic traits

Analyses of quantitative traits among the non-diabetic controls in GWA studies have also identified common variants that modulate fasting glycemia, although these effects do not always appear to translate into an increased risk of T2DM (Figure 12.3).

Fasting plasma glucose (FPG) levels are tightly regulated within a narrow physiologic range by a feedback mechanism that targets a particular fasting glucose setpoint for each individual. Disruption of normal glucose homeostasis and substantial elevations of FPG are hallmarks of T2DM and typically result from sustained reduction in pancreatic β-cell function and insulin secretion [178,179]. Elevated FPG levels within the normal range are an independent risk factor for T2DM and are associated with increased risk of mortality in prospective studies [178].
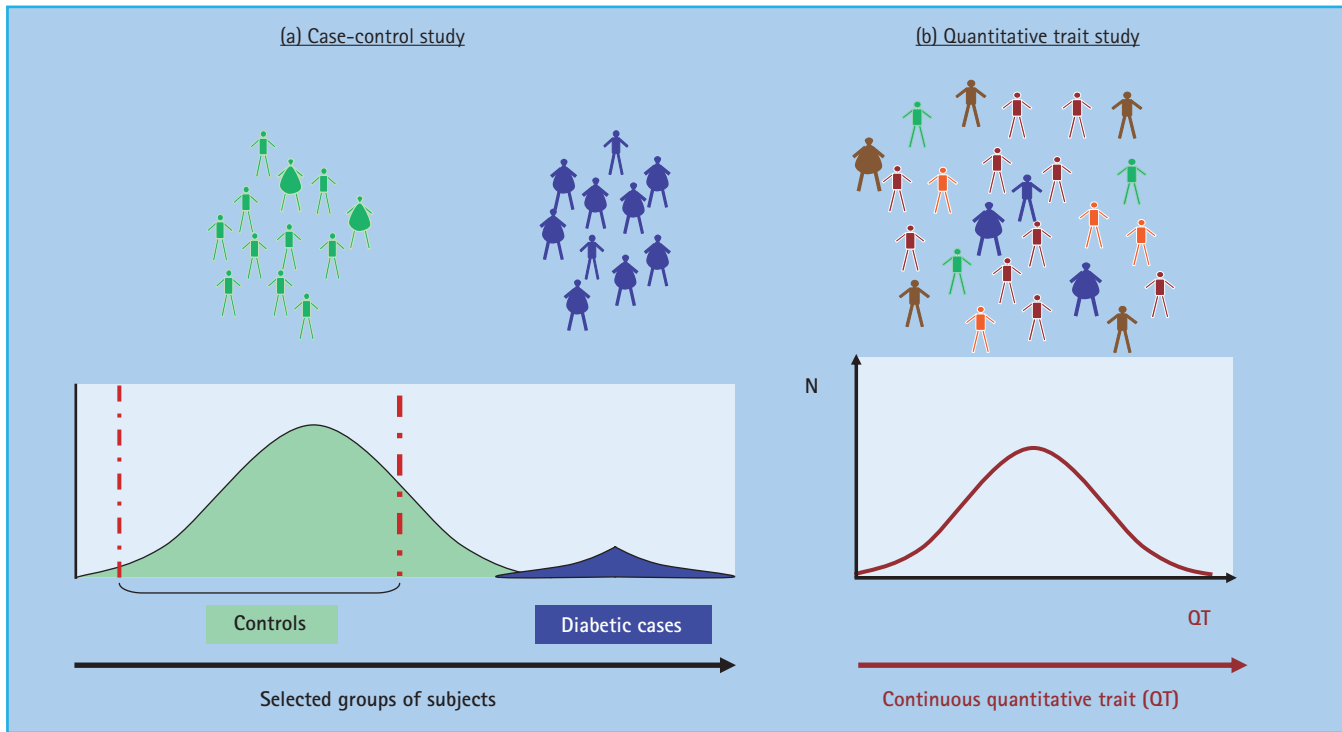
Figure 12.3 Schematic representation of the two major study designs used in the genetic mapping of complex traits and complex diseases. (a) Case–control study to search for genes and gene variants associated with disease risk. (b) Quantitative trait study to correlate genotype effects with modulation of continuous quantitative variables (i.e. values of a biologic measure, measured or calculated metabolic variables).

Even within the healthy non-diabetic population, however, there is substantial variation in fasting glucose levels; around one-third of this variation is genetic. There is growing evidence to suggest that common variants contributing to variation in FPG are largely distinct from those associated with major disruptions of β-cell function that predispose to established T2DM. Common sequence variants in the *GCK* (glucokinase) promoter and around genes encoding glucokinase regulatory protein (*GCKR*) and the islet-specific glucose-6-phosphatase (*G6PC2*) [180] have been associated with individual variation in FPG, whereas weak effects on T2DM risk were found. Three large independent studies in European populations, however, have recently demonstrated that non-coding variants in *MTNR1B* (encoding melatonin receptor 1B) are consistently associated with increased FPG levels (0.07 mmol/L per G-allele of rs10830963, the strongest signal observed at the locus), reduced β-cell function as measured by the homeostasis model assessment (HOMA-B index) and increased T2DM risk (OR of 1.09 for the same G-allele) [181–183], and with impairment of early insulin response to both oral and intravenous glucose and with faster deterioration of insulin secretion over time [183,184]. *MTNR1B* encodes one of the two known melatonin receptors and, as well as being highly expressed in the brain (mainly in the suprachiasmatic nucleus that controls the circadian rhythm) and retina, MT2 is also transcribed in human islets and sorted β-cells [181,185]. The study by Lyssenko *et al.* [183] showed an

increased expression of MT2 in islets of individuals without diabetes carrying the risk allele and of individuals with T2DM, and that insulin released from clonal β-cells in response to glucose was inhibited in the presence of melatonin. These data support a direct role of MT2 in mediating the inhibitory effect of melatonin on insulin secretion, and that the circulating hormone melatonin, which is released from the pineal gland in the brain to regulate the circadian rhythm by translating photoperiodic information from the eyes to the brain, is involved in the pathogenesis of T2DM. These pathogenic effects are likely to be exerted via a direct inhibitory effect on β-cells. In view of these results, blocking the melatonin ligand-receptor system could be a therapeutic option in T2DM.

The total variance in FPG attributable to these four signals is approximately 1.5%, indicating that additional loci remain to be found. The ongoing MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) transnational study group, which represents a collaborative effort to combine data from multiple GWAs to identify additional loci that affect glycemic and metabolic traits, and other studies from the Northern Finland Birth Cohorts, the ENGAGE consortium (including 20 000 individuals in multiple European cohorts and family studies) or other large cohorts, case–control studies and clinical trials from Europe and the USA, will continue to deliver novel association signals in additional loci, which may highlight novel pathways involved in regulation of common metabolic traits.

One recent study has reported a novel association between glycated hemoglobin (HbA$_{1c}$) levels and intronic variants of *HK1* encoding the hexokinase type 1 isoform predominantly found in erythrocytes, although it is also expressed in other human tissues that depend strongly on glucose utilization for their physiologic functioning such as brain and muscle [186]. Three other loci – *GCK*, *G6PC2* and *SLC30A8* – have been identified in this study as being significantly associated with HbA$_{1c}$ levels in individuals without diabetes. While genetic variants of *GCK* and *G6PC2* are known to influence FPG levels in people without diabetes, the association with the diabetes gene *SLC30A8* has not been previously identified. Because intra-erythrocyte glucose concentration is thought to be the main determinant of HbA$_{1c}$ rate and HK1 is the initial and rate-limiting enzymatic step in erythrocyte glucose metabolism after glucose transporter mediated-diffusion, it is likely that variation in HK1 activity would affect glycation. Alternatively, genetic variations at HK1 might modulate systemic glucose metabolism, raising the possibility that these variations are associated with the risk of incident diabetes. Indeed, HK1 is expressed in muscle, an insulin-sensitive tissue recognized for its importance in glucose metabolism. Whether the differences in hemoglobin glycation associated with genetic polymorphisms are paralleled by differences in glycation in other tissues that are thought to underlie long-term complications of diabetes remains an open question.

Serum lipids are important determinants of cardiovascular diseases and are related to morbidity [187]. The high heritability of circulating lipid levels is well established, and earlier studies of individuals with extreme lipid values or families with Mendelian forms of dyslipidemias have reported the involvement of numerous genes and respective proteins in lipid metabolism [188]. Recent GWA studies mostly carried out in samples enriched for T2DM cases have implicated a total of 19 loci controlling serum high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides (TG). The loci include the genes encoding *ABCA1*, *APOB*, *CELSR2*, *CETP*, *DOCK7*, *GALNT2*, *GCKR*, *HMGCR*, *LDLR*, *LIPC*, *LIPG*, *LPL*, *MLXIPL*, *NCAN*, *PCSK9* and *TRIB1*, and three genomic regions with multiple associated genes including *MVK-MMAB*, *APOA5-APOA4-APOC3-APOA1* and *APOE-APOC1-APOC4-APOC2* [188,189].

Many of these have also been consistently associated with lipid levels in candidate gene studies reported over the past 30 years; many of the same loci with common variants have already been shown to lead to monogenic lipid disorders in humans and/or mice, suggesting that a spectrum of common and rare alleles at each validated locus contributes to blood lipid concentrations [188].

More recently, larger scale GWA studies have identified additional loci for lipid levels [190,191], and sequencing of these loci should pinpoint all associated alleles. For three loci (*HMGCR* encoding HMGCoA, the drug target for statins, *NCAN* and *LPL*) effect sizes differed significantly by sex (i.e. gene–sex interactions) [190]. The construction of genetic risk profiles suggests that the cumulative effect of multiple common variants contributes to polygenic dyslipidemia [191].

With a full catalog of DNA polymorphisms in hand, a panel of lipid-related variants can be studied to provide clinical risk stratification and targeting of therapeutic interventions.

Combined genetic effects of multiple susceptibility gene variants and clinical implications

GWA studies have dramatically increased the number of common genetic variants that are robustly associated with T2DM. A possible clinical use of this novel information is to identify individuals at high risk of developing the disease in the general population, so that preventative measures may be more effectively targeted (Figure 12.4).

A first study in a UK population showed that individuals combining T2DM risk alleles in *TCF7L2*, *PPARG* and *KCNJ11* had an OR of 5.71-fold compared with those with no risk alleles to develop the disease [192]. Then, using a similar case–control design, a second study in a French population reported that after adjustment for age, BMI and gender, subjects with at least 18 risk alleles (representing 14.5% of French subjects with T2DM) had

Figure 12.4 Additive effects of common genetic variants (i.e. SNPs) on T2DM risk in the population. The distribution of normoglucose-tolerant (NGT, indicated by red bars) individuals and individuals, with diabetes (T2DM, indicated by yellow bars) is shown according to the number of at-risk alleles in each class (for a total of 15 single nucleotide polymorphisms genotyped in each individual, from the best replicated variants following the results of genome-wide association studies). The study was performed in 4232 patients with diabetes and 4595 normoglycemic adult subjects. Adapted from Cauchi *et al.* [193].
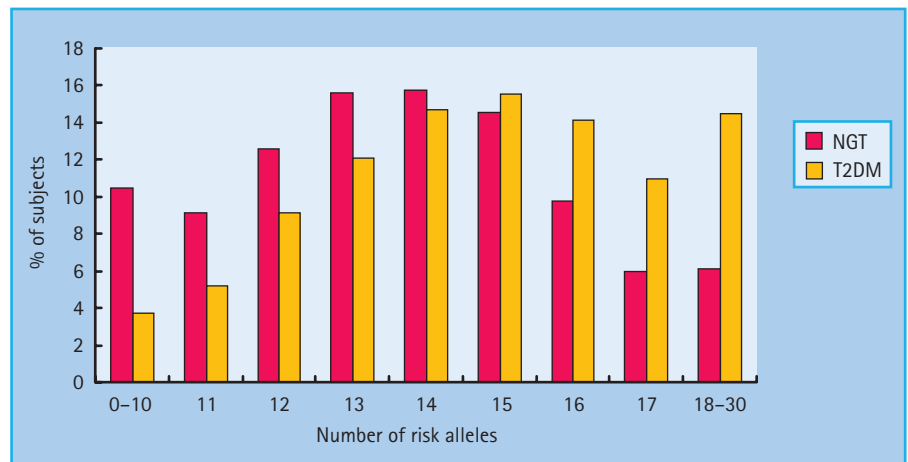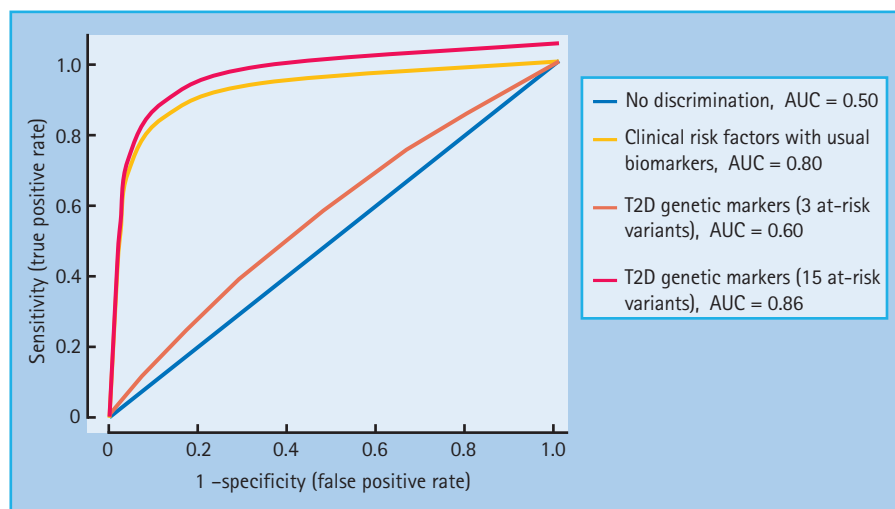
Figure 12.5 The predictive value of type 2 diabetes gene variants. Examples of receiver operator characteristic (ROC) curves for a test including classic clinical risk factors (in green) or three genetic variants at-risk for T2DM (in orange) or 15 genetic variants at-risk for T2DM (in red). The area under the ROC curve (AUC) is a measure of the discriminatory power of the test evaluating the predictive power of defined clinical factors or genetic variants (a test with no discriminatory power would have an AUC of 0.50). For details see [46,192–196].

approximately ninefold higher risk of developing T2DM compared with the reference group (with an area under the receiver operator characteristic (ROC) curve, a measure of discriminative accuracy, of 0.86; Figure 12.5) [193].

Two more recent population-based studies using a longitudinal design with prospectively investigated cohorts have examined the predictive value of a genotype score in addition to common risk factors for prediction of T2DM [194,195]. Meigs *et al.* [194] reported that a genotype score based on 18 risk alleles predicted new cases of diabetes in the community but provided only a slightly better prediction of risk than knowledge of common clinical risk factors alone [195]. A similar conclusion was drawn in the paper by Lyssenko *et al.* [196], along with an improved value of genetic factors with an increasing duration of follow-up, suggesting that assessment of genetic risk factors is clinically more meaningful the earlier in life they are measured. They also showed that β-cell function adjusted for insulin resistance (using the disposition index) was the strongest predictor of future diabetes, although subjects in the prediabetic stage presented with many features of insulin resistance. It is also noteworthy that many of the variants that were genotyped appear to influence β-cell function. The addition of DNA data to the clinical model improved not only the discriminatory power, but also the reclassification of the subjects into different risk strategies. Identifying subgroups of the population at substantially different risk of disease is important to target these subgroups of individuals with more effective preventative measures. As more genetic variants are now identified, tests with better predictive performance should become available with a valuable addition to clinical practice.

Pharmacogenetics of T2DM in adults

An early focus of T2DM pharmacogenetic studies has been to examine the effect of *PPARG* P12A on the response to thiazolidinediones, because the nuclear receptor PPAR-γ is the known drug target of thiazolidinedione medications. In the TRIPOD study, one-third of subjects showed no increase in insulin sensitivity in response to troglitazone (400 mg/day); however, the P12A variant did not explain this failure [197]. Similarly, in the DPP study, no effect of *PPARG* P12A or five other polymorphisms was observed on response to troglitazone therapy in 340 participants randomized to this thiazolidinedione [198]. Knowledge of allelic variation at this locus does not seem to offer a rationale for therapeutic choices. The impact of the *KCNJ11/* genetic variant (E23K) on the effectiveness of sulfonylurea therapy is also unclear. In the study by Sesti *et al.* [199], which genotyped the E23K variant in 525 white patients with T2DM, carriers of the 23K allele have a relative risk of failure (defined as FPG >300 mg/dL) of 1.45 compared with E23E homozygotes, and the risk allele was also associated with an earlier onset of diabetes and worse metabolic control in non-responders. These results stand in contrast to those of the UK Prospective Diabetes Study (UKPDS), in which no significant association was found between the E23K variant and response to sulfonylurea therapy in 364 patients with newly diagnosed T2DM [200]. Interestingly, in the DPP study, metformin was effective in lowering the risk of diabetes for the E23E homozygotes, but failed to protect carriers of the risk K-allele. In addition, K23K homozygotes randomized to metformin did not show the expected improvement in insulin sensitivity at 1 year [201].

In regard to a possible drug–*TCF7L2* genotype interaction, the GoDARTs study genotyped 6516 UK participants for the rs7903146 variant and found that the TT homozygote case and control subjects had a higher HbA$_{1c}$, with the TT cases with diabetes being more likely to require oral medication or insulin than the CC homozygotes [202]. This suggests that *TCF7L2* risk variants may be associated with increased disease severity and therapeutic failure. The same group reported the effect of *TCF7L2* genotypes on therapeutic response in 901 patients with diabetes treated with sulfonylurea and 945 patients treated with metformin. Carriers of the risk *TCF7L2* variants were more likely not

to respond to sulfonylurea but to respond to metformin therapy as measured by a HbA_{1c} >7% within 3–12 months after treatment initiation [203]. This finding further supports the hypothesis that *TCF7L2* variation is important in β-cell function. The DPP study reported that the lifestyle intervention was effective in reducing the genetic risk conferred by the high-risk homozygous genotype in the participants with impaired glucose tolerance and elevated fasting glucose [204].

Another example in which genetic variability may have a significant impact on therapy response is the organic cation transporter 1 (*OCT1*), which participates in the hepatic uptake of metformin, a widely used biguanide, and was shown to be highly polymorphic in humans. A clinical study by Shu *et al.* [205] reported that genetic variation in *OCT1* affects the metformin therapeutic action and the response to the drug.

Conclusions and perspectives

Our knowledge of the genetic architecture of T2DM has greatly improved with the new GWA findings, and this will have promising implications on new diagnostics and therapeutics for T2DM. It is likely, however, that other yet unidentified genes may contribute to the genetic risk of T2DM, because only a part of the inheritance of T2DM in families has so far been explained. It should be emphasized that the current GWA studies are based on the "common disease/common variant" hypothesis, whereas the alternative "allelic heterogeneity" hypothesis holding that susceptibility to common disorders may result from a large number of rare variants, each having a relatively large effect, still has to be tested. Identification of such rare variants, if they exist, will require deep resequencing of T2DM loci (those already established, and novel) in cases of diabetes and in normoglycemic individuals, which is becoming more realistic with the ongoing development of new powerful sequencing methods. Likewise, the contribution of structural variants, such as copy number variants, insertions, deletions and duplications, could also contribute to genetic susceptibility not explained by single nucleotide substitutions. Further more novel approaches looking at epigenetic sites, multitissue genome expression related to genetic traits, metabonomics and metagenomics should bring even more knowledge in the near future.

Together, these discoveries will continue to improve our understanding of the biologic mechanisms that maintain glucose homeostasis, and of still hidden molecular defects leading to chronic hyperglycemia, and could also lead to the development of more specifically targeted antidiabetic drugs or even gene-based therapies. Moreover, pharmacogenetic testing might then be used to predict, for each patient, the therapeutic response to different classes of drugs. The identification of T2DM genes will also provide the tools for the timely identification of high-risk individuals who may benefit from early behavioral or medical intervention to prevent the development of diabetes. An important reduction in diabetes-related morbidity and mortality could be then expected, along with a reduction in the costs of the treatment of diabetes and its complications.

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