Genetic determinants and molecular pathways in the pathogenesis of Type 2 diabetes

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ABSTRACT

T2DM (Type 2 diabetes mellitus) has reached epidemic proportions worldwide, exerting major health consequences at an individual and public health level alike. Unfortunately, the molecular pathophysiology of diabetes remains incompletely understood, impairing progress towards more effective prevention and treatment strategies. Although the rapid increase in the prevalence of insulin resistance and T2DM over the past several decades highlights a major environmental contribution related to overnutrition, obesity and inactivity, susceptibility is likely to reflect individual differences in complex gene–environment interactions. In the present review, we focus on mediators of genetic and environmental risk for T2DM at a molecular level.

INTRODUCTION

T2DM (Type 2 diabetes mellitus) in the United States and around the world has reached epidemic proportions. At present, 17.9 million people in the United States have been diagnosed with diabetes, with an additional 5.7 million undiagnosed [1]. Worldwide, the incidence of diabetes is projected to continue to rise exponentially, reaching 366 million by 2030 [2]. Diabetes also exacts enormous personal tolls, among them long-term complications of cardiovascular disease, retinopathy, neuropathy and nephropathy.

Intimately linked with the rise in T2DM prevalence is the burgeoning epidemic of obesity around the world, particularly in developed societies. It is estimated that 400 million individuals worldwide have obesity (2005 estimate) [3]. Despite expenditure of billions of dollars on diets and health clubs, rates of obesity continue to increase exponentially [4]. In 2004, 17.1% of children aged 2–19 years old in the United States were overweight and 32.2% of adults over 20 years of age were obese [4]. These findings are particularly alarming, as obesity is a major risk factor for insulin resistance and T2DM. Current findings indicate that 57 million Americans suffer from pre-diabetes (fasting blood glucose between 100 and 125 mg/dl) [5]; many more individuals have insulin resistance and unappreciated risk for T2DM. An important public health goal, then, should be to identify individuals at high risk for the development of T2DM in order to implement prevention and early intervention strategies, including exercise, modest weight loss and specific medication [6,7].

Key words: endoplasmic reticulum stress, inflammation, mitochondrion, obesity, pancreatic β-cell, Type 2 diabetes.

Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; CNS, central nervous system; DPP-IV, dipeptidyl peptidase-IV; ER, endoplasmic reticulum; FTO, fat mass and obesity-associated; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HSP, heat-shock protein; IGF-2, insulin-like growth factor-2; IL-6, interleukin-6; IRE-1, inositol-requiring enzyme-1; IR, insulin receptor; βIRKO, β-cell IR knockout; FIRKO, fat-specific IR knockout; JNK, c-Jun N-terminal kinase; KCNJ11, encoding Kir 6.2, a member of the inwardly rectifying potassium channel family; LBW, low birthweight; LIRKO, liver-specific IR knockout; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acid; NF-κB, nuclear factor-κB; ORP150, oxygen-regulated protein 150; PPAR, peroxisome-proliferator-activated receptor; PERK, PKR (double-stranded-RNA-dependent protein kinase); KCNJ11, encoding Kir 6.2, a member of the inwardly rectifying potassium channel family; LBW, low birthweight; LIRKO, liver-specific IR knockout; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acid; NF-κB, nuclear factor-κB; ORP150, oxygen-regulated protein 150; PPAR, peroxisome-proliferator-activated receptor; PERK, PKR (double-stranded-RNA-dependent protein kinase); PGC-1, PPARγ co-activator-1; PPARγ, encoding PPARγ; RBP-4, retinol-binding protein-4; S1, insulin sensitivity; T2DM, Type 2 diabetes mellitus; TCF7L2, encoding transcription factor 7-like 2; TNF-α, tumour necrosis factor-α; WGA, whole-genome association.

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Multiple risk factors are involved in the pathogenesis of T2DM, including classical genetic risk (family history) as well as a prominent contribution from multiple environmental risk factors. These include suboptimal intrauterine environment, which can impact development of key tissues in metabolic homeostasis, as well as postnatal factors, including overnutrition, obesity, inactivity and aging. Together, these factors can lead to progression of insulin resistance and β-cell dysfunction, both of which are required to ultimately produce clinical T2DM. Unravelling the complex pathophysiology of T2DM is complicated by the secondary effects of ‘glucolipotoxicity’ (hyperglycaemia and hyperlipidaemia).

**Risk Factors for Diabetes and Progression to T2DM**

Risk factors for the development and/or progression of T2DM include (i) genetics/family history, resulting in alterations in DNA sequence [8–14]; and (ii) both pre- and post-natal environmental factors, including suboptimal intrauterine environment [15,16], LBW (low birthweight) [15,17], obesity [18,19], inactivity [20], gestational diabetes [21] and advancing age [22]. Each of these risk factors can, via largely undefined mechanisms, lead to skeletal muscle, adipose and hepatic insulin resistance, and/or β-cell dysfunction. Ultimately, insulin resistance accompanied by inadequate insulin secretory responses results in postprandial and fasting hyperglycaemia. In turn, diabetes-related hyperglycaemia and associated metabolic abnormalities can further alter signal transduction and gene expression (glucolipotoxicity), thus contributing to a vicious cycle [23,24] (Figure 1).

**Family History**

The importance of genetic risk factors is exemplified by the high concordance of T2DM in identical twins [25], the strong influence of family history and ethnicity on risk, and the identification of DNA sequence alterations in both rare and common forms of T2DM [11,14]. Low S(I) (insulin sensitivity) and low S(G) (insulin-independent glucose effectiveness), both measures of glucose disposal, predict T2DM development several decades later in family-history-positive individuals, but not in family-history-negative individuals, indicating that family history may contribute to specific alterations in systemic physiology distinct from other risk factors [26,27].

Previous genetic studies have relied on family studies and candidate gene approaches to identify potentially pathogenic genes [8,10,13]. More recently, population studies have employed whole-genome scans to identify variation at multiple loci potentially contributing to T2DM risk ([28–36], and discussed below further).

**Intrauterine Environment**

The nutritional environment during both pre- and postnatal life is increasingly recognized as a significant contributor to the pathogenesis of T2DM. Both epidemiological and animal studies have linked a suboptimal nutritional environment during pregnancy and LBW with increased susceptibility to diabetes and obesity during adult life [37–41]. In this context, individuals who are both small at birth and have rapid and excessive postnatal catch-up growth are at the highest risk level [42–44]. Such findings have led to the formulation of the developmental programming hypothesis, which proposes that insults or stimuli acting during critical windows of development, including fetal and/or early postnatal periods, can...
produce permanent alterations in cell/tissue structure and function [45]. Conversely, offspring of pregnancies complicated by gestational diabetes are at greater risk of macrosomia and the development of obesity and T2DM during adult life [16]. The specific molecular mechanisms mediating risks linked to intrauterine life remain unknown, but may include epigenetic regulation of development and gene expression, including alterations in histone modification and methylation [46,47].

**Obesity**

Although increased intake of calorie-dense foods and decreased energy expenditure are clear contributors to the development of obesity, the mechanisms responsible for either obesity or its effects on systemic metabolism have not been completely elucidated [19]. Adipose tissue is now recognized as an important endocrine tissue, which modulates systemic metabolism by releasing NEFAs (non-esterified fatty acids; ‘free fatty acids’) and glycerol, hormones [e.g. leptin, adiponectin, resistin and RBP-4 (retinol-binding protein-4)] [48], and pro-inflammatory cytokines such as TNF-α (tumour necrosis factor-α), IL-6 (interleukin-6), and MCP-1 (monocyte chemotactic protein-1) [49–51]. With obesity, patterns of adipose tissue metabolism are altered, with impaired production of adiponectin and increased release of NEFAs and pro-inflammatory cytokines, a pattern which may contribute to systemic insulin resistance. These relationships are particularly prominent for intra-abdominal adipose tissue, contributing to the heightened risk associated with abdominal obesity [52].

Conversely, reduced quantity and/or function of adipose tissue, as observed in lipodystrophy syndromes, are also associated with insulin resistance. A common denominator between obesity and lipodystrophy syndromes is the ectopic accumulation of lipid metabolites in skeletal muscle and liver, which can contribute to tissue insulin resistance, particularly when energy demand is low (e.g. inactivity).

**Aging**

Life expectancy from birth has increased from approx. 45 years in the early 1900s to approx. 75 years for men and 80 years for women today [53]. These trends have certainly contributed to the increasing prevalence of age-related diseases, including obesity and T2DM [54], perhaps related to aging-associated loss of lean body mass and increased adipose tissue [55,56]. In addition, pre-adipocyte capacity to replicate, differentiate and store lipids declines with age [57]. This, too, may contribute to increased accumulation of ectopic lipids in muscle and liver, and induction of insulin resistance.

**Inactivity**

Decreased physical activity also contributes to T2DM risk [20,58]. Exercise is critical for maintenance of muscle oxidative function and systemic insulin sensitivity. Both acute exercise and chronic exercise training increase mitochondrial gene expression and oxidative capacity, via pathways activating AMP kinase, calcium/calmodulin-dependent protein kinase and the expression of the PGC-1 [PPAR (peroxisome-proliferator-activated receptor) γ co-activator-1] family of genes controlling oxidative mitochondrial function [59–62]. Fibre type may also contribute to genetic-dependent and activity dependent differences in muscle oxidative function [63,64]. Moreover, decreased PGC-1 expression may contribute to increased type Iib fibre content in non-diabetic individuals with a family history of diabetes [65]. Although fibre type may therefore contribute to muscle metabolic phenotypes, individuals with obesity and T2DM have impaired oxidative capacity and increased lipid content in all fibre types; thus such fibre-type distribution differences cannot fully explain muscle metabolic phenotypes linked to diabetes risk [63].

**Tissue-Specific Metabolic Defects in T2DM: Lessons from Human and Animal Studies**

Multiple defects in physiology have been identified in T2DM, including insulin resistance in muscle and adipose tissue, increased hepatic glucose production, impaired insulin secretion, decreased secretion and action of intestinal incretin hormones, and altered balance of CNS (central nervous system) pathways controlling food intake and energy expenditure. Therefore, when considering genetic and environmental factors in diabetes risk, it is critical to assess the individual and collective impact of these defects on tissue-specific metabolism and net whole-body physiology (Figure 2).

**Skeletal muscle**

Skeletal muscle is the largest insulin-sensitive organ in humans; thus insulin resistance in this tissue has a major impact on whole-body glucose homoeostasis. An early phenotype of individuals at high risk for the development of T2DM is reduced insulin-stimulated glucose disposal, as measured by the hyperinsulinæmic–euglycaemic clamp (low M) or by intravenous glucose tolerance testing (low S3) [26,66]. Moreover, the ability of insulin to activate signal transduction events, alter expression of selected candidate genes and stimulate muscle glycogen synthesis is impaired in high-risk subjects [66–68]. Accumulation of intramyocellular lipid is another key feature of muscle insulin resistance and may play a pathogenic role via the down-regulation of insulin signal transduction [69].

Although insulin resistance with decreased glucose disposal and glycogen synthesis is a consistent finding across populations, previous studies require a re-examination of our concepts of the primacy of muscle insulin signal...
transduction in the pathogenesis of insulin resistance. For example, although disruption of GLUT4, the glucose transporter which mediates the final step in glucose uptake into muscle, results in severe insulin resistance [70], the absence of IRs (insulin receptors) in skeletal muscle in mice (MIRKO mice (muscle IR knockout mice)) surprisingly causes minimal glucose intolerance [71]. Although there are always caveats in the application of rodent results to human disease, these findings demonstrate that glucose uptake into skeletal muscle is clearly critical for maintenance of glucose homoeostasis, but that novel mechanisms are likely to play important roles.

Adipose

As noted above, adipose tissue is now recognized not only as an energy storage depot, but also as a complex active endocrine tissue which can modulate whole-body metabolic physiology. In humans, either adipose tissue excess (obesity) or deficiency (lipodystrophy) is associated with insulin resistance and T2DM.

The importance of insulin signalling in adipose tissue has been highlighted by the phenotype of FIRKO mice (fat-specific IR knockout mice) [72]. FIRKO mice have markedly reduced fat mass, and are protected from obesity and glucose intolerance. Adipocytes from FIRKO mice also have marked heterogeneity in cell size and protein expression patterns, and inappropriately elevated serum leptin levels, suggesting previously unrecognized roles of insulin in regulation of adipose tissue biology [73].

One adipocyte secretory protein, RBP-4, has recently been linked to insulin resistance. Serum RBP-4 levels are increased in normoglycaemic insulin-resistant humans in parallel with many features of the metabolic syndrome and reduced by exercise training in parallel with improved insulin sensitivity [48]. Interestingly, animal results suggest that RBP-4 may play a pathogenic role, as administration of RBP-4 to mice induces insulin resistance, whereas treatment with fenretinide (promoting urinary clearance of RBP-4) normalizes insulin sensitivity in mice fed a high-fat diet [74].

Liver

The liver plays a central role in carbohydrate, protein and fat metabolism. It is critical for maintaining glucose homoeostasis via (i) storage of glucose as glycogen during fuel availability; and (ii) metabolism of glycogen and synthesis of glucose from non-carbohydrate sources such as amino acids (gluconeogenesis) during periods of fasting. Insulin acts directly on the liver by binding to hepatic IRs, activating insulin signalling pathways to modify gene expression and inhibit glucose production.

Inappropriate hepatic glucose production is a key feature of T2DM in humans [75], perhaps mediated by lipid accumulation, insulin resistance and excessive counter-regulatory hormone effect, including glucagon [76]. Rodent results also support an important role for the liver in diabetes pathogenesis. For example, LIRKO mice (liver-specific IR knockout mice) develop insulin resistance, glucose intolerance, impaired insulin suppression of hepatic glucose production and altered patterns of
Pancreatic islets

T2DM is characterized by progressive deterioration in \( \beta \)-cell function over time, leading to impaired insulin secretion and progression of hyperglycaemia. Islet function is reduced by at least 50% at diagnosis [79], and autopsy studies have shown that \( \beta \)-cell mass is reduced by approx. 60% in individuals with T2DM [80]. Many factors may contribute to progressive \( \beta \)-cell dysfunction, including glucotoxicity, lipotoxicity, pro-inflammatory cytokines, hyperleptinaemia, impaired incretin secretion and action [81], and islet amyloid accumulation [80]. Insulin resistance at the level of the \( \beta \)-cell may also contribute to impaired \( \beta \)-cell function [82]. Mice lacking IR specifically in \( \beta \)-cells (\( \beta \)IRKO mice (\( \beta \)-cell IR knockout mice)) develop progressive glucose intolerance due to selective loss of glucose-stimulated insulin secretion [83]. Remarkably, this closely resembles human T2DM because the lack of an insulin secretory response is specific for glucose, whereas the response to other secretagogues, such as arginine, is preserved. Thus the \( \beta \)IRKO mouse recapitulates many features of the \( \beta \)-cell functional defects underlying human T2DM and provides support for the hypothesis that insulin resistance may also underlie \( \beta \)-cell dysfunction.

\( \beta \)-Cell growth and function is also regulated by circulating factors, including insulin, glucose, the incretin hormones GLP-1 (gastric inhibitory peptide-1) and GIP (gastric inhibitory peptide) [81], leptin [84], and others. Postprandial GLP-1 responses are reduced in T2DM and may contribute to impaired postprandial insulin secretion [85] and potentially to reduced islet mass [86]. The importance of GLP-1 in diabetes pathogenesis has been underlined by recent therapeutic success with GLP-1 agonists [87] and DPP-IV (dipeptidyl peptidase-IV) inhibitors [88]. GIP secretion is preserved in T2DM, but functional response is impaired [81]. Additional complex inter-tissue interactions may also influence \( \beta \)-cell function, as suggested by the increase in \( \beta \)-cell mass in the LIRKO mouse [77].

Pancreatic \( \alpha \)-cells also contribute to diabetes pathogenesis via secretion of glucagon, a key counter-regulatory hormone secreted during the fasting state and during hypoglycaemia [89]. Absolute or relative glucagon excess may contribute to the increased glucose production and fasting hyperglycaemia characteristic of T2DM [76]. Thus targeting glucagon pathways, e.g. to reduce glucagon levels and/or its action, may be a potential therapeutic intervention in T2DM [90]. Interestingly, GLP-1 agonists (exenatide), DPP-IV inhibitors (e.g. sitagliptin) and the amylin analogue pramlintide also reduce glucagon secretion [87,88] and may contribute to their therapeutic efficacy.

Gastrointestinal tract and systemic metabolism

The gastrointestinal tract is increasingly recognized as a major contributor to complex mechanisms regulating appetite, satiety and motility, all of which are important for the regulation of whole-body insulin sensitivity. In addition to the incretins GLP-1 and GIP (discussed above), ghrelin, cholecystokinin, peptide YY and FGF-21 (fibroblast growth factor-21) are important intestinal peptides influencing systemic metabolism [91–94]. The synthesis of enterohepatic recirculation of bile acids may also contribute to regulation of systemic metabolism. Bile acids have been demonstrated to stimulate peripheral energy expenditure via modulation of tissue deiodinase activity and thus cellular thyroid hormone action [95].

CNS

Increasing evidence supports a role for neuronal sensing of energy intake, fuel availability and regulation of tissue-specific and whole-body metabolism [96–98]. IR signalling within the CNS may also regulate whole-body metabolism. For example, neuron-specific inactivation of the IR [NIRKO (neuronal IR knockout)] in mice produces obesity, mild insulin resistance, hyperinsulinaemia, hyperleptinaemia and hypertriglyceridaemia [99]. Determining whether brain insulin resistance is also present in humans will be an important focus of future clinical research studies.

SYSTEMIC METABOLIC DEFECTS IN T2DM

Inflammation

Although it is clear that tissue-specific defects contribute to T2DM pathophysiology, studies have emphasized common molecular events mediating defects in multiple tissues. A prime example is the body of evidence linking obesity to the induction of systemic inflammation. The activity of JNK (c-Jun N-terminal kinase)- and NF-\( \kappa \)B (nuclear factor \( \kappa \)B)-mediated inflammatory pathways are up-regulated in obese individuals, in association with increased expression of downstream cytokines, such as TNF-\( \alpha \) and IL-6, among others [50]. Indeed, robust predictors of the progression to T2DM risk include increased levels of IL-6 and CRP (C-reactive protein), a protein produced by the liver in response to systemic inflammation [100].

Adipose tissue is probably the central player and site of activation of systemic inflammation. More than 10 years ago, Hotamisligil and co-workers [101] demonstrated that adipocytes produced TNF-\( \alpha \) and could contribute to insulin resistance. Subsequent studies from multiple investigators have shown that high-fat feeding and obesity can induce inflammation within adipose tissue [102,103], with production of IL-6, MCP-1 and other inflammatory factors.
mediators. At a molecular level, this may be mediated through increased adipocyte death and remodelling [104], leading to recruitment of pro-inflammatory effector cells, increased oxidative stress and increased activity of transcription factors, including NF-κB, AP-1 (activating protein-1) and EGR-1 (early growth response-1) [105].

NF-κB has emerged as a key therapeutic target to reduce inflammatory processes associated with T2DM. Mouse models demonstrate that activation of NF-κB in liver can induce insulin resistance [106], whereas reduction in NF-κB signalling can reverse insulin resistance [107]. In humans, inhibition of NF-κB using salicylates is a promising new strategy for management of both T2DM and ‘pre-diabetes’ [108,109].

ER (endoplasmic reticulum) stress

Newly synthesized proteins are folded to attain their proper three-dimensional structures and undergo additional post-translational modification inside the ER, a network of intracellular membranes. Obesity, viral infections, toxins and other environmental stressors can all trigger ER stress. ER stress appears to be mediated by three primary effectors, including IRE-1 (inositol-requiring enzyme-1)/ERN-1 (ER to nucleus signalling-1), PERK (PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase) and ATF-6 (activating transcription factor-6), which initiate downstream responses to relieve ER stress [110]. For example, there is evidence of ER stress, including increased JNK activity and PERK phosphorylation, in adipose tissue from both genetic and diet-induced obesity mouse models [111]. Unfortunately, these cascades also disrupt insulin signalling, a feature potentially exacerbated by the reductions in heat-shock chaperone proteins in T2DM [112].

ER stress may also contribute to impaired β-cell function, as demonstrated during the progression of both T1DM (Type 1 diabetes mellitus) and T2DM, and Wolfram syndrome [113–115]. Indeed, IRE-1α activity is high in the β-cell, and IRE-1 signalling has major effects on insulin biosynthesis [113]. A study of islets from humans with T2DM also demonstrated that glucose-mediated induction of BiP (immunoglobulin heavy-chain-binding protein) and XBP-1 (X-box-binding protein-1) was increased compared with control islets, suggesting that T2DM may be characterized by increased susceptibility to ER stress [116].

Intriguingly, therapeutic reduction in ER stress may also be beneficial for T2DM. Overexpression of the ER chaperone protein ORP150 (oxygen-regulated protein 150) or HSP72 [72 kDa HSP (heat-shock protein)] in mice can improve insulin resistance and glucose tolerance [117]. Conversely, expression of antisense ORP150 in the liver of normal mice decreased insulin sensitivity [118,119]. More recently, treatment with two independent chemical chaperones, PBA (4-phenyl butyric acid) and TUDCA (taurine-conjugated ursodeoxycholic acid), has been shown to enhance ER responses and improve insulin sensitivity [120], and enhancement of HSP responses by heat shock or pharmacological activation of HSF (heat-shock factor) can also decrease JNK activation and reduce insulin resistance [117].

Mitochondrial dysfunction

Over the past few years, multiple lines of experimental evidence have converged to provide support for the hypothesis that mitochondrial dysfunction is intimately linked with the complex pathophysiology of T2DM. Genomic analysis of skeletal muscle biopsy samples from diverse populations has demonstrated that a dominant pattern associated with diabetes is reduced nuclear-encoded mitochondrial gene expression [121–123]. These findings have been extended by enzymatic and NMR spectroscopy studies further linking T2DM with decreases in mitochondrial oxidative activity [124], impaired basal and insulin-stimulated ATP synthesis [125,126] and reduced numbers of subsarcolemmal mitochondria [124]. Importantly, similar patterns are also observed in some populations of insulin-resistant, but completely normoglycaemic, individuals [121,127]. Thus mitochondrial oxidative dysfunction is a key feature of T2DM, potentially contributing to metabolic inflexibility, reduced lipid oxidation and increased accumulation of intramyocellular lipid. Whether this phenotype plays a pathogenic role, particularly in the absence of obesity or aging, remains unclear. Indeed, recent functional analysis in isolated mitochondria indicates that respiratory chain function is normal in non-obese patients with T2DM [128]. Adding complexity, experimental reduction in mitochondrial OXPHOS (oxidative phosphorylation) expression and function [via targeted deletion of AIF (apoptosis-inducing factor)] in an animal model results in enhanced insulin sensitivity [129]. Thus additional studies will be required to determine whether lipid oxidation, the tricarboxylic acid cycle or other oxidative pathways are primary pathogenic sites of the oxidative defect in T2DM or secondary to insulin resistance.

Many of the mitochondrial oxidative genes dysregulated in both T2DM and ‘pre-diabetes’ are regulated by the transcriptional coactivators PGC-1α and PGC-1β [121]. These findings suggest a potential role for both genetic and environmental factors in mediating diabetes risk by modifying the expression or activity of these transcriptional regulators. Indeed, expression of PGC-1α and PGC-1β is reduced in both skeletal muscle and adipose tissue from individuals with T2DM, obesity and insulin resistance [112,121,130]. Genetic variation, epigenetic factors, aging, impaired fitness and lipid excess may also contribute to such reductions in PGC-1 and related oxidative metabolic gene expression [131,132]. Similar patterns are observed during experimental induction of
obesity and insulin resistance in mice by high-fat feeding, and in cultured cells exposed to saturated fatty acids [125]. Thus decreased expression of PGC-1 may reflect an integrated transcriptional response to both genetic variation and environmental stressors, including obesity, inactivity and intracellular lipid excess, and contribute to the metabolic inflexibility characteristic of obesity or established T2DM. Moreover, mitochondrial dysfunction would also be predicted to impair insulin secretion.

Whether or how these genes contribute to ‘pre-diabetes’ remains unclear at present, as mitochondrial function (assessed by NMR) is impaired in healthy insulin-resistant individuals without T2DM, whereas the expression of PGC-1α and mitochondrial DNA content is normal [127]. Together, these findings indicate that additional, as yet unidentified, upstream regulators must contribute to impaired oxidative capacity and the risk of T2DM.

**EXPERIMENTAL APPROACHES TO UNDERSTANDING THE PATHOGENESIS OF T2DM**

**Genetic analysis of candidate genes**

The ‘thrifty gene hypothesis’ suggests that human evolution has selected for genes promoting efficient food collection and nutrient storage in order to promote survival during periods of famine. In the present era of food excess, these genes would be disadvantageous because they may contribute to energy storage, impaired energy expenditure and the risk of T2DM [133].

Many candidate genes have been identified on the basis of function related to insulin action in muscle, adipose or liver. A key example is the gene encoding PPARγ (PPARG) initially identified as a nuclear receptor key for adipogenesis [134]. Subsequent genetic studies and meta-analyses confirmed that the P12A polymorphism is associated with insulin sensitivity and influences diabetes risk [135,136], with odds ratios of 1.15–1.50 [137,138]. Although the molecular mechanisms responsible for these relationships have not been fully elucidated, the PPARG P12A substitution may reduce adipogenesis [139].

Similarly, genes influencing β-cell function have been identified as T2DM-susceptibility genes. For example, the E23K polymorphism in the ATP-sensitive potassium channel Kir 6.2 (KCNJ11) is consistently associated with disease risk [140,141], probably as a result of reduced insulin secretion [141]. It is also interesting that mutations in KCNJ11, ABCC8A [SUR1 (sulfonylurea receptor 1)] and INS (insulin) genes have also been recently linked to neonatal diabetes [142,143].

Positional approaches to candidate gene identification have been largely unsuccessful, with the notable exception of the genes CAPN10 (calpain 10) [10] and TCF7L2 (transcription factor 7-like 2; [13], and discussed below).

**Genome-wide screening**

Although candidate gene studies have been informative, such approaches are inherently limited. Recent improvements in high-throughput identification of genetic variants have made WGA (whole-genome association) studies feasible for the study of complex diseases. Although such approaches are less biased with regard to presumed functions or chromosomal locations of causal variants, limitations remain, primarily with regard to study design and identification of control and diseased individuals. For example, individuals without T2DM may have substantial insulin resistance, yet be misclassified as non-diabetic if detailed metabolic analysis cannot be performed. Moreover, if young individuals are evaluated, metabolic function may be normal at study, but individuals may in fact carry risk alleles which place them at risk for aging-related phenotypes, including T2DM. Despite these limitations, the flurry of recent WGA studies in late 2007 has provided interesting insights into the genetic basis of T2DM. These include the WTCCC (Wellcome Trust Case Control Consortium) scan [28], DGI (Diabetes Genetics Initiative) [29], FU-SION (Finland–United States Investigation of NIDDM Genetics) [138] and others [30–36]. Although each identified multiple potential genes of interest, including PPARG and KCNJ11, we will note several which were consistently associated in multiple studies.

The transcription factor TCF7L2 was first linked to T2DM risk using a positional approach [13], but this was followed rapidly by identification of the gene as high-ranking in WGA studies as well. Although genotype-based risk varies between studies, homozygosity for the high-risk allele confers approx. a 2-fold higher risk of T2DM [13]. These findings have been replicated in Caucasian and Mexican-American populations [12,144]. TCF7L2 polymorphisms increase the risk of T2DM progression among subjects with impaired glucose tolerance [12], perhaps via effects on islet expression of TCF7L2 and incretin-mediated insulin secretion [145]. However, given its broad patterns of expression, including the hypothalamus, and major role in the Wnt signalling pathway and development, it remains unclear whether TCF7L2-related diabetes risk can be solely attributed to effects on pancreatic function.

Additional novel T2DM-susceptibility loci were identified within or close to (i) SLC30A8 (encoding a zinc transporter and member of solute carrier family 30), potentially linked with β-cell insulin secretion, (ii) a haplotype block encompassing the HHEX/IDE/KIF11 (haematopoietically expressed homeobox/insulin-degrading enzyme/kinesin 5) genes [138], (iii) CDKAL1 (cyclin-dependent kinase 5 regulatory subunit associated protein-like 1), a widely expressed gene with sequence similarity to cell-cycle regulatory proteins, (iv) a region near CDKN2A/CDKN2B (cyclin-dependent kinase inhibitor 2A and 2B respectively), genes regulating...
cell-cycle progression, (vi) IGF2BP2 [IGF-2 (insulin-like growth factor-2)-mRNA-binding protein 2], encoding a protein regulating the translation of IGF-2 [28–30], and (vii) FTO (fat mass and obesity-associated), via its effect on BMI (body mass index) [146]. FTO has been identified as a member of the Fe(II)- and 2-oxoglutarate-dependent oxygenase family, which may catalyse the demethylation of single-stranded DNA and is highly expressed in the CNS; however, its functional role in mediating obesity risk remains unknown [147].

Taken together, what have we learned from these studies about T2DM pathophysiology? First, it is striking that all but one of the associated variants were located in non-coding regions. Whether these findings reflect variation in regulatory elements producing distant effects on gene expression (e.g. microRNA) remains unknown at present. Secondly, current functional annotations would suggest that many of the identified variants are located near genes which may affect β-cell function. This may indeed reflect study design, which focused on individuals with established T2DM (and thus β-cell dysfunction, by definition); identification of genes predisposing to T2DM through insulin-resistance effects will probably require analysis of quantitative traits and/or alternative subject cohorts for which insulin resistance is the primary phenotype. Furthermore, the majority of genes are actually widely expressed and thus may have roles in tissues not typically evaluated in clinical studies (including the CNS). Finally, each of the variants identified by WGA studies is associated with only a small increase in diabetes risk; in one analysis, even if an individual carried risk alleles at all ten identified loci, the incremental disease risk (over non-carriers) was rather small, with risk rising only from 5 to 20% [138]. Although these results may also reflect incomplete coverage of the human genome or study design (as noted above), it is also intriguing to speculate that DNA-sequence variants may not entirely account for family history effects. By design, sequence-based approaches utilized to date cannot assess the potential contributions of epigenetic variation, developmental patterning, differential splicing and shared familial environments in mediating risk linked to family history.

**Genomic approaches and the mitochondrial hypothesis**

Genomics, or the comparative analysis of mRNA expression, has reached maturity over the past decade and has been widely utilized to study patterns of expression characteristic of disease states or disease risk. Such approaches are particularly valuable for the study of complex diseases integrating both genetic and environmental risk, as is the case for T2DM. Moreover, the small tissue sample size required for genomic analysis has allowed the study of gene expression patterns from biopsies from healthy subjects with T2DM or at risk of diabetes, prior to disease onset.

As noted above, genomic studies have contributed significantly to the ‘mitochondrial hypothesis’, implicating mitochondrial oxidative dysfunction in metabolic inflexibility, reduced lipid oxidation and increased accumulation of intramyocellular lipid characteristic of insulin resistance [121,122,124,125]. A key unanswered question is whether these defects are primary, i.e. important in disease pathogenesis, or secondary to obesity, lipid accumulation [131] and/or the metabolic milieu of T2DM [123]. Additional studies in both muscle and adipose tissue from human subjects will be required to test whether defects in mitochondrial gene expression are also a feature of isolated insulin resistance early in the course of progression to diabetes.

Microarray approaches have also been utilized to identify candidate genes and pathways dysregulated in islets from humans with T2DM [148]. In one study employing isolated islets, the dominant pattern observed was marked down-regulation of expression of the transcription factor ARNT (aryl hydrocarbon receptor nuclear translocator)/HIF-1β (hypoxia-inducible factor-1β), accompanied by altered expression of genes related to glucose sensing and transcriptional control [148]. Interestingly, targeted reduction in ARNT also altered insulin secretion in both cultured cells and ARNT-deficient mice. It will be interesting to similarly evaluate diabetes-related gene expression patterns specifically in β-cells isolated from human islets using laser capture microdissection methods [149].

**Proteomic and metabolomic approaches to T2DM**

Despite major advances in genetic and genomic data over the past decade, these techniques have major limitations in the analysis of complex metabolic diseases such as T2DM, given the additional levels of regulation of translation and post-translational modification, signal transduction and metabolic pathways in target tissue cells. Recent advances in the sensitivity and throughput of proteomic technologies will probably allow application of these techniques more broadly to the pathogenesis of diabetes in the near future [150–152]. Indeed, early reports of plasma proteomic analysis have revealed differences in patterns even in family-history-positive individuals [153].

Analysis of metabolites, including lipids, in both plasma and tissue samples will also be a valuable part of the research toolbox for human translational studies. Indeed, metabolomic analysis indicates that excessive fatty acid uptake into mitochondria, but incomplete β-oxidation, is characteristic of insulin-resistant rodents [154].
CONCLUDING REMARKS

Recent scientific findings have underlined the importance of both genetic and environmental contributions to the risk of T2DM. Multi-faceted approaches to understanding this complex disease have led to several key hypotheses of pathogenesis, including: (i) a critical, and potentially dominant, role for both pre- and post-natal environmental factors, (ii) impaired regulation of nuclear-encoded mitochondrial gene expression, ER stress and systemic inflammation in the aetiology of insulin resistance, (iii) β-cell insulin resistance as a contributor to insulin secretory dysfunction and the onset of overt hyperglycaemia, (iv) altered CNS regulation of whole-body metabolism, and (v) altered intestinal hormone regulation of insulin secretion and T2DM risk.

Given that the epidemic of obesity, insulin resistance and T2DM is a major public health crisis worldwide, it is absolutely critical to define further the complex web of genetic and environmental factors contributing to this complex disease in order to develop more effective strategies for both prevention and treatment.

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