REVIEW

Monogenic syndromes of abnormal glucose homeostasis: clinical review and relevance to the understanding of the pathology of insulin resistance and β cell failure

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Type 2 diabetes mellitus is caused by a combination of insulin resistance and β cell failure. The polygenic nature of type 2 diabetes has made it difficult to study. Although many candidate genes for this condition have been suggested, in most cases association studies have been equivocal. Monogenic forms of diabetes have now been studied extensively, and the genetic basis of many of these syndromes has been elucidated, leading to greater understanding of the functions of the genes involved. Common variations in the genes causing monogenic disorders have been associated with susceptibility to type 2 diabetes in several populations and explain some of the linkage seen in genome-wide scans. Monogenic disorders are also helpful in understanding both normal and disordered glucose and insulin metabolism. Three main areas of defect contribute to diabetes: defects in insulin signalling leading to insulin resistance; defects of insulin secretion leading to hypoinsulinaemia; and apoptosis leading to decreased β cell mass. These three pathological pathways are reviewed, focusing on rare genetic syndromes which have diabetes as a prominent feature. Apoptosis seems to be a final common pathway in both type 1 and type 2 diabetes. Study of rare forms of diabetes may help ion determining new therapeutic targets to preserve or increase β cell mass and function.

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Diabetes mellitus is a group of metabolic disorders characterised by hyperglycaemia and defective metabolism of lipids and glucose. Diabetes was estimated to affect 177 million people worldwide in 2000 and this figure is projected to increase to 300 million by 2025. Most of this increase in diabetes is accounted for by an increased prevalence of type 2 diabetes.

Type 2 diabetes accounts for approximately 90% of cases of diabetes mellitus worldwide,[1] and is characterised by a combination of defects in insulin secretion and insulin sensitivity. There is a significant genetic component to type 2 diabetes, but genome-wide scans and association studies have proved difficult to reproduce across different populations.[2] Type 1 diabetes is characterised by autoimmune destruction of pancreatic β cells, causing absolute insulin deficiency. The human leucocyte antigen (HLA) gene region contributes 42%, and the insulin gene region 10% to the genetic susceptibility of type 1 diabetes. Other genes must therefore be involved in determining disease risk, each with a small effect but with a collective importance similar to the HLA region.[3] Common to both type 1 and type 2 diabetes is progressive loss of β cell function. In type 1 diabetes this β cell loss is modulated by cytokines and is rapid; in type 2 diabetes progressive insulin resistance leading to increased demand on the β cell, endoplasmic reticulum stress, lipotoxicity, and glucotoxicity cause a more gradual loss of β cell function. The contribution of β cell loss to type 2 diabetes in the human is still controversial. It has been clearly shown that in rodents the β cell mass is variable throughout life, and that replication of mature β cells is an important mechanism for increase in rodent β cell mass,[4] but for obvious reasons this has not been easy to show in humans. Recently, however, two papers from Japan and the USA have shown apparent loss of β cell mass in human subjects at necropsy, interestingly showing differences in normal obese versus diabetic obese subjects.[5 6] If, as seems likely, β cell loss is common to type 1 and type 2 diabetes, the shared pathway for loss of β cell mass in both forms is likely to be apoptosis, although the mechanisms underlying the initiation of apoptosis may differ.[7]

Maturity onset diabetes of the young (MODY) is a group of monogenic diabetes disorders causing 1–2% of cases of diabetes in the United Kingdom.[8] Mutations in one of six MODY genes account for the diabetes in 85% of patients with MODY.[9] Although the phenotypes of MODY syndromes differ, a β cell defect is common to all. Approximately 5% of individuals with diabetes have the condition as part of a constellation of features or syndrome.[10] These syndromes are usually monogenic and may cause insulin deficiency, insulin resistance, or both.

In the last 10 years extensive advances in our understanding of monogenic diabetes disorders have stemmed from tight clinical characterisation

Abbreviations: BSCL, Berardinelli–Seip congenital lipodystrophy; FARR, Friedreich's ataxia with retained lower limb reflexes; FFA, free fatty acids; GSIR, glucose stimulated insulin release; LOFA, late onset Friedreich's ataxia; MODY, maturity onset diabetes of the young; PERK, pancreatic endoplasmic reticulum kinase; PPARγ, peroxisome proliferator activated receptor γ; RMS, Rabson–Mendenhall syndrome; TNDM, transient neonatal diabetes; TRMA, thiamine responsive megaloblastic anaemia

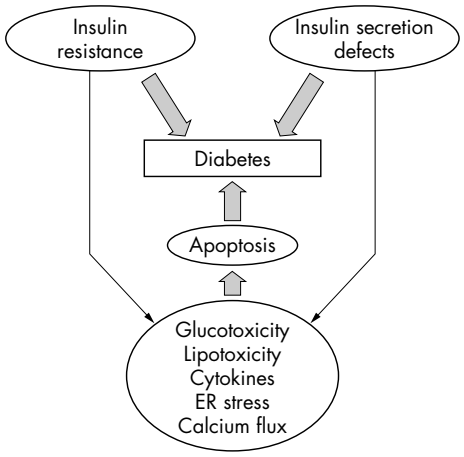

Figure 1 The interaction between insulin resistance, apoptosis, and insulin secretion defects in the aetiology of diabetes mellitus

and the discovery of genes for many of these syndromes. Identification of some of the MODY genes has revealed important components of the insulin secretion pathway, and has led to specific treatments for patients with MODY—for example, patients with HNF-1α gene mutations have been found to be very sensitive to sulphonylurea drugs, and so may be able to stop insulin treatment.[11 12]

In this paper we will argue that the processes contributing to diabetes are threefold: insulin resistance and signalling problems; defects in insulin secretion; and diminished insulin secretion owing to reduction in β cell mass by apoptosis (fig 1). We shall illustrate how rare syndromes of diabetes, and disorders of glucose homeostasis, have helped advance our knowledge of each mechanism, with specific examples (table 1).

INSULIN RESISTANCE

Insulin resistance is an important contributory factor to type 2 diabetes, and is particularly relevant with increasing obesity rates worldwide. In recent years it has been realised that adipose tissue is not an inert tissue as was once thought, but actively secretes hormones.[13] Peroxisome proliferator activated receptor γ has been at the forefront of our understanding of this new endocrine organ. Familial partial lipodystrophy and insulin receptor abnormalities have helped us understand more about the pathways for insulin signalling, and how they can be disrupted.

Peroxisome proliferator activated receptor γ (PPARγ)

PPARγ (OMIM 601487) is a transcription factor predominantly expressed in adipose tissue,[14] but has also been shown to be expressed in small quantities in liver, muscle, and pancreas.[15] It has become clear that PPARγ plays a vital role in the regulation of body fat distribution and insulin sensitivity, acting to promote energy storage as a thrifty response. Thiazolidinediones are a class of antidiabetic drugs

Table 1 Conditions illustrating the contribution of insulin resistance, insulin secretion defects, and apoptosis to the development of diabetes

Presumed mechanism	Condition	Gene and location	Other features
Insulin resistance	PPARγ	PPARγ (3p25)	• Hypertension • Hypertriglyceridaemia
	Congenital generalised lipodystrophy	BSCL1 (9q34.3) BSCL2 (11q13)	• Congenital lipodystrophy • Hypertriglyceridaemia • Hyperandrogenism
	Insulin receptor defects	INSR (19p13.2)	• Fasting hypoglycaemia • Postprandial hyperglycaemia • Lipodystrophy and dysmorphism • Progressive diabetes
Insulin secretion defects	Glucokinase defects	GCK (7p15–p13)	• MODY • PNDM • PHHI
	Kir6.2 defects	KCNJ11 (11p15.1)	• Developmental delay • Epilepsy • PHHI
Apoptosis	Wolcott–Rallison	EIF2AK3 (2p12)	• Epiphyseal dysplasia • Developmental delay • Renal/hepatic impairment
	Wolfram	WFS1 (4p16.1) ?WFS2 (4q22–24)	• Deafness • Optic atrophy • Diabetes insipidus
	Friedreich's ataxia	FRDA1 (9q13)	• Ataxia • Pyramidal signs • Loss of leg reflexes
	Werner syndrome	WRN (8p12–11.2)	• Premature aging • Sarcomas
	Thiamine responsive megaloblastic anaemia	SLC19A2 (1q23.3)	• Megaloblastic anaemia • Sensorineural deafness

GCK, glucokinase; MODY, maturity onset diabetes of the young; PHHI, persistent hyperinsulinaemic hypoglycaemia of infancy; PNDM, permanent neonatal diabetes.

that act through PPARγ stimulation to improve hypertension, blood glucose control, and insulin sensitivity in patients with type 2 diabetes.[16] The complex effects of PPARγ are modulated by several ligands (most notably the thiazolidinediones), the tissue expression of PPARγ, and the specific activity of PPARγ. Our understanding of PPARγ effects has been advanced by study of mutations in the PPARγ gene (PPARG) in humans, tissue selective knockout of PPARγ, and study of the effects of PPARγ ligands on body composition and insulin sensitivity.

The effects of mutations in PPARG in humans illustrate the complexity of the role of PPARγ. An activating mutation in PPARG is associated with obesity but preserved insulin sensitivity.[17] Inactivating mutations in PPARG cause a form of lipodystrophy with insulin resistance.[18–20] These findings suggest that increasing activity of PPARγ is inversely proportional to insulin sensitivity, albeit at the expense of weight gain, and indeed this is the effect of thiazolidinediones.[21] However, a common polymorphism in PPARG (Pro12Ala), which decreases the activity of PPARγ, is associated with high insulin sensitivity and decreased susceptibility to type 2 diabetes.[22 23] How can activation and attenuation of PPARγ both be associated with improved insulin sensitivity? The solution may be twofold. First, stimulation of PPARγ causes small adipocyte proliferation[24] while loss of function of PPARγ causes decreased lipogenesis and increased fatty acid oxidation[24]; both processes result in decreased adipocyte hypertrophy and decreased free fatty acids, and hence increased insulin sensitivity.[24] Second, PPARγ activity relates linearly to fat mass accretion but not to insulin sensitivity,[25] suggesting that other tissues are involved in the regulation of insulin sensitivity. Murine models have allowed elimination of PPARγ from selected tissues to explore this suggestion further.

Murine models of non-specific PPARG mutations compare well with human phenotypes.[26 27] Adipose tissue specific PPARγ knockout mice have severe lipodystrophy[28 29] but normal glucose tolerance. Knockout of liver PPARγ causes an increase of free fatty acids (FFA), increased adiposity, and insulin resistance.[30] Muscle knockout of PPARγ leads to raised FFA, adiposity, and insulin resistance.[31 32] These results indicate that the interplay between all three tissues is important in maintaining glucose and lipid homeostasis. The absolute importance of adipose tissue in insulin sensitivity is illustrated by the fact that thiazolidinediones cause increased insulin sensitivity in both liver and muscle knockouts but not the adipose tissue knockout mouse, although the blood glucose response remains.

The selective adipose tissue PPARγ −/− mouse knockout shows that thiazolidinedione effects on glucose (but not insulin resistance) are independent of adipose tissue. PPARγ activation reduces islet cell hyperplasia[33] and is also thought to reduce islet cell apoptosis—in part by reducing lipid deposition and inhibiting nitric oxide synthesis in the β cell and in part by reducing free fatty acids by repartitioning adipose tissue and stimulating production of small insulin sensitive adipocytes.[15] PPARγ has also been shown to upregulate glucokinase[34] and the Glut 2 glucose transporter,[35] key components in glucose sensing, in both pancreas and liver, thus increasing insulin release and hepatic insulin sensitivity.

PPARγ appears to play a vital role in fat mass accretion, and independently in the regulation of insulin sensitivity. Increasing understanding of PPARγ has led to attempts to modulate rather than absolutely stimulate PPARγ. Thiazolidinediones are potent PPARγ agonists which are effective at lowering blood glucose and improving insulin resistance in patients with impaired glucose tolerance or type 2 diabetes,[16] and seem to alter fat distribution so that subcutaneous rather than visceral adipose tissue is laid down.[21 36 37] Weight gain on thiazolidinediones can be a problem, however, and experiments with other PPARγ ligands have shown that partial agonists and antagonists of PPARγ can produce the effects on glucose tolerance and insulin resistance without the increase in adipose tissue.[38–40] These agents may prove to be extremely useful antidiabetic drugs.

Berardinelli–Seip congenital lipodystrophy/ congenital generalised lipodystrophy

Berardinelli–Seip congenital lipodystrophy (BSCL; OMIM 269700) is a rare disorder characterised by congenital absence of subcutaneous and visceral fat, with severe insulin resistance that progresses to diabetes in early adolescence.[41] Other features of the condition are acanthosis nigricans, hypertriglyceridaemia, fatty liver, virilisation, and cardiomyopathy.[41] The condition is autosomal recessive; two genes have been isolated by positional cloning which account for 82% of cases.

The first, BSCL1 on chromosome 9q34, encodes 1-acylglycerol-3-phosphate-O-acyltransferase 2 (AGPAT2).[42] AGPAT2 catalyses an important step in the triacylglycerol pathway. AGPAT2 regulates conversion of lysophosphatidic acid to phosphatidic acid. Phosphatidic acid is the substrate for phosphatidylinositol production. Phosphatidylinositol is an important messenger in insulin signalling pathways and so disruption of its production may be the cause of insulin resistance in BSCL.[43]

The second locus, BSCL2 on 11q13,[44] maps to a gene Gng31g ,which encodes a protein, seipin, of unknown function. Unlike AGPAT2, Gng31g is widely expressed in the brain. Patients with Gng31g mutations have learning difficulties (80%), are three times more likely to suffer from cardiomyopathy, have diabetes with onset at a significantly earlier age, and may be at risk of early death when compared with patients with mutations in AGPAT2.[41 45] There is poor genotype to phenotype correlation in BSCL, suggesting that alternative environmental or genetic influences may be important.[46]

Insulin receptor defects

Insulin receptor defects produce a spectrum of insulin resistant phenotypes,[47] ranging from the severe Donohue syndrome (OMIM 246200) to the relatively mild insulin resistance type A (OMIM 147670). Patients with Donohue syndrome (previously known as leprechaunism) have severe intrauterine growth restriction and hyperinsulinaemia with abnormal glucose homeostasis, characterised by fasting hypoglycaemia and postprandial hyperglycaemia,[48] facial dysmorphism, reduced subcutaneous fat, and protuberant abdomen. Patients have <10% of wild type insulin binding, and either premature stop mutations or mutations in the extracellular domain of the receptor. These defects are associated with death occurring before two years of age.[48] In contrast, patients with Rabson–Mendenhall syndrome (RMS) have mutations in the intracellular domain of the receptor, and insulin binding at levels of up to 25% of normal.[48] RMS can be distinguished from Donohue syndrome by the presence of dysplastic gums and teeth, thickened nails, and hirsutism. Children with RMS develop progressive ketoacidotic diabetes, most dying before adolescence.[48] Type A insulin resistance is associated with hirsutism and hyperandrogenism in slim individuals, and with survival well into adulthood.[49]

Insulin affinity studies had shown a defect in insulin affinity in these syndromes before the receptor gene was isolated.[50] Following discovery of the insulin receptor gene on chromosome 19p, homozygous and compound heterozygous mutations were found in several individuals with severe

insulin resistance.[47] These have varied effects on the insulin receptor. Taylor classified these mutations into five classes: those that impair synthesis of receptors; those that impair transport of receptors to the cell membrane; those that decrease receptor affinity for insulin; those that reduce the tyrosine kinase activity of the receptor intracellular domain; and those that accelerate receptor degradation.[47] Initially it was thought that the presence of some insulin receptors was vital for intrauterine survival, but patients with homozygous null mutations have since been described.[48 51] The finding that a heterozygote father of one patient with Donohue syndrome was insulin resistant was of interest for the wider population, but the very rarity of Donohue syndrome mitigates against heterozygous mutations being a common cause of type 2 diabetes.[52] The latest research in these syndromes has shown, by gene expression studies, significant alterations in the expression of genes encoding growth factors (including insulin-like growth factors) and apoptotic factors in patients with Donohue syndrome.[53] Further work in this area may help link the contributions of insulin resistance and loss of β cell mass in diabetes pathology.

INSULIN SECRETION DEFECTS

One of the successes of the 1990s was the unravelling of the genetics of maturity onset diabetes of the young. While most of the genes found for MODY have been transcription factors,[9] the glucokinase gene encodes a key regulatory enzyme, and a newly found neonatal diabetes gene KCNJ11 encodes Kir6.2, a component of the ATP sensitive potassium channel. Glucokinase and Kir6.2 are key components in the β cell insulin secretion pathway. What is particularly interesting is that both activating and inactivating mutations in these genes have been found, and the contrasting phenotypes have led to greater understanding of the molecular biology of the β cell and also to guidelines for treatment in patients with these conditions.

1. Thiamine responsive megaloblastic anaemia
2. Glucokinase defects
3. Friedreich's ataxia
4. Kir6.2 neonatal diabetes
5. Wolcott-Rallison syndrome
6. Wolfram syndrome
7. Werner syndrome

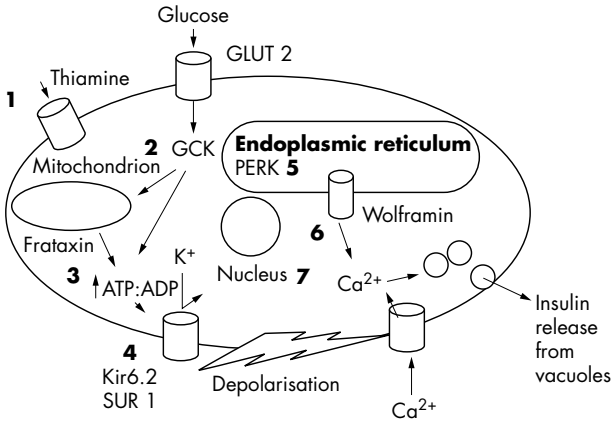

Figure 2 Schematic of the current theory of glucose stimulated insulin release from the pancreatic β cell, showing the proposed sites of action of inherited diabetes. *Current theory of insulin release*: Glucose enters the β cell through the GLUT 2 transporter. Glucose is metabolised in the glycolytic pathway by enzymes including glucokinase (GCK) to produce ATP. Rising intracellular ATP:ADP closes the ATP sensitive potassium channel Kir6.2/SUR1, causing membrane depolarisation. Voltage gated calcium channels open and intracellular calcium flux triggers insulin release.

Glucokinase defects

Glucokinase (GCK) is a key hexokinase enzyme involved in modulation of glucose stimulated insulin release (GSIR). The high $S_{0.5}$, cooperativity with glucose, and lack of inhibition by its product glucose-6-phosphate make it the rate limiting step in GSIR and thus allow it to act as a glucose sensor for the β cell.[54] Glucose enters the cell by way of the GLUT 2 transporter and is then metabolised by glucokinase as the first step of the glycolysis pathway that generates ATP necessary for insulin exocytosis (fig 2). Given the central role of glucokinase it was a prime candidate gene for monogenic diabetes, and heterozygous[55 56] and homozygous and compound heterozygous[57 58] inactivating mutations have been found (OMIM *138079) that cause diabetes, as well as heterozygous activating mutations that cause hyperinsulinism.[59]

Heterozygous inactivating mutations of glucokinase cause a subtype of maturity onset diabetes of the young (MODY2/GCK-MODY). There are now over 190 published mutations in glucokinase in different ethnic populations causing MODY2.[54] MODY2 is a mild form of hyperglycaemia/diabetes; clinically, patients are often asymptomatic and are classically found to have diabetes coincidentally, most commonly during pregnancy. The defect in glucokinase leads to a rise in the plasma level of glucose at which insulin secretion is triggered, from a normal value of ~5 mmol/l. Compensation by the wild type allele means that the rise in GSIR in glucokinase is limited to ~7 mmol/l in all cases, equating to a fasting plasma glucose of 6–8 mmol/l.[54] This raised fasting glucose remains constant throughout life, and can be managed by basic dietary measures alone, in contrast to the transcription factor MODY types where fasting glucose rises with age resulting in complications in the long term.[60]

Homozygous or compound heterozygous inactivating mutations of glucokinase cause permanent neonatal diabetes.[57 58] This is a very rare condition and shows evidence of genotype–phenotype correlation. Mutations in the substrate binding site cause severe increases in the GSIR, resulting in fasting glucose levels of 50 mmol/l or more. Unlike MODY2, the diabetes is insulin dependent, requiring full insulin replacement.[58]

Heterozygous activating mutations in glucokinase cause persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI).[59] PHHI is a heterogeneous disorder caused by defects in any one of Kir6.2[61] or SUR1[62] (components of the ATP sensitive potassium channel of the β cell), GLUD1[63] (an enzyme in the amino acid metabolism pathway in the β cell), or glucokinase.[59] Unlike PHHI caused by abnormalities of Kir6.2/SUR1, in GCK-PHHI there is still a relation between glucose level and insulin production, but it is reset to a lower GSIR than normal.[54] GCK-PHHI responds well to diazoxide which acts to open the potassium channels and inhibit insulin release. GCK-PHHI is an alteration in glucose homeostasis that can range from mild hypoglycaemia, treated in some cases by regular eating alone, to severe persistent hypoglycaemia.[64]

These different mutations in the glucokinase gene cause three separate phenotypes—MODY, permanent neonatal diabetes, and PHHI—each illustrating aspects of glucose stimulated insulin release and providing new areas for targeting with drug treatments in diabetes.

Kir6.2 defects (OMIM *600937)

The β cell potassium channel is intimately involved in the regulation of insulin secretion and is an octomeric complex composed of four inwardly rectifying potassium channels (Kir6.2), and four sulphonylurea receptor subunits (SUR1).[65] Kir6.2 binds ATP to close the channel, and magnesium nucleotides bind to SUR1 causing activation of the channel.[65]

In the fed state, high intracellular glucose generates ATP through glycolysis and closes the potassium channel, leading to cell membrane depolarisation and insulin release (fig 2). Fasting lowers the intracellular ATP:ADP ratio, resulting in opening of the potassium channel and inhibition of insulin secretion.[66] Both Kir6.2 and SUR1 are vital for the correct regulation of insulin secretion, and inactivating mutations in the genes encoding both have been shown to cause autosomal recessive PHHI, characterised by complete loss of GSIR and poor response to diazoxide.[61 62]

Interest in the role of Kir6.2 in insulin secretion led to consideration of the gene for Kir6.2 (KCNJ11) as a candidate gene for type 2 diabetes. An association study in 2486 UK subjects showed an association with type 2 diabetes and the E23K allele of KCNJ11.[54] Following the finding that glucokinase mutations can cause both diabetes and hyperinsulinaemia, mutation analysis showed activating mutations in the KCNJ11 gene encoding Kir6.2 in ~34% of permanent neonatal diabetes cases.[66] This raised the possibility of treatment with oral sulphonylureas rather than insulin injection, and has proved successful in some patients, albeit with short follow up to date.[67] Some cases of neonatal diabetes with KCNJ11 mutations have also shown mild dysmorphism, developmental delay, and epilepsy.[66 68]

APOPTOSIS

One of the evolutionary compromises the β cell appears to have accepted is increased sensitivity to apoptosis; indeed it appears that the balance between β cell loss and replication is one of the ways in which glucose homeostasis is maintained in the normal individual, the overall β cell mass being remarkably variable in response to the demands of pregnancy or obesity.[69] Moreover the β cell deals routinely with substrates and byproducts that are lethal to the cell in raised concentrations. Glucotoxicity, lipid toxicity, free radicals, and cytokines have all been implicated as factors initiating apoptosis.[70] Unregulated apoptosis appears to be a final common pathway for the loss of β cell mass in type 1 diabetes, type 2 diabetes,[70] and possibly MODY.[71] The development of a transgenic mouse model of transient neonatal diabetes (TNDM), in which the human transient neonatal diabetes locus TNDM29 is expressed,[72] has suggested that apoptosis may be an important feature in diabetes development in this disease as well. Humans with TNDM present with diabetes at a median age of three days, but then recover to normal glucose homeostasis at a median of 12 weeks.[73] Follow up has shown that many of these individuals then develop permanent diabetes as adolescents.[73] Fetal transgenic mice have reduced β cell numbers at day 14 of gestation compared with normal mice, but have increased β cell production resulting in normal numbers of β cells by term, and greater than normal β cell numbers as juveniles. However, as adults the transgenic mice have the same number of β cells as their normal peers, suggesting β cell loss as adolescents, and this β cell mass is coupled with impaired glucose tolerance.[72] TNDM29 contains at least one gene, zinc finger protein, that regulates apoptosis and cell cycle arrest (ZAC) and which is a potent apoptotic factor.[74] This model suggests that the balance between β cell neogenesis, apoptosis, and function is key to the remitting and relapsing nature of TNDM.

Although initially it was thought that type 1 diabetes was caused by autoimmune reactions and type 2 by non-immune processes, this division now seems oversimplistic. While cytokine mediated apoptotic pathways, chiefly tumour necrosis factor α (TNFα) and interleukin 1β (IL1β), have been shown to be the leading cause of β cell loss in type 1 diabetes,[75] inflammatory responses also seem to have a bearing on type 2 diabetes. Glucose toxicity to islet cells in high concentrations seems to be mediated by IL1β secreted by the islet cell itself[76] and probably contributes to both type 1 and type 2 diabetes. Some overlap between type 1 and type 2 diabetogenic processes appears to be the cause of the ill defined entity of latent autoimmune diabetes of the adult. Many rare forms of diabetes have apoptosis as an underlying cause, and we will discuss five different mechanisms proposed as causes for β cell apoptosis: Wolcott–Rallison syndrome (endoplasmic reticulum stress); Wolfram syndrome (calcium signalling); Friedreich's ataxia (mitochondria dysfunction); Werner's syndrome (deficient DNA repair); and thiamine responsive megaloblastic anaemia[69] (defective ribose synthesis).

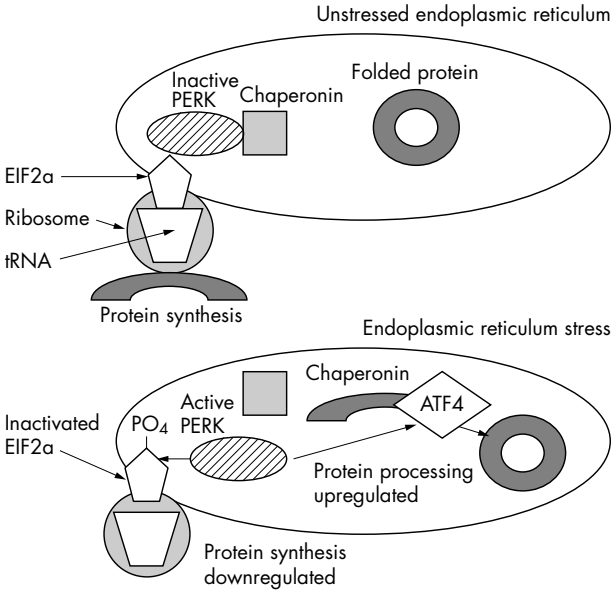

Figure 3 Schematic of the role of pancreatic endoplasmic reticulum kinase (PERK) in the response to endoplasmic reticulum stress. Build up of unfolded protein causes PERK to dissociate from inactivating chaperonins. Activated PERK then phosphorylates EIF2α and activates ATF4, causing reduction of protein synthesis and increase protein processing.

Wolcott–Rallison syndrome

Wolcott–Rallison syndrome (OMIM 226980) is a rare autosomal recessive condition characterised by extremely early onset diabetes, epiphyseal dysplasia, renal impairment, acute hepatic failure, and developmental delay. Diabetes presents in infancy and is associated with β cell loss, leading to insulin deficiency without autoimmune pathology.[77 78] The Wolcott–Rallison gene *EIF2AK3*,[78] which codes for eukaryotic initiation factor 2α kinase 3, is also known as the pancreatic endoplasmic reticulum kinase (EIF2AK3/PERK).[78] PERK regulates the cellular response to endoplasmic reticulum stress.[79] The latter is caused when an imbalance between protein synthesis and processing occurs, such that unfolded protein builds up in the endoplasmic reticulum. This occurs commonly in secretory cells, and in the normal state is controlled by three main processes: first, protein synthesis is reduced; second, protein processing and removal of excess unfolded protein is upregulated; and third, apoptosis occurs.[79] PERK is a vital component of the control of these processes (fig 3). PERK is normally bound in the endoplasmic reticulum to chaperonins that inactivate it.[80] These disassociate from PERK when unfolded protein levels rise in the endoplasmic reticulum. PERK kinase activity allows phosphorylation of eukaryotic initiation factor 2 α (EIF2α), a complex recruiting tRNA to ribosomes. This causes a

reduction in EIF2α activity and thus a suppression of protein synthesis.[79] PERK is also crucially involved in the second step of the cellular response to endoplasmic reticulum stress—the upregulation of translation of activating transcription factor 4 (ATF4), which is involved in protein processing.[79] It can be predicted that loss of PERK activity will lead to uncontrolled apoptosis.

PERK knockout mice are born with apparently normal islet cells, but undergo islet cell loss over the first weeks of life culminating in the development of lethal diabetes.[79] Interestingly PERK −/− mice do not show features of hepatic dysfunction or impaired gluconeogenesis seen in Wolcott–Rallison patients, but this phenotype is seen in mice with a defective EIF2α that is resistant to phosphorylation. PERK is one of four EIF kinases. These findings suggest that in mice, unlike humans, hepatic EIF2α is not exclusively phosphorylated by PERK.[79]

EIF2AK3 is expressed in islet cells, liver, bone, glomerular tissue, and lung in humans,[81] and loss of kinase activity has been demonstrated in patients with Wolcott–Rallison syndrome.[82] Initial findings indicate that *EIF2AK3* is not a common diabetes susceptibility gene in the general diabetes population,[83] but study of Wolcott–Rallison syndrome has highlighted endoplasmic reticulum stress as an important potential factor in the development of diabetes.

Wolfram syndrome

Wolfram syndrome (sometimes known by the acronym DIDMOAD) (OMIM 222300) is an autosomal recessive syndrome in which the association of diabetes with progressive optic atrophy under 16 years of age is diagnostic.[84] Other features are bilateral sensorineural deafness, diabetes insipidus, dilated renal tracts, and truncal ataxia or more protean neurological signs, with the complete phenotype seen in 75% of patients.[84] The diabetes is non-autoimmune and insulin deficient and presents at a mean age of six years. Patients require insulin treatment from the time of diagnosis. The median age of death in Wolfram syndrome is 30 years and development of the full phenotype is seen with increasing age.[85] A gene for Wolfram syndrome (WFS1) was discovered in 1998 on chromosome 4p by two separate groups, using positional cloning[86] and candidate gene approaches.[87] Mutations in WFS1 are present in at least 90% of patients with clinical Wolfram syndrome.[87] WFS1 encodes a protein termed wolframin that has nine putative transmembrane segments; it is exclusively located in the endoplasmic reticulum in the rat[88] and appears either to modulate the action of calcium channels in the endoplasmic reticulum or to actually be a calcium channel itself. Calcium fluxes have been shown to be important in intracellular signalling and in the prevention of apoptosis. It has been proposed that loss of wolframin expression leads to apoptosis in the pancreatic β cells and the neurones of the central nervous system, producing the characteristic Wolfram phenotype.[88]

Very recently, Japanese researchers have disrupted the WFS-1 gene in mice.[89] They have shown that mutant mice have a 23% reduction in glucose stimulated insulin secretion, and develop diabetes. Insulin secretion mediated directly by calcium release from the endoplasmic reticulum was also reduced in these mice. When wolframin was re-expressed in islets from mutant animals, glucose stimulated insulin release and insulin release mediated by calcium release in the endoplasmic reticulum were both restored. In addition, isolated islets from mutant mice showed higher than normal susceptibility to apoptosis when exposed to agents inducing endoplasmic reticulum stress. Wolframin seems to be vital for both calcium mediated insulin release in the endoplasmic reticulum and the prevention of apoptosis induced by endoplasmic reticulum stress.

As with other syndromes discussed in this review, WFS-1 has been suggested as a susceptibility gene in type 2 diabetes. In a study of 327 individuals with type 2 diabetes and 357 controls, the R456-H611 haplotype was significantly more frequent in type 2 subjects.[90] Further studies in other populations will be required to prove the significance of this finding.

Friedreich's ataxia

Friedreich's ataxia (OMIM 229300) is an inherited neurodegenerative disorder. Patients usually present with four limb ataxia, associated with cerebellar signs, absent lower limb reflexes, sensory loss, and pyramidal signs before the age of 20, and the disease is progressive.[91] Diabetes mellitus is seen in around 10% of patients. Clinically there are three subforms of Friedreich's ataxia—classical disease, late onset disease (onset >20 years)(LOFA), and Friedreich's ataxia with retained lower limb reflexes (FARR).[91] The pattern of inheritance in Friedreich's ataxia is autosomal recessive, and the mutated gene FRDA1 has been mapped to chromosome 9q.[92] FRDA1 encodes a protein frataxin which is localised to mitochondria.[93] The genetic defect in most patients is homozygous areas of trinucleotide (GAA) repeat expansion in exon 1 of FRDA,[94] but some patients are compound heterozygotes with an expanded allele and a point mutation in FRDA1.[94] Most normal individuals have 7 to 22 repeats in FRDA1, while patients with Friedreich's ataxia have 200 to 900+.[94] There is a genotype–phenotype correlation: individuals with less than 500 repeats are significantly less likely to develop cardiomyopathy and more likely to have the milder disease variants LOFA and FARR,[91] but there is no relation between repeat length and diabetes.[95] Compound heterozygotes are more likely than homozygotes to have a milder atypical Friedreich phenotype.[95 96] Friedreich's ataxia is the first known recessive trinucleotide repeat disease and, unlike dominant examples, lacks genetic anticipation. It does resemble other trinucleotide repeat diseases in that paternal expanded alleles tend to reduce in size during transmission, consistent with selection pressure for spermatozoa with shorter repeats; however, unlike myotonic dystrophy where maternal repeats expand with each generation, maternal repeats in Friedreich's ataxia can expand or reduce with transmission.[91]

The areas of expanded repeats in Friedreich's ataxia can form a unique structure called sticky DNA.[97] This association of two triplet expansion segments has been shown to reduce frataxin transcription by binding to the DNA and blocking the action of RNA polymerase.[97] Frataxin is localised in human and other species to mitochondria, and in vivo, deficits in mitochondrial function can be seen in patients with Friedreich's ataxia.[98] Fibroblasts from Friedreich patients show increased sensitivity to oxidative stress from hydrogen peroxide or iron, which can be reversed by iron chelators[99] or frataxin transfection.[100] In many cell lines, oxidative stress causes increased intracellular calcium levels and apoptosis. Calcium chelators and apoptosis inhibitors preferentially rescue Friedreich fibroblasts, suggesting that uncontrolled apoptosis maybe the cause for the manifestations of Friedreich's ataxia.[99] Finally, lack of frataxin in knockout mice has been shown to be associated with progressive loss of β cell mass leading to diabetes with reduced insulin secretory capacity.[101]

The association of diabetes in Friedreich's ataxia and the asymptomatic carriage of expanded repeats in heterozygotes has led to suggestions that heterozygote status for this disease might be an important cause of susceptibility to type 2 diabetes. Heterozygous carriers of a triplet expansion are

insulin resistant[102] and those with impaired glucose tolerance have altered β cell characteristics.[103] Studies in German and American populations have suggested an association with type 2 diabetes,[104] but this has not been borne out in other European populations,[103 105] suggesting that Friedreich heterozygote status is not an important risk factor for type 2 diabetes.

Werner syndrome

Werner syndrome (OMIM 277700) is an autosomal recessive disorder characterised by the appearance of premature aging and the development of age associated disorders such as type 2 diabetes mellitus (penetrance up to 90%),[106] osteoporosis, atherosclerosis, and susceptibility to neoplasms; particularly sarcomas. In Werner syndrome, sarcomas are as prevalent as carcinomas, whereas in the normal population carcinomas are 10 times more common.[106] Patients with Werner syndrome become insulin resistant, but initially maintain glycaemia by hypersecretion of insulin. When insulin secretion becomes compromised diabetes develops.[107] In the third decade Werner patients begin to show signs of aging, with hair loss and skin atrophy.[106 108]

The gene for Werner syndrome (*WRN*) was discovered in 1996,[109] and its product has two functions: as a DNA helicase and as an exonuclease. WRN protein is located in the nucleus or nucleolus depending on cell type.[108] All mutations in Werner syndrome result in truncation of the protein and loss of the nuclear localisation signal on the C-terminal end of the protein[110]; thus transport of the protein into the nucleus seems to be critical for the protein function.

Study of proteins that interact with it has shed some light on the function of WRN. WRN interacts with replication protein A (RPA), topoisomerase 1, proliferating cell nuclear antigen, and DNA polymerase δ (pol δ),[108] to enhance polymerase activity.[111] WRN enables pol δ to continue DNA synthesis despite hairpin and tetraplex structures formed by trinucleotide repeats, which would normally stop synthesis.[112] WRN appears to aid DNA replication and repair by unwinding potential blocks in the process. This hypothesis is supported by the interaction of WRN with p53 and MYC. p53 is a tumour suppressor protein and can regulate WRN exonuclease activity.[113] Absence of WRN protein reduces the efficacy of p53 mediated cellular apoptosis, the key to its role in tumour suppression.[114] Mice with both p53 and WRN mutations show accelerated tumorigenesis compared with mice with p53 mutation alone,[115] suggesting a collaborative role for WRN with p53 in the prevention of tumour development. MYC is an oncoprotein which when overexpressed causes genomic instability and prevents cellular senescence. When MYC is added to WRN negative cells, the cells fail to proliferate like normal cells and express cellular markers of senescence.[116] It appears that failure to undergo p53 induced apoptosis leads to a high risk of tumours in the syndrome, while failure to repair the genome results in apoptosis in other cell lines, leading to diabetes and degenerative disease.

Thiamine responsive megaloblastic anaemia (Roger's syndrome)

Thiamine responsive megaloblastic anaemia (TRMA; OMIM 249270) is a rare genetic syndrome where early onset megaloblastic anaemia (which responds to thiamine) is associated with diabetes and sensorineural deafness.[117] The diabetes, which is insulin deficient in nature, is responsive to thiamine in some patients, although all seem to develop an insulin requirement in the long term.[118] Deafness is unresponsive to thiamine.[118] The condition has autosomal recessive inheritance, and three teams found mutations in a novel gene SLC19A2 in 1999 in a total of nine families.[119–121] SLC19A2 encodes a membrane bound thiamine transporter protein known as THTR-1,[121] and mutations causing TRMA have been shown to affect membrane targeting of THTR-1.[122 123] In TRMA cell lines, both cell and mitochondrion have a defect in the high affinity import of thiamine,[124] but the significance of this mitochondrial defect is unclear, as the mitochondrion in TRMA individuals can import thiamine diphosphate (the active form of thiamine) normally.[124] Thiamine is required for creation of thiamine pyrophosphate, a cofactor for several enzymes; one of these is transketolase, the rate limiting enzyme in the non-oxidative pentose shunt pathway for ribose synthesis, vital for nucleic acid production. Studies in fibroblast cell lines have shown that TRMA fibroblasts have normal ribose production in thiamine replete medium, but when exposed to thiamine deficient medium show a marked reduction in ribose synthesis concurrent with an increase in cellular apoptosis.[125] This suggests that mutation in the high affinity thiamine transporter seen in TRMA leads directly to an increased rate of apoptosis in cells that have a high rate of nucleic acid synthesis—that is, those with high turnover such as the marrow, or those with high translation rates such a secretory β cells.

CONCLUSIONS

The advances in molecular genetics and allied molecular sciences over the last 15 years have contributed greatly to our understanding of the normal workings of the β cell, and the underlying causes for disruption of glucose homeostasis. The enormous advances brought about by the human genome project and new techniques such as RNA interference promise a continued accretion of knowledge over the coming years. Diabetes in all its forms seems to stem from three main mechanisms: apoptosis/β cell loss, insulin secretion defects, and insulin resistance. While previous attempts to unify the theories of type 1 and type 2 diabetes have proved to be flawed, abnormal apoptosis appears to be an exciting final common pathway in the development of diabetes, which may also explain the neurodegenerative and cancer susceptibility phenotypes seen in some syndromes associated with diabetes. Perhaps the most illuminating idea is that, far from being a catastrophic event, apoptosis is as much part of homeostatic mechanisms as are classical hormonal negative feedback loops. The study of rare diabetes syndromes gives us the chance to examine disruptions in individual components of the glucose homeostatic balance. This should lead to an understanding of how the mechanism works in health, and thus how minor alterations in the pathway can lead to glucose intolerance and diabetes. The combination of clinician, clinical geneticist, molecular scientist, and pharmacologist forms a powerful team for understanding the basis of diabetes pathogenesis and designing future treatments for this disease.

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