Genes of Type 2 Diabetes in β−Cells

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Abstract/ Synopsis

Type 2 diabetes is complex polygenic metabolic disorder of epidemic proportions. This review provides a brief overview of the susceptibility genes in type 2 diabetes that primarily affect pancreatic β-cells, with emphasis on their function and most relevant polymorphisms. More in detail we focus on Calpain 10, the only susceptibility gene identified thus far through a positional cloning approach in diabetic subjects.

Introduction

Diabetes mellitus is a complex metabolic disorder with epidemic proportions. It currently affects 170 million people worldwide, and its prevalence is dramatically rising. Diabetes develops when the insulin production and secretion are not sufficient to satisfy the metabolic demands of the organism. The insulin hormone lowers the blood glucose levels, and any insufficiency in the insulin secretion and/or action leads to hyperglycemia and diabetes. According to the American Diabetes Association (ADA), diabetes can be classified as type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types of diabetes that cannot be included into any of the previous forms such as maturity-onset diabetes of the young (MODY) (1). In addition, an intermediate group of pre-diabetic subjects is recognized whose glucose levels, although not meeting the criteria for diabetes, are still too high to be considered normal, and is classified as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) (Table 1).

Type 2 diabetes is the most common form of diabetes accounting for approximately 90% of all patients. Its frequent association with other metabolic disorders has given rise to the so called “metabolic syndrome” (2) whose concept has developed in the last 80 years (3, 4), but has been recently questioned (5, 6). While the definitions of the metabolic syndrome by the WHO (7) and the National Cholesterol Education Program’s Adult Treatment Panel III (NCEP:ATP III) (8) are different, there is wide agreement that its essential components include glucose intolerance, obesity, hypertension and dyslipidaemia. Many factors contribute to the development of type 2 diabetes. Often environmental and behavioral factors as well as obesity contribute to its pathogenesis (9). Genetic factors, however, have also been shown to play a key role in the development of

diabetes. Sixty percent of the offspring from diabetic patients is estimated to have abnormal glucose tolerance by the age of 60 years (10). In MODYs the mutation of a single gene that is inherited as an autosomal-dominant trait causes diabetes. In recent years most MODY genes have been identified. The genetic background of type 2 diabetes, which is the most common form of the disease, is instead only partially known. From a genetic point of view, type 2 diabetes is a complex polygenic disorder associated with polymorphisms of multiple genes whose frequency varies among different ethnic groups (11). Many of these genes are involved in glucose sensing or insulin secretion and action, while others are associated with increased susceptibility for metabolic conditions such as obesity and lipid disorders, which in turn, promote diabetes development. This review provides first a brief overview of the genes that confer susceptibility to type 2 diabetes primarily by affecting β-cells, with emphasis on their function. For those genes which do not appear to directly impair β-cell function, such as peroxisome proliferating receptor-gamma (PPAR-γ), beta-3-adrenergic receptor (ADRB3) or adiponectin (ADIPOQ), the reader is referred to specific reviews on the topic (12, 13). The second part of this review summarizes recent advances in our understanding of insulin gene expression, which could be related to the association between polymorphisms in the calpain 10 gene and the increased susceptibility to type 2 diabetes (14).

Type 2 diabetes susceptibility genes of β-cells

Glucose transporter 2 (GLUT2). This transporter is mostly responsible for the entry of glucose in β-cells. It is constitutively expressed at their surface, as well as in liver and intestinal cells. Since its capacity to transport glucose is very high, its opening enables the equilibrium between extracellular and intracellular glucose to be reached very rapidly. GLUT2 is encoded by the SLC2A2 gene that is located on human chromosome 3 and contains 11 exons. Its product is a peptide with 12 membrane spanning domains containing several sites for glucose binding. A single nucleotide polymorphism (SNP) that replaces threonine 110 with isoleucine within the second membrane spanning domain is modestly increased in type 2 diabetic patients. Mutation of valine 197 to isoleucine in the fifth membrane spanning domain impairs glucose transport and has been found in a diabetic patient (15-17).

Glucokinase. Once it is inside the cytosol of β-cells, glucose is phosphorylated to glucose-6-phosphate by an atypical member of the hexokinase family called glucokinase (GCK, or hexokinase IV). The gene GCK is located on human chromosome 7 and contains 12 exons and acts as the "glucose sensor" of β-cells. Activation of GCK only occurs when glucose concentrations are above 5 mM (18). Contrary to the other members of the hexokinase family, GCK is not inhibited by its product, thus enabling GCK to remain active while glucose is abundant. This enzyme regulates therefore the metabolic flux of glucose into β-cells and is the rate-limiting step for glycolysis. This key metabolic pathway produces ATP from the anaerobic and aerobic oxidation of glucose metabolites, and eventually induces the secretion of insulin. The importance of GCK in glucose

homeostasis and diabetes is highlighted by the identification of many GCK mutations that alter the threshold for glucose-stimulated insulin release. On one hand, activating mutations can lower the threshold for insulin release to 1.5 mM, thereby causing persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (19). Inactivating mutations on both GCK alleles, on the other hand, increase the levels of glucose required to stimulate insulin secretion, hence leading to permanent neonatal diabetes (diabetes at birth) (20). Inheriting inactivating mutations on a single GCK allele are responsible instead for mild hyperglycemia and MODY2 (21).

Insulin.

Insulin was the first hormone to be identified (22). Most animals have a single copy of the insulin gene, which in human is located on the short arm of chromosome 11. Rodents have two non-allelic insulin genes (I and II) that encode identical polypeptide chains, but differ in the number of introns and chromosomal location (23). The initial translation product of the insulin mRNA is preproinsulin, which is converted into proinsulin following the co-translational removal of its signal peptide. After its exit from the Golgi complex and sorting into secretory granules (SGs), proinsulin is cleaved by two Ca^{2+} dependent converting proteases termed protein convertase 1/3 (PC1/3) (24-27), and 2 (PC2) (28). These cleavages separate proinsulin into three polypeptides named chain A (21 amino acids), chain B (30 amino acids) and the intervening C-peptide, respectively. Chains A and B remain associated by two disulfide bridges and together form insulin. Several SNPs and mutations have been identified within the insulin gene, which are associated with an increased risk for type 2 diabetes. The phenylalanine at position 24 is highly conserved and its aromatic ring is thought to be important for anchoring insulin to

the insulin receptor (IR, see below). Mutations affecting this residue are associated with diabetes (29). Another point mutation that converts arginine 65 into histidine prevents proinsulin cleavage, thereby causing increased proinsulin secretion and diabetes (30, 31).

ATP-sensitive K^+ channels (K_{ATP} channels). ATP production from glycolysis increases the ATP/ADP ratio within β-cells, hence favoring the binding of ATP to ATP-sensitive K^+ channels (K_{ATP} channels) (32) and their closure. This causes the depolarization of β-cell membranes (33), the opening of voltage-gated Ca^{2+} channels, and the entry of Ca^{2+}. Ca^{2+}, in turn, acts as a second messenger that triggers the exocytosis of insulin SGs. Insulin is secreted in two temporally distinct phases in response to glucose stimulation. The first phase of insulin secretion has its peak ~2-4 minutes after glycemia has reached the threshold value of ~5.5 mM and generally lasts up to 10 minutes. The second phase begins ~20 minutes after glucose stimulation, it reaches its plateau within 15 minutes and usually does not last longer than 60 minutes. Among several pathways inducing glucose-stimulated insulin secretion, the K_{ATP} channel-dependent or triggering pathway is the one responsible for the first phase of insulin secretion, which results from the exocytosis of insulin SGs that are already docked to the plasma membrane.

Most K_{ATP} channels consist of two different subunits - Kir6.2 and SUR1. Kir6.2 and SUR1 genes are adjacent to each other in the short arm of human chromosome 11. K_{ATP} channels are thought to be a complex including four SUR1 and four Kir6.2 proteins. SUR1 is a member of the ATP-binding cassette transporter family of proteins, which use the energy of ATP to transport molecules across membranes, and typically contain two ATP binding domains (also know as nucleotide binding folds (NBF 1 and 2) and two

transmembrane domains. Mutations in Kir6.2 or SUR1 can alter the nucleotide sensitivity and closure probability of K_{ATP} channels. There are mutations reported for NBF1 and NBF2 in SUR1 that favor the closure of the K_{ATP} channel, and are associated with increased insulin release and PHHI (34-36). Especially frequent in Kir6.2 is the replacement of glutamic acid 23 to lysine (E23K), which decreases insulin release (37-39). A SUR1 variant, which is often linked to Kir6.2 E23K, has an alanine instead of a serine at position 1369 (S1369A) and is also associated with defects in insulin release and diabetes.

Insulin receptor (IR). The gene encoding the IR is located on human chromosome 19. It contains 23 exons, and it is primarily expressed in insulin target cells, including hepatocytes, muscle cells, adipocytes and β-cells. The receptor is a heterotetrameric tyrosine kinase consisting of two insulin-binding extracellular α subunits and two predominantly intracellular β subunits (40), linked by disulfide bridges. Insulin binding elicits the kinase activity of the IR, which autophosphorylates first its β subunits and then insulin receptor substrates 1 (IRS1) and 2 (IRS2), which bind the phosphorylated receptor. Phosphorylated IRS1 and IRS2, in turn, act as adaptor proteins for the recruitment of factors associated with two distinct signaling pathways. One pathway is Ras-dependent and leads to the activation of MAP kinases, which are primarily responsible for the mitogenic effect of insulin. The other pathway is Ras-independent and prompts the activation of the serine/threonine kinase AKT/PKB following the generation of phosphatidylinositol 3-phosphates by phosphatidylinositol-3 kinase (PI3K). Among other functions, AKT/PKB triggers the translocation of the glucose transporter GLUT4 to the plasma membrane, thereby allowing glucose uptake by hepatocytes, muscle cells and

adipocytes. In β-cells the IR signaling is mostly relevant to sustain beta-cell mass and insulin secretion (41, 42).

Mutations of the insulin receptor are responsible for leprechaunism, severe insulin resistance (43) with diabetes, acanthosis nigricans (44) and polycystic ovary syndrome (45)**.** Knockout of mouse IR in beta cells was sufficient to reduce the first phase of insulin secretion and led to glucose intolerance (41). Polymorphisms in the IR gene, do not appear to be a common trait among the large majority of patients with type 2 diabetes.

IRS1. The gene encoding IRS1 is located on human chromosome 2. There are several missense variants of the IRS1 gene characterized by amino acid replacement, namely G81R, P512A, S892G and G972R, which have been suggested to be more common among type 2 diabetes patients then in control subjects (46, 47). Glycine 972, in particular, is located in close proximity to the two tyrosine phosphorylation sites that are involved in the binding of PI3K. Its replacement into arginine could therefore impair insulin by affecting the PI3K/AKT pathway (47).

Phosphoinositide-3 kinase (PI-3K). PI-3K has a catalytic and regulatory subunit. The gene encoding the regulatory PI-3K subunit is located on human chromosome 5. This subunit contains two SH2 domains that bind tyrosine phosphorylated residues in motifs that are present in IR (two) and in IRS1 (four). The full activation of the PI-3K requires both SH2 domains to bind the tyrosine-phosphorylated motifs present in the IRS1. The

PI-3K missense variant M326I and the intronic variant SNP42 are associated with increased diabetes risk and have been proposed to affect glucose homeostasis (48-50).

Hepatocyte nuclear factor 1-α (HNF-1α). HNF-1α is a transcription factor that plays a key role in the development and function of the pancreatic β-cells by regulating the expression of many genes, including insulin and HNF4α (51). In addition to β-cells, it is highly expressed in liver and kidney. The HNF1α gene is located on human chromosome 12. The HNF1α protein is active as a homodimer, which is stabilized by the co-factor DCOH (52). A subset of mutations which destabilizes the formation of HNF1α dimers accounts for the deficient insulin production and secretion in MODY3 (53, 54). Due to its role in β-cell development there is growing evidence that HNF1α missense variations may also play a role in the development of type 2 diabetes. Common HNF1α missense variants A98V, S319G and P447L are associated with lower insulin secretion (55), whereas the I27L variant is linked to reduced insulin sensitivity. Recent meta-analysis studies, however, have suggested that common HNF1α variants, with the exception of the rare V98 allele, play only a minor role in type 2 diabetes unless associated with other genetic or environmental predisposing factors (56, 57).

Hepatocyte nuclear factor 4-α (HNF-4α). The gene encoding HNF4α is located on human chromosome 20. It regulates gene expression in liver, pancreatic β-cells, kidney and intestine during embryonic development, but also in adult life. Specifically, it controls the expression of insulin, GLUT2, mitochondrial uncoupling protein-2 and other genes linked to insulin secretion (58). Mutations of this gene cause insulinopenia, thereby indicating that reduced levels of HNF4α lead to β-cell dysfunction (59-61).

In particular, coding genetic variants of HNF4α have been identified in MODY1 patients. Other genetic variants associated with both MODY1 and type 2 diabetes are found in the HNF4α alternative promoter region called P2, where HNF1α binds (62-64, 51).

NEUROD1. NeuroD1 is a basic helix-loop-helix (bHLH) transcription factor that is important for cell fate determination during neurogenesis (65). The NEUROD1 gene is mapped on human chromosome 2 and it is highly expressed in brain as well as in the precursor of several islet cells, including β-cells. Specifically, it regulates insulin gene expression by binding to a critical E-box motif in the insulin promoter (66). Accordingly, NeuroD1 knockout mice display an abnormal pancreatic islet morphogenesis and diabetes (66). Insertion of a cytosine at position 206 in NEUROD1 generates a truncated polypeptide lacking the C-terminal trans-activation domain and is responsible for impaired insulin secretion and MODY6. Replacement of arginine 111 with leucine (R111L) within the bHLH domain, which is responsible for DNA binding, is associated instead with type 2 diabetes.

Calpain-10 (CAPN10)

Unlike the above mentioned type 2 diabetes susceptibility genes, CAPN10 was not identified using a candidate gene approach, but through a unique positional cloning strategy following a genome-wide scanning in diabetic sibling pairs (67, 14). In particular, linkage studies in the Mexican-American population localized a major susceptibility locus for type 2 diabetes on a region of chromosome 2q, which contains the CAPN10 gene. Subsequent analyses revealed that an intronic G/A polymorphism in CAPN10 termed SNP-43 was linked to type 2 diabetes. However, the association of

SNP-43 with the disease was weak (14), except in Pima Indians (68) and African-Americans (69). Inclusion of two additional intronic SNPs, defined as SNP-19 and SNP-63, allowed the further identification of two major CAPN10 haplotypes, which have been termed 112 and 121 and whose combination is associated instead with type 2 diabetes among multiple ethnic groups (14, 70-77). The 112/121 haplotype has a variable population frequency and correlates with reduced levels of CAPN10 mRNA in muscle cells and insulin resistance (14, 68, 71).

Calpains are cytosolic cysteine proteases that catalyze the endoproteolytic cleavage of a wide array of substrates, including cytoskeletal proteins, kinases, phosphatases and transcription factors (78, 79). The human genome includes 14 calpain genes. Most information about calpains has been gathered through studies on calpain-1 and calpain-2, which are ubiquitously expressed and are activated in vitro by low and high levels of Ca^{2+}, respectively. Calpains preferentially recognize bonds between protein domains, thereby leading to the generation of large peptide fragments that retain functionally intact domains. Calpains are regarded therefore as bio-modulators, since the properties of substrate proteins are often modified after calpain hydrolysis (79-82). In addition to type 2 diabetes, deficits in calpain activity have been associated with the development of autosomal recessive limb-girdle muscular dystrophy type 2A (LGMD2A), cataracts, and age-related hypertension (83).

How is CAPN10 contributing to the development of type 2 diabetes? Until today there is no clear answer to this question, especially because the specific function of CAPN10 remains to be elucidated. Inhibition of CAPN10 activity was originally associated with

~60% decrease in insulin-stimulated glucose uptake into cultured mouse adipocytes because of impaired translocation of glucose transporter 4 (GLUT4)-containing vesicles to the plasma membrane (84). Recent studies, however, have shown that deficits of GLUT4 vesicle translocation resulted from the inhibition of proteasome cysteine proteases, rather than calpains (85). In β-cells CAPN10 activation is required for promoting apopotosis in response to ryanodine, fatty acids, and low glucose (86). Increasing evidence indicates that calpains can also regulate β-cell secretion. Exposure of mouse islets to calpain inhibitors lead to increased glucose-dependent insulin release (87), possibly by acting on proteins involved in the exocytosis of the insulin-containing secretory granules. Conversely, calpain inhibition decreased insulin secretion from rat insulinoma cells (88). This diminution may result from the reduced proteolysis of SNAP-25 (89), a protein implicated in the fusion of secretory granules with the plasma membrane. These inhibitors, however, can affect other calpains in addition to CAPN10. Recent studies, in particular, have pointed to the role of calpain-1 (Figure 1) in the up-regulation of insulin production through the cleavage of the receptor tyrosine phosphatase-like protein ICA512/IA-2 (90). ICA512 is enriched in the membrane of insulin secretory granules (91) and is involved in the regulation of insulin secretion (92). Upon secretory granules exocytosis, ICA512 is transiently inserted into the plasma membrane where its cytoplasmic domain is proteolyzed by calpain-1. The ICA512 cytosolic fragment resulting from this cleavage is then targeted to the nucleus, where it up-regulates the expression of insulin (90) and other secretory granule components (our unpublished observations)(Figure 2). Through this novel pathway β-cells could directly adjust the production of secretory granules to their consumption. Hence, it would be interesting to determine whether ICA512 is also cleaved by CAPN10, and if so, whether

this process is linked to the increased susceptibility to type 2 diabetes in carriers of the 112/121 haplotype.

Conclusions

Diabetes and its complications represent one of the major threats to public health worldwide. Increasing evidence in human and animal models indicate that deficient β-cell function is an essential factor for the development of the disease. Genetic studies have led to the identification of numerous susceptibility genes for type 2 diabetes, most of which regulate β-cell glucose metabolism, gene expression and/or insulin secretion. Hence, the need for elucidating further the physiology of β-cells in normal and pathological conditions, and hopefully alleviate the burden of diabetes, could not be more compelling.

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Figure legends

Figure 1. Schematic representation of CAPN 1, CAPN4 and CAPN10 domain structure.

Figure 2. Schematic representation of the ICA512 signaling pathway.

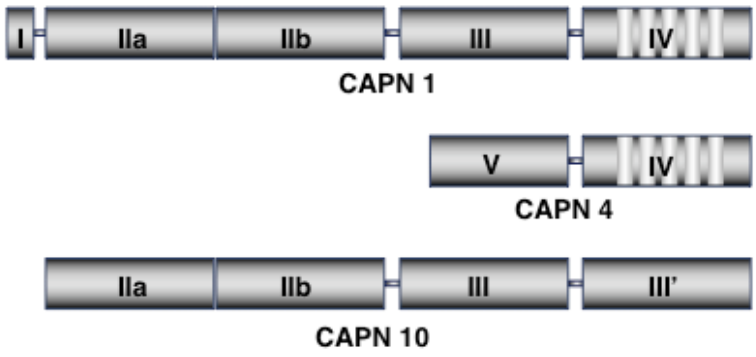

Fig. 1

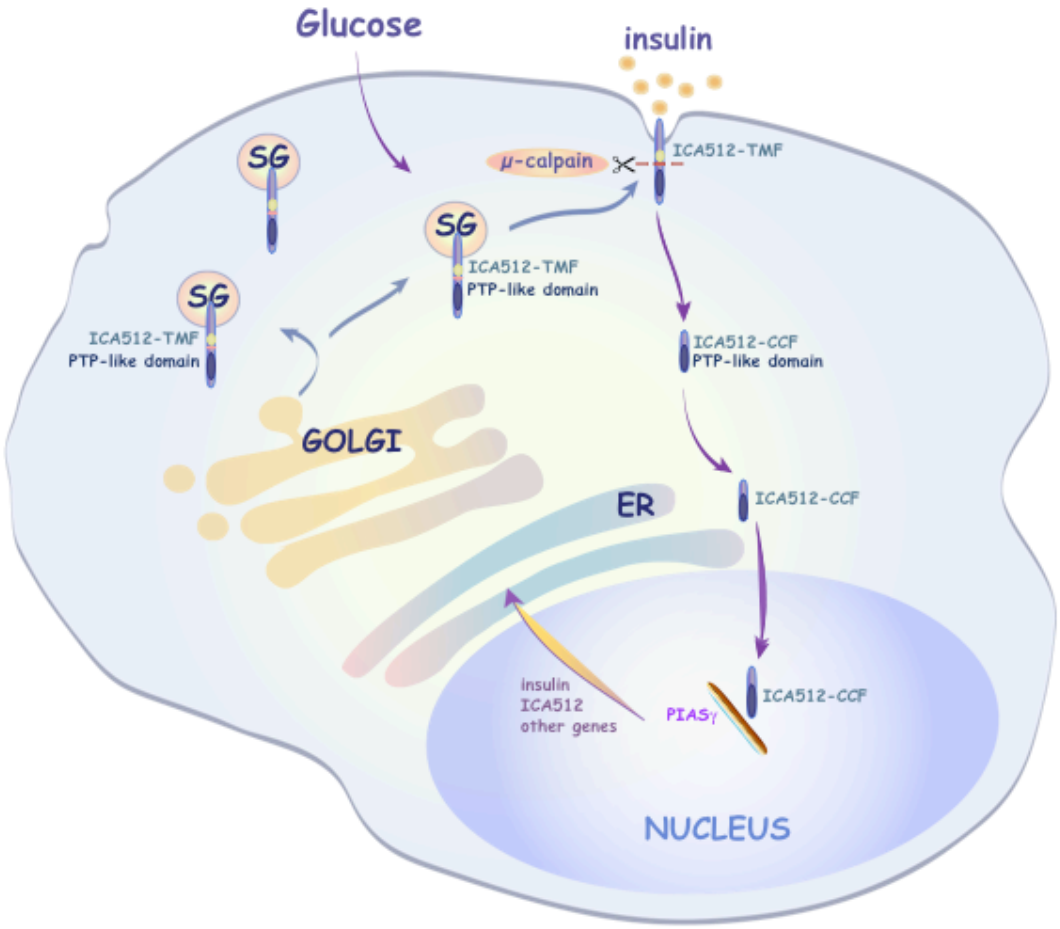
Glucose
insulin
SG
μ-calpain
ICA512-TMF
SG
ICA512-TMF
PTP-like domain
SG
ICA512-TMF
PTP-like domain
ICA512-CCF
PTP-like domain
GOLGI
ER
ICA512-CCF
insulin
ICA512
other genes
PIASγ
ICA512-CCF
NUCLEUS

Fig. 2

Table 1 - Criteria for diagnosis of diabetes

Normoglycemia	[1]Impaired fasting glucose (IFG) [2]Impaired glucose tolerance (IGT)	Diabetes melitus
FPG* < 100mg/dl	[1]FPG ≥ 100mg/dl and <126mg/dl	FPG ≥ 126mg/dl
2h PG** < 140mg/dl	[2]2h PG ≥140mg/dl and <200mg/dl	2h PG ≥ 200mg/dl

*FPG - Fasting plasma glucose
**PG - Post 75g glucose load