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Commentary

Implications of genome wide association studies for the understanding of type 2 diabetes pathophysiology

John R. Petrie [a,*], Ewan R. Pearson [b], Calum Sutherland [b]

[a] BHF Glasgow Cardiovascular Research Centre, University of Glasgow, 126 University Place, Glasgow G11 6TA, United Kingdom
[b] Biomedical Research Institute, Ninewells Hospital, University of Dundee, Dundee DD1 9SY, United Kingdom

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ABSTRACT

The rapid rise in prevalence of type 2 diabetes mellitus (T2DM) has been driven by changes in environmental factors – primarily increased caloric intake and reduced energy expenditure – resulting in reduced whole body insulin sensitivity (often termed insulin resistance). Insulin resistance has been proposed to be a major driver of progression to T2DM. However, of 38 individual susceptibility loci for T2DM recently identified by genome wide association studies, by far the majority code for proteins involved in β-cell function. In this review, we discuss the possible reasons for the paucity of insulin resistance genes and ask whether the new genetic susceptibility data should focus attention on β-cell targets in the development of therapies for T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a condition characterised by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from the pancreatic β-cell. The development of the disease has genetic, environmental and intra-uterine pathophysiological determinants [1]. The rapid rise in prevalence has been driven over recent decades by changes in environment, including the lifestyle of populations (urban migration, calorie excess, physical inactivity), reflected in closely associated increases in the prevalences of obesity and T2DM [2].

At the time of writing, 38 individual susceptibility loci for T2DM have been identified by genome wide association studies [3,4,5]. The majority code for proteins involved in β-cell function, including glucose-sensing, proinsulin processing, and insulin secretion. It has been suggested on this basis that insulin resistance plays a less important role than previously thought in T2DM pathophysiology, and a less critical one than impaired insulin secretion.

In this mini-review, we discuss this question in the context of recent advances in the understanding of the physiology of glucose metabolism in order to determine whether the classical understanding of T2DM pathophysiology should be revised and more focus placed on the β-cell in the development of therapies for T2DM. In particular, we consider the extent to which the difficulty in identifying insulin resistance genes to date reflects limitations of study design, inadequate physiological assessment of insulin resistance or the complex underlying pathophysiology of insulin resistance (i.e. multiple parallel compensatory pathways).

2. Genetic discoveries in T2DM

If insulin resistance and impaired insulin secretion play equal roles in T2DM pathophysiology, it might be predicted that the genetic loci determining the inherited component of T2DM should occur at (or adjacent to) SNPs coding proteins involved in insulin secretion and insulin action in approximately equal measure.

Nearly all of the recent discoveries have used genome wide association study (GWAS) techniques to identify single nucleotide polymorphisms (SNPs) that exist at higher frequency in DNA from people with established T2DM (“cases”) than in non-diabetic individuals (“controls”). Where the physiological roles of these variants have so far been determined, the majority encode proteins linked with the β-cell. For example, of 19 validated T2DM genes, 14 have been shown to influence glucose or incretin stimulated insulin secretion (reviewed in [6]). In addition, these variants have relatively large effects on diabetes risk compared with other variants, with the seven variants with the greatest association with diabetes risk (*TCF7L2, CDKAL1, HHEX, CDKNA/2B, IGF2BP2, SLC30A8, JAZF1*) all affecting β-cell insulin secretion.

Abbreviations: GWAS, genome wide association study; HOMA, homeostasis modelling assessment; IGI, insulinogenic index; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes.

* Corresponding author. Tel.: +44 (0) 141 330 3325; fax: +44 (0) 141 330 6972.
E-mail address: john.petrie@glasgow.ac.uk (J.R. Petrie).

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Table 1
38 validated type 2 diabetes associated variants and impact on glucose-insulin homeostasis.

Chromosome	SNP	*Nearest gene*	Description	Reduced Beta cell function	Reduced insulin action
1	rs10923931	*NOTCH2*	Notch Homolog 2		
1	rs340874	*PROX1*	Prospero homeobox 1		
2	rs780094	*GCKR*	Glucokinase (hexokinase 4) regulator		Yes
2	rs11899863	*THADA*	Thyroid adenoma associated	Yes	
2	rs243021	*BCL11A*	B-cell CLL/lymphoma 11A (zinc finger protein)		
2	rs7578426	*IRS1*	Insulin receptor substrate 1		Yes
3	rs13081389	*PPARG*	Peroxisome proliferator-activated receptor gamma		Yes
3	rs6795735	*ADAMTS9*	ADAM metallopeptidase with thrombospondin type 1 motif, 9	Yes	
3	rs11708067	*ADCY5*	Adenylate cyclase 5	Yes	
3	rs1470579	*IGF2BP2*	Insulin-like growth factor 2 mRNA binding protein 2	Yes	
4	rs1801214	*WFS1*	Wolfram syndrome 1 (wolframin)	Yes	
5	rs4457053	*ZBED3*	Zinc finger, BED-type containing 3		
6	rs10440833	*CDKAL1*	CDK5 regulatory subunit associated protein 1-like 1	Yes	
7	rs2191349	*DGKB-TMEM195*	Diacylglycerol kinase, beta	Yes	
7	rs849134	*JAZF1*	JAZF zinc finger 1	Yes	
7	rs4607517	*GCK*	Glucokinase	Yes	
7	rs972283	*KLF14*	Kruppel-like factor 14		Yes
8	rs896854	*TP53INP1*	Tumor protein p53 inducible nuclear protein 1		
8	rs3802177	*SLC30A8*	Solute carrier family 30 (zinc transporter), member 8	Yes	
9	rs10965250	*CDKN2A/B*	Cyclin-dependent kinase inhibitor 2A/B	Yes	
9	rs13292136	*CHCHD9*	Coiled-coil-helix-coiled-coil-helix domain containing 9		
10	rs12779790	*CAMK1D*	Calcium/calmodulin-dependent protein kinase ID	Yes	
10	rs5015480	*HHEX/IDE*	Hematopoietically expressed homeobox/insulin-degrading enzyme	Yes	
10	rs7903146	*TCF7L2*	Transcription factor 7-like 2	Yes	
11	rs2334499	*Imprinted region*			
11	rs231362/rs163184	*KCNQ1*	Potassium voltage-gated channel, KQT-like subfamily, member 1	Yes	
11	rs5215	*KCNJ11*	Potassium inwardly-rectifying channel, subfamily J, member 11	Yes	
11	rs1552224	*CENTD2*	Centaurin, delta 2	Yes	
11	rs1387153	*MTNR1B*	Melatonin receptor 1B	Yes	
12	rs1531343	*HMGA2*	High mobility group AT-hook 2		*Possible*
12	rs4760790	*TSPAN8*	Tetraspanin 8	Yes	
12	rs7957197	*HNF1A*	Hepatic nuclear factor 1 alpha		
15	rs11634397	*ZFAND6*	Zinc finger, AN1-type domain 6		
15	rs8042680	*PRC1*	Protein regulator of cytokinesis 1		
16	rs11642841	*FTO*	Fat mass and obesity associated		Yes
17	rs4430796	*HNF1B*	Hepatic nuclear factor 1 beta	Yes	
19	rs10423928	*GIPR*	Gastric inhibitory polypeptide receptor	Yes	
X	rs5945326	*DUSP9*	Dual specificity phosphatase 9		*Possible*

SNP, single nucleotide polymorphism.

In contrast, the only T2DM variants found in this initial analysis to be functionally associated with insulin resistance are in *PPARG* [7], *IRS-1* [8], and *FTO* [9], (the latter by virtue of its effect on obesity). However, a recent meta-analysis from the DIAGRAM+ consortium added 12 additional variants associated with T2DM, making a total of 38 established risk variants [3]. Of these 12, three are likely to be associated with impaired insulin action – KLF14 and possibly HMGA2 and ADAMTS9 [3]. Additionally DUSP9, encoding mitogen-activated protein kinase phosphatase-4, which regulates insulin action in mice [10], was also associated with diabetes risk, although not with fasting insulin or the homeostasis modelling assessment (HOMA) measure of insulin resistance (HOMA-IR) (see Table 1).

3. Why the paucity of genes involved in insulin resistance relative to β-cell function?

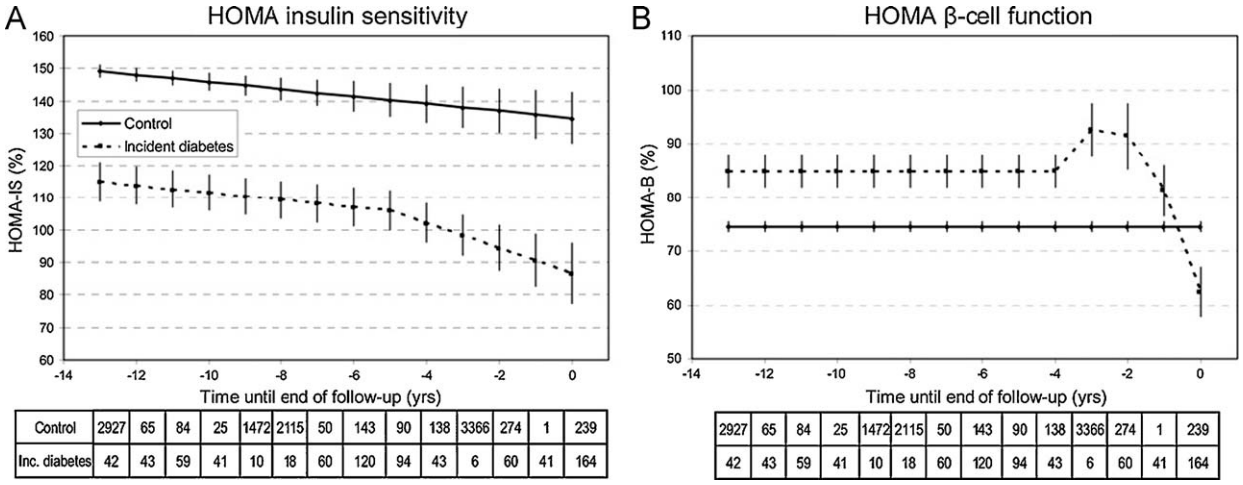
3.1. Study design

There are many potential reasons why genes of insulin action are less represented in these studies. The first is simply that they may have a smaller effect size, with a resultant requirement for even larger cohorts to discover them. Thus, the total sample size in DIAGRAM+ was more than 43,000 and 25% of the additional genes identified in this study were related to insulin action i.e. the percentage of genes involved in insulin action is greatest in the largest study. Even so, there are still notably more T2DM genes linked to insulin secretion than insulin resistance.

In addition there may be two intrinsic difficulties with the case control study approach. Firstly, no attempt is made to select the control population other than for absence of diabetes; as discussed later, control subjects are likely to be drawn from a population across a spectrum of insulin resistance, and the inclusion of insulin resistant controls will decrease the power of any study aiming to discover insulin resistant genes in the cases. Secondly, a diagnosis of diabetes requires a degree of β-cell decompensation, so it is perhaps not surprising that this approach will identify genes associated with β-cell function more readily than insulin resistance.

Therefore, a potentially better approach is to study the genetics of insulin resistance per se rather than the development of T2DM. This approach was used in the Meta-analysis of Glucose and Insulin Consortium (MAGIC) study, which used glucose, insulin and HOMA derived indices of insulin resistance and secretion as continuous traits [5]. It involved a GWAS of 46,186 non-diabetic participants and a follow up of 25 loci in 76,558 additional subjects. Sixteen loci were found to be associated with fasting glucose and a measure of insulin secretion (HOMA-B), yet only two loci (*GCKR* and *IGF1*) were associated with fasting insulin and a measure of insulin resistance (HOMA-IR).

In contrast to common variants that increase risk by 10% or 20% in a population (as identified by GWAS), single gene mutations



Control	2927	65	84	25	1472	2115	50	143	90	138	3366	274	1	239
Inc. diabetes	42	43	59	41	10	18	60	120	94	43	6	60	41	164

Fig. 1. Homeostasis model assessment insulin sensitivity (HOMA2-%S) and HOMA β-cell function (HOMA2-%B) trajectories (panels A and B) before the diagnosis of diabetes mellitus or the end of follow-up in 505 incident diabetes cases compared to 6033 non-diabetic controls. Insulin sensitivity declines rapidly in those who subsequently develop diabetes, with an initial compensatory rise in β-cell insulin secretion, before a failure in β-cell compensation prior to developing diabetes. Reproduced with permission from reference [16].

inherited in a Mendelian manner are associated with a considerable effect size. In rare cases of extreme insulin resistance, families have been described with severe defects in insulin signalling. In these families, mutations have been described in the insulin receptor e.g. Type A insulin resistance, Donohue syndrome (MIM ID #246200); and in key insulin signalling proteins *AKT2* [11] and *AS160* [12]. Lipodystrophies have also been described due to mutations in *PPARG* and *LMNA/C*, and more recently due to defects in the lipid droplet gene *CIDEC* [13]. It should be noted that these severe insulin resistance syndromes are only rarely associated with diabetes, because of pancreatic β-cell compensation. However, these cases provide evidence for the existence of genetic mutations that can severely alter insulin sensitivity. It remains possible therefore that the paucity of insulin resistance genes found by GWAS may be at least in part explained by the relative difficulty of accurately measuring small variations in insulin sensitivity compared to measuring small changes in insulin secretion in large populations.

3.2. Accurate assessment of insulin secretion and sensitivity in large populations

3.2.1. T2DM pathophysiology

Within any population there exist marked variations in sensitivity to insulin-mediated glucose disposal: for example in middle-aged mostly non-obese adults participating in the Relationship between Insulin Sensitivity and Cardiovascular (RISC) study, clamp-measured insulin sensitivity varied sixfold [14].

Glucose homeostasis depends in large part on production of appropriate quantities of insulin by pancreatic β-cells correctly timed around nutrient ingestion. In the evolution of an individual case of T2DM, it is generally considered that sensitivity to insulin-mediated glucose disposal and insulin suppression of hepatic glucose production diminishes over time (e.g. as a result of increasing adiposity), with an initial compensatory increase in insulin secretion from β-cells to achieve glucose homeostasis. At this stage, which may be asymptomatic and prolonged, absolute insulin concentrations measured in plasma may be higher than the reference range. For an individual developing T2DM, a plot against time of total insulin secretion across a standard oral glucose tolerance test (OGTT) is therefore an inverted "U"-shape as β-cells (teleologically) fail to maintain compensation [15]. As compensation becomes less effective ("β-cell exhaustion"), even in the absence of a further deterioration of insulin sensitivity, either impaired glucose tolerance or impaired fasting glucose will develop before finally, the threshold is crossed for a diagnosis of T2DM (as defined by current WHO/ ADA glucose criteria). This trajectory of increase in insulin resistance, β-cell compensation and subsequent failure is nicely demonstrated in the Whitehall II study, a prospective follow up of London civil servants (Fig. 1) [16]. In this model, insulin resistance plays an early (pre-diabetic) and important part in the development of T2DM, possibly even inducing β-cell failure due to the strain of prolonged compensation.

It should be noted that there are only a few studies, for example in specific high risk populations such as Pima Indians, in which detailed, serial long-term metabolic assessments have been conducted on individuals progressing from normal glucose tolerance through a pre-diabetic state towards frank T2DM [17]. Longitudinal understanding of changes in insulin resistance and β-cell function in the development of T2DM have largely been inferred from elegant cross-sectional studies involving intensive physiological characterisation (e.g. euglycaemic and hyperglycaemic clamp) [18], and from larger cohort studies based on less precise empirically derived surrogate measures (e.g. HOMA-IR and HOMA-B) [19].

It is therefore not surprising that the respective roles of β-cell dysfunction and insulin resistance in T2DM pathophysiology remain imprecisely defined. Studies including intensive phenotyping tend to be cross-sectional with sample small numbers of cases from a particular population or ethnic group; while studies based on imprecise read-outs are carried out on a much larger scale. A common view is therefore that there are a range of possible intermediate routes (and velocities of progression) between normal glucose tolerance and T2DM, ranging between two extremes i.e. between a profound state of insulin resistance with compensatory insulin hypersecretion and a state of intact insulin sensitivity with profound β-cell dysfunction. A recent study reported that healthy individuals with a positive family history of type 2 diabetes (and therefore a high lifetime risk of T2DM) had *lower* insulin concentrations during a glucose tolerance test than negative family history controls closely matched for clamp-derived insulin sensitivity [14]. This clearly supports the notion that individuals exist with a primary β-cell defect who are at a high risk of developing T2DM.

Whether the evolution of frank T2DM depends on "primary" β-cell failure or "secondary" decompensation, it is likely that there

are individual differences in susceptibility to these processes that may at least in part be genetically determined.

3.2.2. Measurement of insulin resistance

Insulin has complex actions on numerous post-receptor pathways regulating protein, fat and carbohydrate metabolism in multiple tissues (including muscle, liver, adipose tissue, and endothelium); in many of these there is considerable redundancy and hence scope for compensatory upregulation of parallel pathways (Fig. 2). However, insulin sensitivity is usually measured globally in terms of whole body insulin-mediated glucose uptake. The most direct methods involve an intravenous insulin infusion: for example, the euglycaemic clamp technique (regarded as the gold standard), which is simple and intuitive although time-consuming, invasive and labour-intensive. Only two "clamp" studies of reasonable size (RISC & ULSAM) have been performed to date [20,21], although other techniques involving insulin infusion without glucose clamping – the steady state plasma glucose method (SSPG) and the minimal model method – are also informative [22,23]. In the Insulin Resistance Atherosclerosis Study (IRAS) which used minimal model derived measures of insulin sensitivity, a recent genome wide association study in 279 individuals without diabetes, and subsequent replication in a study of 814 individuals, identified some variants associated with insulin resistance in both the discovery and replication cohorts. However, these did not achieve genome wide significance (best p-value 1×10^{-4}), highlighting a lack of power in a relatively small (albeit intensively phenotyped) cohort. [24].

As none of these direct techniques are suitable for use on a large enough scale to provide the power necessary, GWAS have been forced to rely mainly on surrogate indices of insulin resistance based on fasting or OGTT measurements of glucose and insulin. In the MAGIC physiology meta-analysis, insulin sensitivity data were extracted from the participating studies in the units in which they were originally reported (e.g. Matsuda and Stumvoll indices) [4]; where data were available from more direct techniques (RISC, ULSAM), z-scores were calculated to permit data pooling. Correlation coefficients for the relationship between insulin sensitivity measured using surrogate markers and clamp-derived insulin sensitivity ranged from only 0.4 for RISC to 0.6 for ULSAM.

Thus, it is possible that insulin resistance genes remain undiscovered because of: (i) lack of study populations of sufficient size well characterised for insulin resistance; (ii) imprecision of surrogate measures of insulin resistance in available populations; and (iii) loss of statistical power in "data-pooling" exercises. In order to identify more "insulin resistance" genes, it would be necessary to compare larger numbers of (non-diabetic) well-characterised insulin resistant "cases" and insulin sensitive "controls". These issues provide a major challenge for any clinical trial team, and are in stark contrast to those involved in measuring β-cell function.

3.2.3. Measurement of β-cell dysfunction

β-cells within the pancreatic islets of Langerhans contain apparatus for a series of metabolic processes critical to appropriate insulin secretion/glucose homeostasis: (i) transporting glucose into the cell (via the glucose transporter GLUT-2); (ii) sensing ambient glucose concentrations (via the enzyme glucokinase and the generation of ATP); (iii) synthesising insulin and C-peptide from pre-proinsulin (via prohormone convertase 1, prohormone convertase 2 and carboxypeptidase H); (iv) packaging insulin in secretory vesicles; and (v) releasing stored insulin in response to membrane depolarisation (following closure of ATP-sensitive potassium channels). In contrast to the events critical to post-receptor insulin action, the steps involved in insulin secretion are primarily limited to a single organ (the beta cells of the pancreas) and although complex, occur largely in a canonical sequence resulting in a single outcome (Fig. 2) – hence perturbation at any stage in this sequence will almost inevitably result in decreased release of the hormone into the portal circulation. In contrast, variations in function of a single gene product involved in insulin signalling are unlikely to have an effect on all aspects of insulin action and hence would not present with major effects on glucose metabolisms (Fig. 2).

In clinical research, β-cell function is usually described for each individual study participant by a simple empirically derived index: e.g. HOMA2-% β, which is calculated from fasting insulin and glucose concentrations [25]. In the MAGIC meta-analysis, as insulin and glucose levels across OGTT were available for all studies, β-cell function was expressed using a slightly more complex (but still empirically derived) parameter, the "insulinogenic index" or IGI [26]. This is calculated by dividing the change in serum insulin concentration during the first 30 min of an OGTT by the change in blood glucose over the same period – or in some cases by the fasting or 30-min blood glucose to avoid negative values A validation study by Pacini *et al.* reported a global r-value of 0.67 for the relationship between IGI and a more complex parameter of insulin secretion (derived from an intravenous glucose tolerance test) in pooled data from people with and without diabetes [27].

HOMA-2%β has recently been criticised by Reaven, arguing cogently that when glucose values are high the formula requires extraordinarily high concomitant insulin concentrations for "normal" β-cell function to be reported i.e. there is an inherent bias in attributing hyperglycaemia to β-cell dysfunction rather than insulin resistance [28]. Elegant analyses conducted over the last decade by Mari et al. have gone beyond empirical approaches using indices of β-cell function derived directly from physiological data using mathematical modelling [29]. The model-derived components include "β-cell glucose sensitivity" which describes the slope of the relationship between the rate of insulin secretion and ambient glucose concentrations (negative, near linear). This slope decreases with deteriorating glucose tolerance and can be derived from insulin and glucose values across an OGTT.

These more detailed concepts have permitted greater appreciation of the independence of β-cell function and insulin sensitivity in glucose handling as independent biological processes and a greater acceptance of the importance of β-cell dysfunction early in the development of T2DM [30]. It may be that application of GWAS techniques to more accurate parameters of β-cell function might permit more detailed insights into the physiological roles of the products of gene loci identified. However, it remains the case that use of simple indices such as HOMA2-%β and IGI in GWAS studies to date has been sufficient for the discovery of a large number of "β-cell" T2DM genes, which in itself represents a form of validation of these measures.

3.3. Complex processes involved in insulin action

As detailed earlier, clinical assessment of insulin sensitivity primarily relies on measurement of blood glucose and insulin, either in the fasted condition or under hormonal or nutrient "clamp" conditions. While the secretion of insulin is almost exclusively controlled by the functional state of the β-cell there are a large number of other tissues involved in maintaining proper response to changes in nutrients such as glucose. In addition there are multiple counter-regulatory mechanisms in the body to cope with changes in hormonal and nutrient exposure. In other words, mammals have evolved to keep a very tight control on blood glucose concentration and it is highly likely that multiple molecular problems would have to occur simultaneously to alter whole body insulin sensitivity significantly.

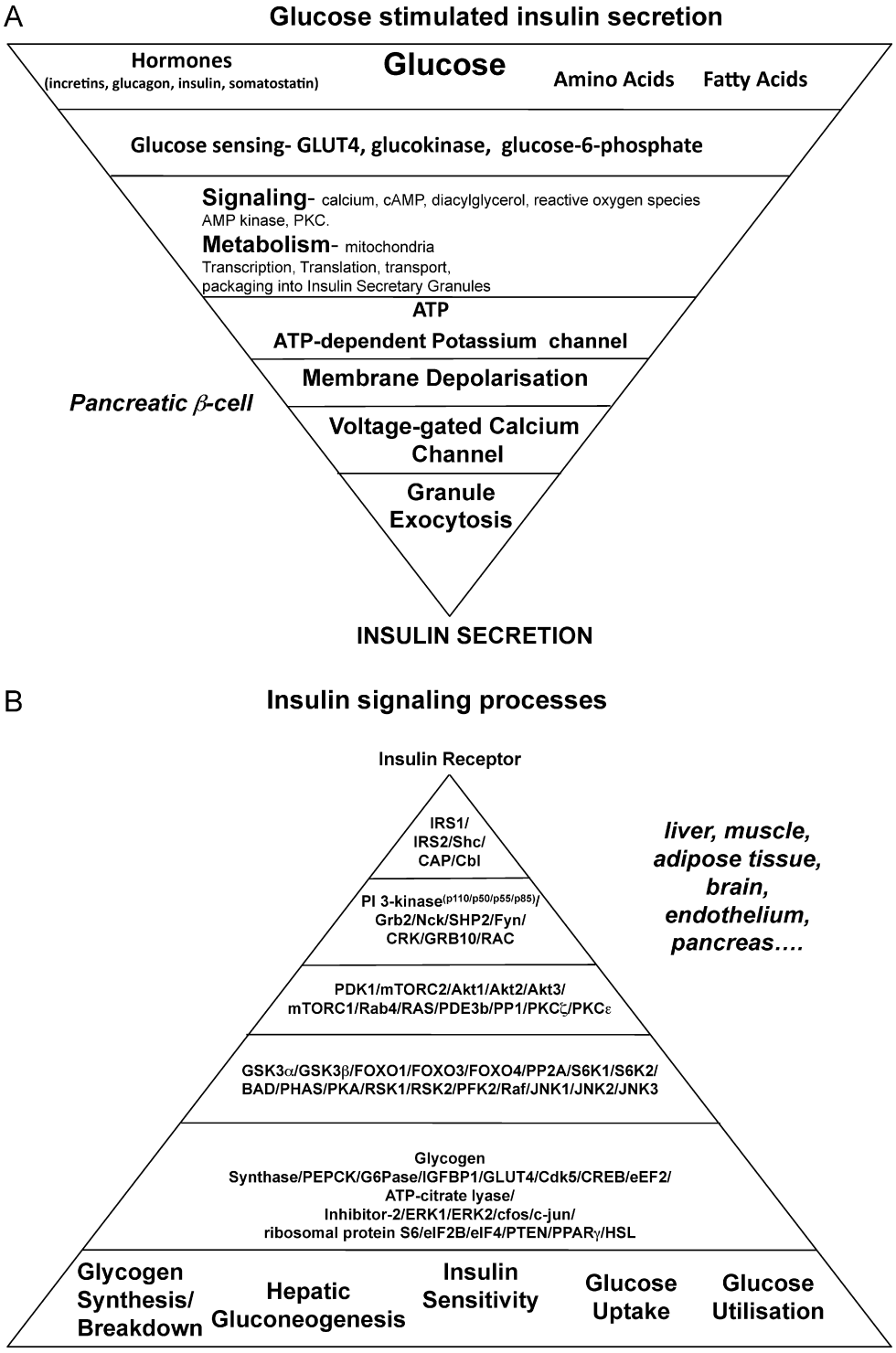


Fig. 2. Diagrammatic illustration of the processes that contribute to insulin secretion in the pancreatic β-cell (A) and insulin signalling in the various tissues involved in glucose metabolism (B). In the former, complex signal transduction processes converge on a single measurable output, namely release of insulin from pancreatic β-cells. In the latter, insulin action diverges from the insulin receptor to multiple outputs in many tissues with summative effects on glucose metabolism and insulin sensitivity. In theory, this means that overall sensitivity of the system to insulin can remain unchanged in response to variations in single components. Abbreviations: Akt – protein kinase B (isolated from the retrovirus AKT8); BAD – bcl-2 antagonist of cell death; CAP – cbl adaptor protein; Cbl – oncogene named after C asitas B-lineage L ymphoma; Cdk – cyclin dependent kinase; CREB – cAMP response element binding protein; eEF – eukaryotic elongation factor; eIF – eukaryotic initiation factor; ERK – extracellular signal regulated kinase; FOXO – forkhead box O; G6Pase – glucose-6-phoasphatase; GLUT – glucose transporter; Grb – Growth factor receptor binding protein; GSK – glycogen synthase kinase; HSL – hormone sensitive lipase; IGFBP – Insulin like growth factor binding protein; IRS – insulin receptor substrate; JNK – c-jun kinase; mTORC – mammalian target of rapamycin complex; Nck – non-catalytic region of tyrosine kinase adaptor protein 1; PDE – phosphodiesterase; PDK – 3-phosphoinositide-dependent kinase; PEPCK – phosphoenolpyruvate carboxykinase; PFK – phosphofructokinase; PHAS – eukaryotic translation initiation factor 4E binding protein 1; PI – phosphoinositide; PKA – protein kinase A; PKC – protein kinase C; PP – protein phosphatise; PPAR – peroxisome proliferator-activated receptor; PTEN – phosphatase and tensin homologue; RSK – p90 ribosomal S6 protein kinase; S6K – p70 ribosomal S6 protein kinase; Shc – src homology domain containing; SHP – small heterodimer partner.

There are therefore a number of levels of complexity impacting on the discovery of T2DM susceptibility genes related to insulin resistance:

(i) Removal of glucose from the blood is primarily achieved by insulin induction of glucose uptake into muscle. This involves insulin sensing and signalling within individual muscle cells, mobilisation of GLUT4 transporters to the cell membrane and conversion of glucose to glycogen for storage [31]. Each of these processes has strict regulatory mechanisms that respond to more than just the amount of insulin the cells are exposed to (e.g. glycogen content, exercise, adrenaline, hypoxia, lipids, etc.).
(ii) Glucose can be removed from the blood by adipose tissue and is also a fuel source for most cells in the body. At the same time endogenous glucose production in the liver is suppressed by insulin [32], but also by other nutrients (including glucose), and the liver is the primary site of insulin removal from the blood. Therefore there are at least three major organs that contribute directly to the level of glucose and insulin in the blood, and which work in concert to cope with variations in nutrient load or requirement, as well as to induce counter-regulatory pathways to limit rebound in any given response. It is now known that many of the proteins involved in these actions work in a tissue-specific fashion, and that most of the intracellular molecular pathways involved have inherent redundancy (Fig. 2), with the ability to mask minor changes in the activity of the proteins involved [33,34].
(iii) Whole-body insulin resistance could arise from hepatic, muscle or adipose insulin resistance or combinations thereof. It is known that rats on a high fat diet based on lard will develop primarily hepatic insulin resistance, whereas a palm oil diet severely affects muscle insulin sensitivity [35]. If there are parallels within the human population, then any clinical study group is likely to represent a mixture of sub-types of tissue insulin resistance that are rarely, if ever, assessed.
(iv) Many T2DM susceptibility genes affect β-cell survival, growth or development (and hence β-cell mass); while the clinical outcome of disrupting these processes is likely to be insulin insufficiency and ultimately diabetes, genetic susceptibility for loss of muscle, liver or adipose tissue (the main organs regulating insulin resistance) is likely to have distinct clinical phenotypes, i.e. insulin resistance will not be the major concern.

Taken together, it seems therefore reasonable to believe that minor changes in a single enzyme or protein function due to a single nucleotide polymorphism are unlikely to generate defects in blood glucose and insulin concentrations across a population as a major clinical outcome. This is in contrast to insulin secretion where relatively minor effects due to gene polymorphism on β-cell viability, survival or function would, over time, have a measurable effect on the rate of insulin secretion from pancreatic islets, and present clinically as hyperglycemia.

4. Biological plausibility and population attributable risk

Whether a case-control or quantitative trait GWAS design is used, the nature and likely function of proteins encoded by the genetic loci identified is imputed from information held *in silico* in databases of the human genome sequence. It must be remembered that "diabetes genes" account for only a small proportion of the overall risk of T2DM. For example, it was calculated in 2008 by Lango et al. that individuals with more than 24 known risk alleles had an odds ratio of 4.2 (95% CI 2.11–8.56) for developing T2DM with respect to those who had "only" 10–12 risk alleles – and these "high-risk" individuals formed only around 1% of the study population [36]. The overall impact of genetic risk is unlikely to have increased significantly with the discovery of further risk alleles in the intervening two years. Patel et al. have recently described a prototype "environment-wide association study" methodology with which they have reported novel environmental factors for T2DM – including environmental pollutants – with effect sizes at least comparable to the best loci yet found by GWAS [37]. This serves to highlight the continuing importance of environmental factors in the pathophysiology of T2DM in terms of their overall contribution to risk, and that research in this area should not be neglected.

5. Conclusion

We would propose that it is highly probable that more insulin resistance than β-cell dysfunction T2DM susceptibility genes remain undiscovered at the present time, most likely due to problems associated with study design and the complex nature of physiological responses to nutrients and insulin. In addition, it must be understood that even with 38 genes identified relevant to T2DM pathophysiology, the risk conferred by these combined genes accounts for only a small proportion of overall risk. It must be remembered that the rapid changes in T2DM incidence and prevalence observed in recent decades are a result of the interaction of a stable genetic background with a rapidly-changing environment. Future intervention at newly-discovered insulin secretion controlling loci should improve β-cell function allowing a more robust defence against environmental insult. Targeting oxidative stress, metabolic stress and low grade inflammation may provide fruitful avenues. However, novel therapeutic approaches, whether pharmacological or non-pharmacological, which can target the effects of diet-induced obesity on tissue-specific insulin resistance in the early pathogenesis of T2DM remain a central and invaluable goal of research aiming to halt the rapidly-increasing prevalence of T2DM and its complications worldwide.

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