EClinicalMedicine 13 (2019) 14–20

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

EClinicalMedicine
Published by THE LANCET

Review

Network medicine-travelling with the insulin receptor: Encounter of the second type

Martial Boutchueng-Djidjou [a,1], Robert L. Faure [b,*]

[a] Département of Pediatrics, Faculty of Medicine, Laval University, CHU de Québec Research Center, Québec City G1V4G2, Canada
[b] Centre de Recherche du CHU de Québec, Laboratoire de Biologie Cellulaire, local T3-55 2705, Boulevard Laurier Québec, QC, G1V4G2

ARTICLE INFO

Article history:
Received 10 April 2019
Received in revised form 8 May 2019
Accepted 18 July 2019
Available online 25 July 2019

Keywords:
Type 2 diabetes
Type 2 diabetes disease module

SUMMARY

Important progress has been made in understanding many aspects of insulin action in the last 10 years. Attention will be focused here on the physical protein interaction network of the internalized insulin receptor (IR) and its relationships with the genetic architecture of type 2 diabetes mellitus (T2D). The IR recognizes signals from the outside (circulating insulin) and engages the insulin signaling response. Within seconds, the IR is also involved in insulin internalization and its subsequent degradation in endosomes (physiological clearance of insulin). A T2D disease module sharing functional similarities with insulin secretion in pancreatic islets was recently identified in the close neighborhood of the internalized IR in liver. This module brought a new light on the apparent functional heterogeneity of numerous genes at risk to T2D by linking them to a few noncanonical layers of signaling feedback loops. These findings should be translated into a better understanding of the primary mechanisms of the disease and consequently a more precise sub-classification of T2D, ultimately leading to precision medicine and the development of new therapeutical drugs.

Contents

1. The insulin receptor: Tyrosine-kinase activation and trafficking

Insulin elicits all of its known physiological effects by binding to the insulin receptor (IR) at the cell surface of target cells [1]. The mature IR is a heterotetramer composed of two extracellular α-subunits involved in insulin binding and two cytosolic-oriented β-subunits that contain the tyrosine kinase domain. Crystallographic studies have revealed that the extracellular domain adopts an inverse V shape in a folded-over, compact conformation [2,3]. The juxta-membrane region of the β-subunit contains three tyrosine residues, Tyr-965, Tyr-972 and Tyr-984. Tyr-965 and Tyr-972 are involved in insulin-mediated endocytosis and the recruitment of signaling proteins. The remaining part of the intracellular domain is formed by an N-terminal lobe linked with a large C-terminal lobe. The C-terminal lobe contains the catalytic loop (residues 1130–1138) and the activation segment. The catalytic loop contains the kinase activation RD motif conserved between a majority of kinases and the three regulatory tyrosine residues Tyr-1158, Tyr-1162 and Tyr-1163 [1–3]. The phosphorylated kinase domains of IR and IGF1R form a specific dimeric arrangement involving an exchange of the juxtamembrane region proximal to the kinase domain [4].Studies on the whole molecular complexes, and using the single-particle cryo-electron microscopy method, recently revealed an intramolecular mechanism of activation similar to the epidermal growth factor (EGF) receptor (EGFR) [5].

Following insulin binding at the cell surface, there is also internalization of the tyrosine kinase-activated complexes into the endosomal apparatus [1,6]. Here, a decision is made either to recycle back the tyrosine-dephosphorylated and ligand-free receptor to the cell surface,

* Corresponding author.
E-mail addresses: martial.boutchueng-djidjou@moffit.org (M. Boutchueng-Djidjou), robert.faure@crchudequebec.ulaval.ca (R.L. Faure).
[1] Present address: H. Lee Moffit Cancer Center and Research Institute, Tampa, FL, USA.

https://doi.org/10.1016/j.eclinm.2019.07.007

preparing for another cycle of insulin binding and kinase activation, or to a transport in late compartments for eventual late recycling or, ultimately, degradation of the active complexes within lysosomal compartments [6]. While the fate of internalized insulin has been well characterized, particularly in liver parenchyma, which is the main site of insulin clearance in physiological concentrations of circulating insulin [6,7], the molecular mechanisms underlying IR routing and signaling are relatively unknown when compared with the EGFR [6,8]. The original experimental repertoire including morphological analysis on fixed hepatocytes and in vitro assays has shown that the internalized IR-insulin complexes are distributed through prelysosomal sorting centers that are sensitive to acidotropic and microtubule-disrupting agents [9,10]. A slower recycling route originating from late compartments without apparent involvement of multivesicular bodies was also depicted in cultured hepatocytes [11]. Concomitant biochemical characterization of insulin and EGF revealed the presence of signaling molecules in mixed hepatic Golgi/endosome (G/E) fractions suggesting the presence of a signaling activity [6]. Studies mainly done on the EGFR in cell lines favored [12–16], or challenged [17–19], the concept of endosomal signaling. These different results are now explained by the diversity and plasticity of endosomes [20,21], which are also perceived as quantal signal decoding devices [22,23].

As for the EGFR, IR tyrosine kinase activity appears to be the crucial regulator selecting ligand-dependent movements [1,6]. A system in which each receptor-tyrosine kinase (RTK) is able to induce its own structure for internalization seems unrealistic, unless the different receptors share common elements. While the topology of the endosomal apparatus may be subjected to large variations between one experimental model and another, sorting is apparently achieved with large tubulovesicular compartments whose contents are continuously modified by the entry and exit of small 70–80 nm vesicles [24,25]. These sorting compartments enable the continuous transport of cargos and receptors separately resolving security problems inherent to complexity and energy. This is analogous to a cellular version of the Aldrin Mars Cycler (AMC), where large spaceships perpetually cycle back and forth between the orbits of Earth and Mars with only minor trajectory adjustments on each cycle, without requiring a significant amount of propellant. The spaceship does not stop when it flies by a planet. The astronauts have to board a small but speedy space taxi that catches up with the cycler. The system thus enables the transport of cargos and humans, separately resolving the costs inherent to security problems when cargo and astronauts are mixed, as experienced with the past shuttle program [26].

2. The endosomal IR protein-proteins interaction network (PPIN) forms a T2D disease module

A PPIN (named GEN), constructed from a G/E fractions proteome, not only reproduced the general topology of endosomes, but also contained an IR subnetwork (named IRGEN) that is characterized by a marked enrichment in elements associated with T2D risk [27]. To date, genetic studies, including genome-wide association studies (GWAS), have identified a number of functionally heterogeneous common variants spread across the whole genome and explaining approximatively 10–20% of the T2D heritability [28–30]. The concept of network medicine based on the notion of preferential attachment inherent to scale-free networks implies that behind each cellular function there is a network consisting of genes, transcription factors, RNAs, proteins and metabolites. This understanding forces us to view diseases as the breakdown of a given module, that is also sensitive to societal factors (e.g., modern chronic overnutrition state), rather than a single or large group of genes [31]. It has rarely been possible, however, to translate such a massive amount of information on mutations and their associations with disease into primary mechanisms and therapeutic insights as well as the mechanisms underlying genotype–phenotype relationships [32]. For T2D, most of the common associated variants are located at enhancers in pancreatic islets [28], suggesting that this is a preferred location to search for a disease module. This marked enrichment in genes associated with T2D risk in IRGEN [27] suggested however that such a regulated network exists in the liver and that it is cofunctional in islets. These results prompted to ask to what extent new gene candidates linked to the known disease genes through the physical

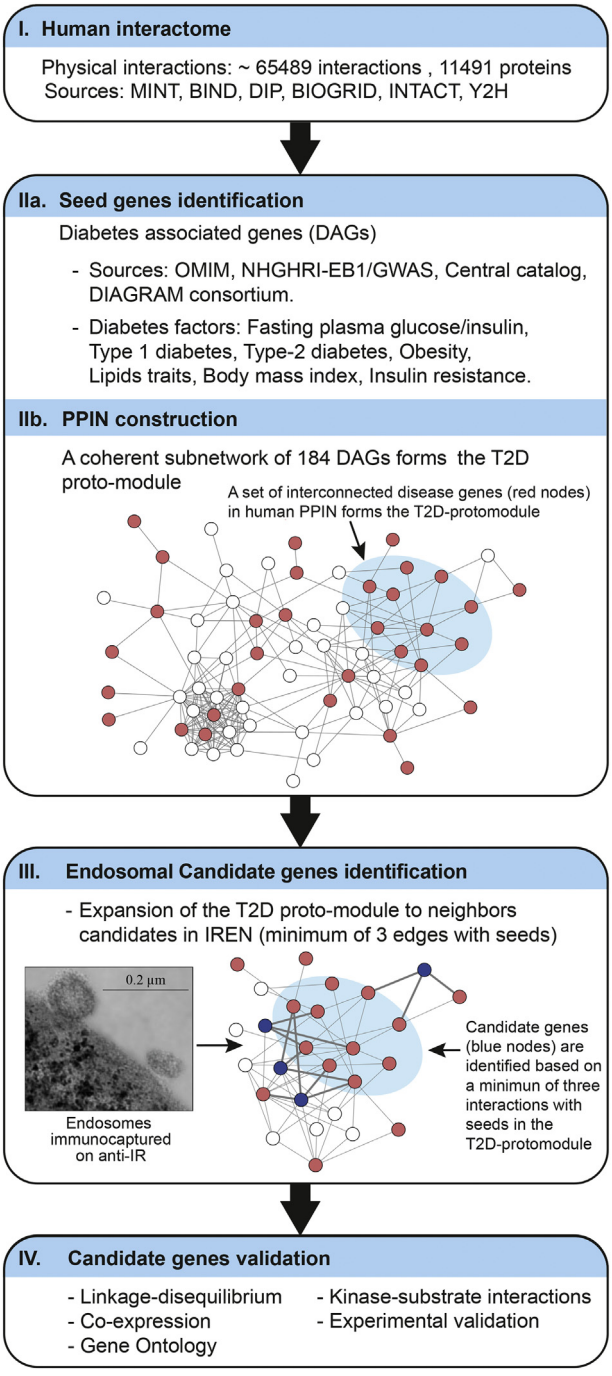


Fig. 1. A module-based approach to identify type 2 diabetes-relevant diagnostic and therapeutic candidate genes that tend to co-localize in the endosomal interactome. Disease-associated genes tend to co-localize in the human physical-protein interaction network (PPIN), forming a proto-module (blue oval). The proto-module expands physically in the IR-containing endosomes PPIN (IREN, 88% of the nodes associated with the type 2 diabetes genetic risk). Proteins having at least three interactions are considered as high candidates (blue nodes) and are validated by experimental methods (adapted from Boutchueng et al. PLoS One 2018;13:e0205180).

association of their products can be dynamically identified by diffusion [31], in the more specific environment of IR-containing endosomes. To accomplish this task, IR-containing vesicles were captured from the same insulin-stimulated G/E fractions and a local physical PPIN covering early and later events of IR routing at a 50% insulin saturating dose was constructed. By integrating a highly confident T2D protomodule (OMIM and GWAS), a T2D disease module was thus identified (named IREN) by diffusion. Proteins physically linked to "diseased" proteins were validated as candidates on the basis of at least three interactions (instead of a filter of one interaction), intracellular colocalisation, coexpression and localisation on genomic risk areas [33] (Fig. 1). The obtained hypothesis-free IREN topology is remarkably robust and organized around a few major central hubs, including the cell cycle kinase Cdk2, the IR itself (an internal control), PTPLAD1, Rab5c, and the V-ATPase components (Fig. 2). Of interest, the T2D-protomodule functional specialization expands in IREN (to include cell cycle, trafficking, signaling components, reactive oxygen species components) [33]. "Hub bottlenicity" (also named centrality) is also thought to dictate essentiality and constitutes the dynamic component of a regulated network [34,35]. This is particularly the case for IREN, given its responsivity to acute insulin stimulation. Cdk2, which displays the highest centrality and is a high-confidence candidate associated with T2D genetic risk, indeed readily associates with key elements including the IR, PTPLAD1, Rab5c, tubulin and actin cytoskeletons all containing appropriate phosphorylation sites. In such a network, PTPLAD1, in the same incoherent input, controls IR tyrosine phosphorylation and other key interactions [33] (Fig. 2). Insulin-dependent Cdk2/cyclinE complexes were previously reported, and they were functionally related with a capacity to decrease vesicle fusion events in vitro [36]. Cdk2 was also mechanistically linked with candidate proteins controlling insulin clearance, including CEACAM1, SHP-1 (PTPN6) and β-catenin [37,38]. On the other hand, targeted Cdk2 deletion in the pancreas was reported as inducing glucose intolerance primarily by affecting glucose-stimulated insulin secretion [39]. Similar to the secretory pathway, the endosomal apparatus consists of multiple compartments linked via anterograde and retrograde transport [40,41]. The endocytic and secretory pathways thus share regulatory elements of the cell division machinery either in a *pause to decide* strategy (similar to checkpoints of the cell division cycle) for appropriate routing or, alternatively, for regulated relief of the continuous braking action of Cdk2 in fusion events.

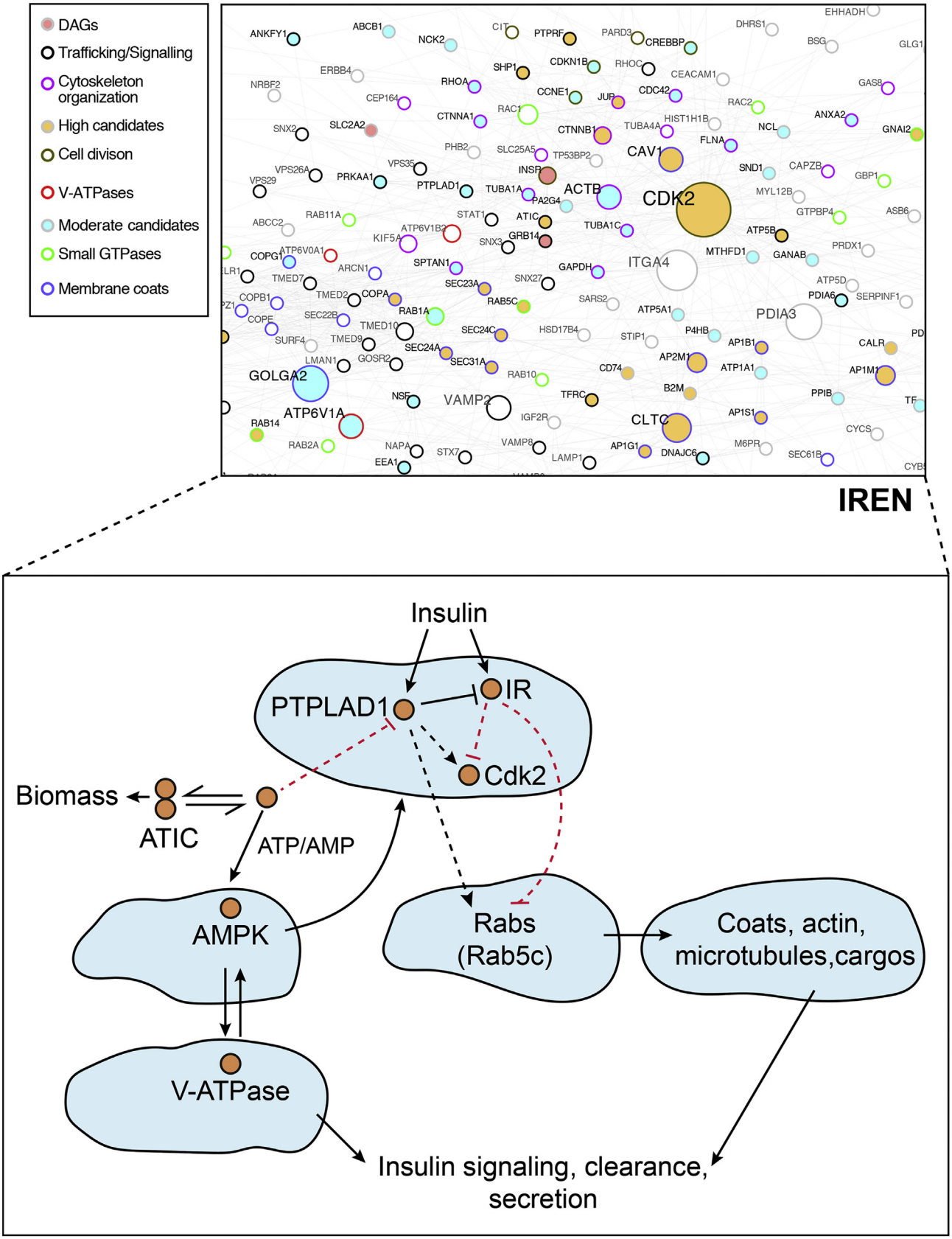
PTPLAD1 itself is particularly sensitive to insulin as it translocates readily from a yet-to-be-fully characterized intracellular compartment to the plasma membrane following insulin stimulation, and then it internalizes along with the IR [27]. Insulin-regulated PTPLAD1 also has an interesting consequence for signaling, as formation of ligand-dependent quanta (clusters of less than 100 p-RTK) with short delay in endosomal vesicles is considered an emergent property of endosomes [22]. In addition to the presence of a number of confidently known substrates for Cdk2 and AMPK in IREN, the IR appears to be a pleiotropic tyrosine kinase with the presence of high-confidence tyrosine phosphorylation sites found in PTPLAD1, ATP6V1E1, AMPK, ATIC, and Rab5c. In addition, the tyrosine phosphorylation of the candidate ANXA2 at Y24 by IR would be a key component of IR traffic through CEACAM1/β-catenin and actin [42]. As a result, the overlay with delay of Cdk2, Rabs (Rab5C, Rab11A), actin and microtubules dependent feedback would convert insulin inputs into robust oscillations consistent with simulation models of signaling [43–46]. PTPLAD1 would thus be the insulin-dependent eraser in the same insulin input (Fig. 2).

3. The endosomal T2D disease module connects signaling with trafficking and metabolism and shares functional similarities with the islets secretory pathway

Following insulin stimulation, the proteins ATIC, PTPLAD1 and AMPK associate within seconds with the tyrosine kinase-activated IR as well as control its tyrosine kinase activity and traffic [27]. ATIC is a key rate-limiting metabolic enzyme involved in the de novo production of purines, which are building blocks of DNA and RNA (biomass) but are also found in ATP, GTP, ADP, AMP, cyclic AMP, NADP, SAM and coenzyme A and thus are not only a source of energy for living cells but also the cofactors for numerous metabolic and signaling enzymes. AMPK is the energy sensor (adenylates) engaging catabolic versus anabolic processes in response to decreases in the ATP/AMP ratio [47]. AMPK activation also results in the phosphorylation of the cargo-binding kinesin light chain, KCL2, of Kif5 and in the disruption of Kif5 association with PI3K and Akt [48] which would be necessary for early and late endocytosis events [49]. Kif5 is involved in the Rab5-dependent movement of vesicles towards either the plus or the minus end of microtubules [50]. Hence, an insulin-sensitive module formed minimally by the apparently unrelated proteins ATIC and AMPK is aware of the state of activation of the IR and acts locally in a concerted manner with PTPLAD1 (IR tyrosine dephosphorylation), Rab5 and kinesins (trafficking) [27]. A concrete problem for the cell concerns its energy sources, and it seems that the cell has found an efficient way to link in a safe and economic manner the continuous local energy demand to a manufacturing center. ATP is the source of energy for the cell, and its level is controlled in part by ATIC (synthesis). The fact that the ATIC substrate, and antidiabetic, AICAR can activate AMPK [51] emphasizes the idea that all the conditions are present to autoregulate the IR module in concert with insulin inputs. This ATIC circuitry linking signaling with metabolism (Fig. 3) suggests the presence of a morpheeic mechanism [52] whereby ATIC monomers, in equilibrium with dimers [53], interact physically in the node, and ATIC dimers support the two last steps of the de novo purine biosynthetic metabolic pathway. Such morpheeic activity linking signaling with metabolism was already reported for the embryonic isoform rate-limiting glycolytic enzyme PKM2 [54].

The ATP-consuming proton pumping activity, mediated by V-ATPase, is key for appropriate insulin clearance in physiological concentrations of insulin [6,7]. By contrast with ligands such as EGF and prolactin, insulin readily dissociates from the IR in the acidic lumenal pH environment and is subsequently degraded by a lumenal protease activity that is now more related to the acidic cathepsin D [6] than to the neutral insulin-degrading enzyme (IDE) [55]. Amylin, a peptide which is co-secreted with insulin was, however, reported as a good substrate for IDE [56]. V-ATPase was found connected with ATIC and AMPK [33] and the widely used anti-diabetic drug metformin, targeting the mitochondrial production of ATP [51], was recently shown to act also on the endolysosomal system through V-ATPase and AMPK [57,58], indicating the presence of connections between the T2D disease module and drug therapy. Additional layers of feedback loops are anticipated as a robust

Fig. 2. A representation of the T2D disease module and associated layers of feedback loops in endosomes. The action of insulin occurs via a receptor tyrosine kinase (IR) located at the surface of target cells. Following insulin binding there is also, within seconds, internalization of the tyrosine kinase-activated complexes into the endosomal apparatus. Shown is the physical protein interaction network (PPIN) forming the disease module (IREN). The general topology of IREN is based on few major hubs, with the kinase Cdk2 displaying the highest centrality. Candidates (yellow and blue colors and black characters) and DAGs (diabetes associated genes, pink color and black characters) form a single-connected disease module of 94 nodes (33% of IREN nodes) with 330 interactions (28,7% of IREN interactions). An expansion to the first level of adjacent nodes results in a connected subnetwork of 272 nodes (88% of nodes) covering 92% of interactions (1070 out of 1147 I.E. interactions). The functional groups are represented according to the colors of the borders indicated in the legends. During its travel in the endosomal compartment (blue color, lower inset), the IR meet with i) proteins having heteregenous functions in signaling, metabolism, membrane transport, cargos ii) These mechanisms linking signaling with metabolism and trafficking are highly associated with the T2D genetic risk and can be cofunctional for insulin secretion (islets), clearance (liver) and action (insulin targets).



phosphorylation signal that was readily abolished by V-ATPase inhibition was reconstituted in endosomes, suggesting the presence of an endosomal homeostatic pathway, informing the cell that the lumenal acidification process is optimized [33].

4. Perspectives

We now know that hyperglycemia can be caused by a combination of genetic and environmental factors that affect circulating insulin

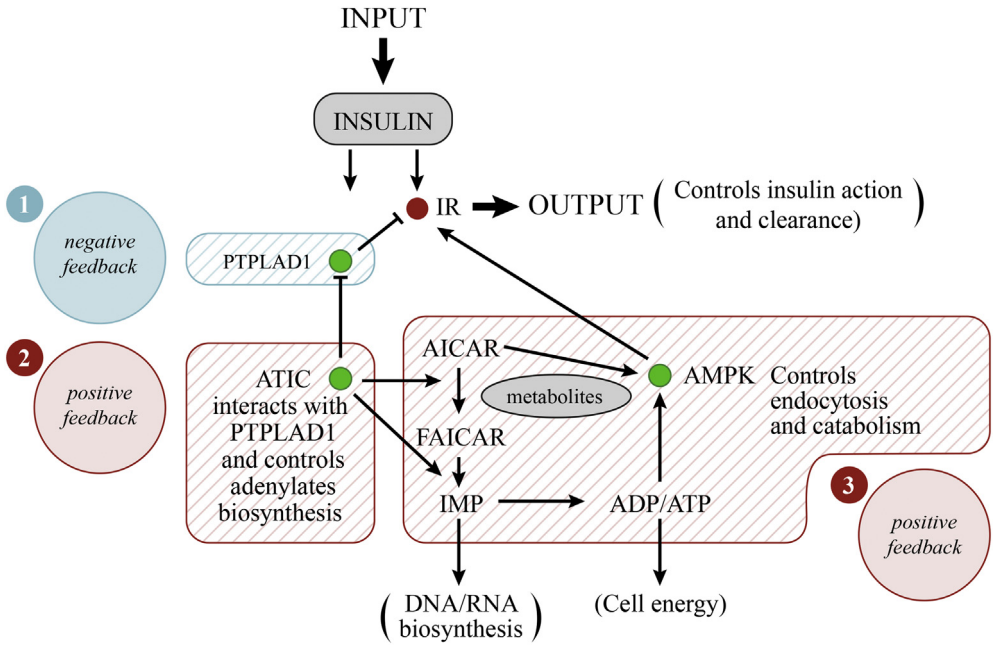


Fig. 3. A representation of the IR/ATIC/PTPLAD1/AMPK circuitery. Insulin inputs in a double incoherent mode: IR autophosphorylation plus PTPLAD1 recruitment at the cell surface (1, blue color) are converted into robust oscillations in output through the overlay of two positive feedback systems driven by the metabolic enzyme ATIC. (2, red color), a local interacting loop counteracting the action of PTPLAD1. (3, red color), adenylates production. Variations in ATIC levels or in adenylates synthesis or a decline in ATP levels independent of de novo purine biosynthesis increase the AMPK activity and IR endocytosis (adapted from Boutchueng et al. Mol Cell Proteomics 2015;14:1079–92).

concentration or action [59,60]. The liver is a major organ that controls insulin action on metabolic homeostasis. Circulating glucose availability is regulated through the insulin dependent reversible storage of glucose and glycogen as well as increased lipogenesis with canonical insulin signaling pathways [60]. Since approximately 50% of the insulin secreted by the pancreas is removed after its first pass by the liver before reaching the peripheral circulation [7], hepatic extraction through insulin-mediated endocytosis is also viewed as an adaptive mechanism that could relieve the stress on pancreatic β-cells imposed by insulin resistance [61–64]. Alternatively or in addition, a moderate chronic hyperinsulinemia due to a reduction in insulin clearance may be the primary mechanism resulting in insulin resistance [65,66]. How the identification of a T2D disease module hidden in the close neighborhood of the internalized IR can be of importance for a better understanding of the primary mechanism of the disease, precision medicine and new drug therapy? A priori, hepatic endosomes might not be the best place to find a module as, consistent with a beta cell-centric classification model [67], the T2D genetic risk is indeed more associated with reduced beta cell function [28]. This suggests a disease model where T2D cases lie across a continuous distribution with regard to beta cell dysfunction versus insulin resistance aetiologies. At the opposite end of the spectrum, obese cases presumably need fewer diabetes risk variants to push them towards diabetes, as they are already under strain from the modern physiological impact of obesity and insulin resistance. The T2D disease module clearly fits in this model as it contains elements related with the T1D, islets physiology, and also new signaling pathways that are more related with insulin resistance, trafficking and metabolism. What is the relationship of these noncanonical pathways found in the T2D disease module and other pathways? Insulin resistance may be viewed as an evolutionary conserved homeostatic response favoring catabolism over anabolism in the conditions of overnutrition. This is not favored by the fact that the adipocyte becomes also insulin resistant overtime leading to several late complication including lipid accumulation elsewhere and subsequent insidious signaling consequences including inflammatory processes [60]. Insulin resistance could also result from pathological activated kinases that are unrelated with the normal insulin response (e.g., PKCs, JNK) but similar enough to the kinases mediating normal negative feedback. This could only have occurred if the actual overnutrition state rarely or never appeared in the evolutionary history to not exert selective pressure against the use of unrelated signaling elements. Several evidence indicate the pathways identified in the T2D disease module belong to the first category as they are highly associated with the genetic disease risk [33]. Their evolutionary conservation is reminiscent, for example, of the integrated stress response (ISR), which is an organelle homeostatic response, that can be modulated by a small interactor (ISRIB) with the potential for future therapy of complex diseases [68,69]. Insulin resistance might be also an essential endosomal response that limit anabolic processes to nutrient oversupply.

The genetic architectures of human disorders are typically classified into two main categories: complex traits, such as T2D, displaying a polygenic architecture arising from numerous low-effect common variants, and rare traits that tend to have high-effect monogenic variants [70,71]. To date, genetic studies have identified a number of functionally heterogeneous T2D common variants [28–30]. The presence of rare T2D variants with high causalities [72], has not been well established by GWAS yet [30,73], except in an homogeneous cohort of Latino patients [74], and therefore there is still a necessity to understand the complexity of GWAS data better, for example by stratifying more lean, prediabetic and obese patients [75–77]. Variants frequently influence multiple phenotypes, often in unexpected ways [78]. The notion of inter-connected diseases also implies a knowledge of the comorbidities that exist between complex and Mendelian diseases [31]. As such, genes that are disrupted in Mendelian disorders are dysregulated by noncoding variants in complex traits [78,79] as exemplified by beta-thalassemia and T2D [78]. The comorbidity between T2D and other complex diseases such as cancer, neuropathies [31] and Mendelian diseases [78] can be accurately examined with regard to the T2D disease module. The hypothesis of phenotype-specific enrichment of Mendelian disorders around GWAS variants should also allow a greater resolution in identifying gene-phenotype relationships and achieve the goals of precision medicine [78,79]. It finds a particular echo in T2D, where a majority of variants are found in non-coding regions [73], and

furthermore many variants identified in coding regions have been reclassified as *false leads* [30].

Diabetes is presently classified into two main categories, type 1 and type 2 diabetes, but type 2 diabetes is particularly heterogeneous in terms of genetics, clinical presentation and outcomes. An important goal for clinicians and researchers is to classify subtypes of diabetes for lean and obese patients in order to more accurately select therapies and predict clinical complications [80–82]. Given the complexity of such a task, it would make sense to start from a class of well-defined interactions, and this is exactly what the combination of hypothesis-free methods with hypothesis-driven approaches offers with the description and validation of a T2D disease module. Recently, a soft clustering of genetic loci associated with T2D allowed the identification of two groups related with insulin deficiency, and three related with insulin resistance [83]. A data-driven cluster analysis based on six additional variables (glutamate decarboxylase antibodies, age at diagnosis, BMI, HbA1c, b-cell function and insulin resistance) allowed the identification of five overlapping clusters of patients within a cohort with different disease progression and risk of complications, thus pointing out an avenue for precision medicine [82]. Clustering can be refined with the integration of a T2D disease module that helps to link to a primary mechanism for each group of patients (insulin signaling response, clearance and production). It would be then possible by using a T2D disease module panel, containing minimally genes of the protomodule (e.g., HNF4A, IR, IGF1, IGF1R) and highly confident candidates (e.g., Cdk2, ATIC, Rab5C, PTPLAD1, ATP6V1A) (Fig. 1) [33], to subclassify smaller cohorts (50–100) of patients to help diabetologists in their day-to-day practice. Moreover, the T2D disease module can be used to explain how, in the presence of high glucose, alteration of endosomal response is functional to avoid excessive nutrient accumulation inside the cells.

Finally, a better knowledge of the T2D disease module will facilitate the appropriate use of existing antidiabetic therapies and enable the development of new drugs. Removing nodes would be a too drastic strategy to study and rewire a network positively [84]. Instead, the use of small surface interactors (edgetic approach) [85,86] seems relevant because gene essentiality in humans is more based on complexity and haploinsufficiency as exemplified for components of the cell cycle [87], PTPLAD1, which is physically connected to a Golgi essentialome [88]; beta-catenin, a transcription factor also involved in the dynamics of the actin cytoskelton [38], and ATIC itself, which is associated with the severe AICAR-ribosiduria syndrome [89]. In this regard, it was recently shown that the inhibition of ATIC homodimerization with a small surface interactor induces the activation of AMPK and improves glucose tolerance [90], supporting the idea that the T2D disease module can be positively rewired with *edgetic molecules*.

Declaration of Competing Interest

There is no conflicts of interest to declare.

Acknowledgements

The research program in the R. Faure laboratory was funded by the National Sciences and Engineering Research Council of Canada (NSERC discovery grants: 155751) and the Fondation du CHU de Québec. M. B-D acknowledges funding from the Fondation du CHU de Québec and the CRCHUQ. The authors tank Sabine Elowe (Université Laval) and four anonymous referees for their comments on the manuscript.

References

[1] Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. Nat Rev Mol Cell Biol 2018;19(1):31–44.
[2] Hubbard SR. Structural biology: insulin meets its receptor. Nature 2013;493(7431):171–2.
[3] Ward CW, Menting JG, Lawrence MC. The insulin receptor changes conformation in unforeseen ways on ligand binding: sharpening the picture of insulin receptor activation. Bioessays 2013;35(11):945–54. https://doi.org/10.1002/bies.201370111.
[4] Cabail MZ, Li S, Lemmon E, Bowen ME, Hubbard SR, Miller WT. The insulin and IGF1 receptor kinase domains are functional dimers in the activated state. Nat Commun 2015;6:6406.
[5] Scapin G, Dandey VP, Zhang Z, et al. Structure of the insulin receptor-insulin complex by single-particle cryo-EM analysis. Nature 2018;556(7699):122–5.
[6] Bergeron JJ, Di Guglielmo GM, Dahan S, Dominguez M, Posner BI. Spatial and temporal regulation of receptor tyrosine kinase activation and intracellular signal transduction. Annu Rev Biochem 2016;85:573–97.
[7] Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. Endocr Rev 1998;19(5):608–24.
[8] Zeigerer A, Wuttke A, Marsico G, Seifert S, Kalaidzidis Y, Zerial M. Functional properties of hepatocytes in vitro are correlated with cell polarity maintenance. Exp Cell Res 2017;350(1):242–52.
[9] McClain DA. Mechanism and role of insulin receptor endocytosis. Am J Med Sci 1992;304(3):192–201.
[10] Authier F, Posner BI, Bergeron JJ. Insulin-degrading enzyme. Clin Invest Med 1996;19(3):149–60.
[11] Carpentier JL, Gazzano H, Van Obberghen E, Fehlmann M, Freychet P, Orci L. Intracellular pathway followed by the insulin receptor covalently coupled to 125I-photoreactive insulin during internalization and recycling. J Cell Biol 1986;102(3):989–96.
[12] Vieira AV, Lamaze C, Schmid SL. Control of EGF receptor signaling by clathrin-mediated endocytosis. Science 1996;274(5295):2086–9.
[13] Schenck A, Goto-Silva L, Collinet C, et al. The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development. Cell 2008;133(3):486–97.
[14] Sorkin A, McClure M, Huang F, Carter R. Interaction of EGF receptor and grb2 in living cells visualized by fluorescence resonance energy transfer (FRET) microscopy. Curr Biol 2000;10(21):1395–8.
[15] Coumailleau F, Furthauer M, Knoblich JA, Gonzalez-Gaitan M. Directional Delta and Notch trafficking in Sara endosomes during asymmetric cell division. Nature 2009;458(7241):1051–5.
[16] Fortian A, Sorkin A. Live-cell fluorescence imaging reveals high stoichiometry of Grb2 binding to the EGF receptor sustained during endocytosis. J Cell Sci 2014;127(Pt 2):432–44.
[17] Damke H, Baba T, Warnock DE, Schmid SL. Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J Cell Biol 1994;127(4):915–34.
[18] Sousa LP, Lax I, Shen H, Ferguson SM, De Camilli P, Schlessinger J. Suppression of EGFR endocytosis by dynamin depletion reveals that EGFR signaling occurs primarily at the plasma membrane. Proc Natl Acad Sci U S A 2012;109(12):4419–24.
[19] Brankatschk B, Wichert SP, Johnson SD, Schaad O, Rossner MJ, Gruenberg J. Regulation of the EGF transcriptional response by endocytic sorting. Sci Signal 2012;5(215):ra21.
[20] Collinet C, Stoter M, Bradshaw CR, et al. Systems survey of endocytosis by multiparametric image analysis. Nature 2010;464(7286):243–9.
[21] Mayor S, Parton RG, Donaldson JG. Clathrin-independent pathways of endocytosis. Cold Spring Harb Perspect Biol 2014;6(6).
[22] Villasenor R, Nonaka H, Del Conte-Zerial P, Kalaidzidis Y, Zerial M. Regulation of EGFR signal transduction by analogue-to-digital conversion in endosomes. Elife 2015;4.
[23] Wang T, Martin S, Nguyen TH, et al. Flux of signalling endosomes undergoing axonal retrograde transport is encoded by presynaptic activity and TrkB. Nat Commun 2016;7:12976.
[24] Gruenberg J. The endocytic pathway: a mosaic of domains. Nat Rev Mol Cell Biol 2001;2(10):721–30.
[25] van Ijzendoorn SC. Recycling endosomes. J Cell Sci 2006;119(Pt 9):1679–81.
[26] Aldrin B. Mission to march: My vision for space exploration. . 1st Edition ed. Washington DC: National Geographic society; 2015; 20036.
[27] Boutchueng-Djidjou M, Collard-Simard G, Fortier S, et al. The last enzyme of the De Novo Purine synthesis pathway 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) plays a central role in insulin signaling and the Golgi/endosomes protein network. Mol Cell Proteomics 2015;14(4):1079–92.
[28] Visscher PM, Wray NR, Zhang Q, et al. 10 years of GWAS discovery: biology, function, and translation. Am J Hum Genet 2017;101(1):5–22.
[29] Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat Genet 2018;50(11):1505–13.
[30] Mahajan A, Wessel J, Willems SM, et al. Refining the accuracy of validated target identification through coding variant fine-mapping in type 2 diabetes. Nat Genet 2018;50(4):559–71.
[31] Barabasi AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease. Nat Rev Genet 2011;12(1):56–68.
[32] Menche J, Sharma A, Kitsak M, et al. Disease networks. Uncovering disease-disease relationships through the incomplete interactome. Science 2015;347(6224):1257601.
[33] Boutchueng-Djidjou M, Belleau P, Bilodeau N, et al. A type 2 diabetes disease module with a high collective influence for Cdk2 and PTPLAD1 is localized in endosomes. PLoS One 2018;13(10):e0205180.
[34] Yu H, Kim PM, Sprecher E, Trifonov V, Gerstein M. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. PLoS Comput Biol 2007;3(4):e59.
[35] Morone F, Makse HA. Influence maximization in complex networks through optimal percolation. Nature 2015;524(7563):65–8.

[36] Gaulin JF, Fiset A, Fortier S, Faure RL. Characterization of Cdk2-cyclin E complexes in plasma membrane and endosomes of liver parenchyma. Insulin-dependent regulation. J Biol Chem 2000;275(22):16658–65.
[37] Caron D, Savard PE, Doillon CJ, et al. Protein tyrosine phosphatase inhibition induces anti-tumor activity: evidence of Cdk2/p27(kip1) and Cdk2/SHP-1 complex formation in human ovarian cancer cells. Cancer Lett 2008;262(2):265–75.
[38] Fiset A, Xu E, Bergeron S, et al. Compartmentalized CDK2 is connected with SHP-1 and beta-catenin and regulates insulin internalization. Cell Signal 2011;23(5):911–9.
[39] Kim SY, Lee JH, Merrins MJ, et al. Loss of cyclin dependent kinase 2 in the pancreas links primary beta-cell dysfunction to progressive depletion of beta-cell mass and diabetes. J Biol Chem 2017;292(9):3841–53.
[40] Rothman JE. Mechanisms of intracellular protein transport. Nature 1994;372(6501):55–63.
[41] Wandinger-Ness A, Zerial M. Rab proteins and the compartmentalization of the endosomal system. Cold Spring Harb Perspect Biol 2014;6(11):a022616.
[42] Caron D, Boutchueng-Djidjou M, Tanguay RM, Faure RL. Annexin A2 is SUMOylated on its N-terminal domain: regulation by insulin. FEBS Lett 2015;589(9):985–91.
[43] Dueber JE, Mirsky EA, Lim WA. Engineering synthetic signaling proteins with ultrasensitive input/output control. Nat Biotechnol 2007;25(6):660–2.
[44] Brandman O, Meyer T. Feedback loops shape cellular signals in space and time. Science 2008;322(5900):390–5.
[45] Tsai TY, Choi YS, Ma W, Pomerening JR, Tang C, Ferrell Jr JE. Robust, tunable biological oscillations from interlinked positive and negative feedback loops. Science 2008;321(5885):126–9.
[46] Novak B, Tyson JJ. Design principles of biochemical oscillators. Nat Rev Mol Cell Biol 2008;9(12):981–91.
[47] Oakhill JS, Steel R, Chen ZP, et al. AMPK is a direct adenylate charge-regulated protein kinase. Science 2011;332(6036):1433–5.
[48] Amato S, Liu X, Zheng B, Cantley L, Rakic P, Man HY. AMP-activated protein kinase regulates neuronal polarization by interfering with PI 3-kinase localization. Science 2011;332(6026):247–51.
[49] Shpetner H, Joly M, Hartley D, Corvera S. Potential sites of PI-3 kinase function in the endocytic pathway revealed by the PI-3 kinase inhibitor, wortmannin. J Cell Biol 1996;132(4):595–605.
[50] Nielsen E, Severin F, Backer JM, Hyman AA, Zerial M. Rab5 regulates motility of early endosomes on microtubules. Nat Cell Biol 1999;1(6):376–82.
[51] Shaw RJ, Cantley LC. Cell biology. Ancient sensor for ancient drug. Science 2012;336(6083):813–4.
[52] Jaffe EK. Morpheeins–a new structural paradigm for allosteric regulation. Trends Biochem Sci 2005;30(9):490–7.
[53] Vergis JM, Bulock KG, Fleming KG, Beardsley GP. Human 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/inosine 5′-monophosphate cyclohydrolase. A bifunctional protein requiring dimerization for transformylase activity but not for cyclohydrolase activity. J Biol Chem 2001;276(11):7727–33.
[54] Semenova G, Chernoff J. PKM2 enters the morpheein academy. Mol Cell 2012;45(5):583–4.
[55] Villa-Perez P, Merino B, Fernandez-Diaz CM, et al. Liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance, without affecting insulin clearance in mice. Metabolism 2018;88:1–11.
[56] Bennett RG, Duckworth WC, Hamel FG. Degradation of amylin by insulin-degrading enzyme. J Biol Chem 2000;275(47):36621–5.
[57] Kim J, You YJ. Regulation of organelle function by metformin. IUBMB Life 2017;69(7):459–69.
[58] Chen J, Ou Y, Li Y, Hu S, Shao LW, Liu Y. Metformin extends C. elegans lifespan through lysosomal pathway. Elife 2017;6.
[59] Sladek R. The many faces of diabetes: addressing heterogeneity of a complex disease. Lancet Diabetes Endocrinol 2018;6(5):348–9.
[60] Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev 2018;98(4):2133–223.
[61] Sidrauski C, Chapman R, Walter P. The unfolded protein response: an intracellular signalling pathway with many surprising features. Trends Cell Biol 1998;8(6):245–9.
[62] Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306(5695):457–61.
[63] Harding HP, Ron D. Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes 2002;51(Suppl. 3):S455–61.
[64] Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell 2012;148(5):852–71.
[65] Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J. Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? Diabetes Care 2008;31(Suppl. 2):S262–8.
[66] Ader M, Stefanovski D, Kim SP, et al. Hepatic insulin clearance is the primary determinant of insulin sensitivity in the normal dog. Obesity (Silver Spring) 2014;22(5):1238–45.
[67] Schwartz SS, Epstein S, Corkey BE, Grant SF, Gavin 3rd JR, Aguilar RB. The time is right for a new classification system for diabetes: rationale and implications of the beta-cell-centric classification schema. Diabetes Care 2016;39(2):179–86.
[68] Tsai JC, Miller-Vedam LE, Anand AA, et al. Structure of the nucleotide exchange factor eIF2B reveals mechanism of memory-enhancing molecule. Science 2018(6383):359.
[69] Zyryanova AF, Weis F, Faille A, et al. Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. Science 2018;359(6383):1533–6.
[70] Schork NJ, Murray SS, Frazer KA, Topol EJ. Common vs. rare allele hypotheses for complex diseases. Curr Opin Genet Dev 2009;19(3):212–9.
[71] Chong JX, Buckingham KJ, Jhangiani SN, et al. The genetic basis of Mendelian phenotypes: discoveries, challenges, and opportunities. Am J Hum Genet 2015;97(2):199–215.
[72] Kadowaki T, Bevins CL, Cama A, et al. Two mutant alleles of the insulin receptor gene in a patient with extreme insulin resistance. Science 1988;240(4853):787–90.
[73] Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. Nature 2016;536(7614):41–7.
[74] Estrada K, Aukrust I, Bjorkhaug L, et al. Association of a low-frequency variant in HNF1A with type 2 diabetes in a Latino population. JAMA 2014;311(22):2305–14.
[75] Perry JR, Voight BF, Yengo L, et al. Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet 2012;8(5):e1002741.
[76] Wray NR, Wijmenga C, Sullivan PF, Yang J, Visscher PM. Common disease is more complex than implied by the core gene omnigenic model. Cell 2018;173(7):1573–80.
[77] Cannon ME, Mohlke KL. Deciphering the emerging complexities of molecular mechanisms at GWAS loci. Am J Hum Genet 2018;103(5):637–53.
[78] Blair DR, Lyttle CS, Mortensen JM, et al. A nondegenerate code of deleterious variants in Mendelian loci contributes to complex disease risk. Cell 2013;155(1):70–80.
[79] Freund MK, Burch KS, Shi H, et al. Phenotype-specific enrichment of Mendelian disorder genes near GWAS regions across 62 complex traits. Am J Hum Genet 2018;103(4):535–52.
[80] Collaboration NCDRF. Effects of diabetes definition on global surveillance of diabetes prevalence and diagnosis: a pooled analysis of 96 population-based studies with 331,288 participants. Lancet Diabetes Endocrinol 2015;3(8):624–37.
[81] Li L, Cheng WY, Glicksberg BS, et al. Identification of type 2 diabetes subgroups through topological analysis of patient similarity. Sci Transl Med 2015;7(311):311ra174.
[82] Ahlqvist E, Storm P, Karajamaki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6(5):361–9.
[83] Udler MS, Kim J, von Grotthuss M, et al. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: a soft clustering analysis. PLoS Med 2018;15(9):e1002654.
[84] Kovacs IA, Barabasi AL. Network science: destruction perfected. Nature 2015;524(7563):38–9.
[85] Sahni N, Yi S, Taipale M, et al. Widespread macromolecular interaction perturbations in human genetic disorders. Cell 2015;161(3):647–60.
[86] Cui H, Zhao N, Korkin D. Multilayer view of pathogenic SNVs in human Interactome through in silico Edgetic profiling. J Mol Biol 2018;430(18 Pt A):2974–92.
[87] Bartha I, di Iulio J, Venter JC, Telenti A. Human gene essentiality. Nat Rev Genet 2018;19(1):51–62.
[88] Blomen VA, Majek P, Jae LT, et al. Gene essentiality and synthetic lethality in haploid human cells. Science 2015;350(6264):1092–6.
[89] Marie S, Heron B, Bitoun P, Timmerman T, Van Den Berghe G, Vincent MF. AICA-ribosiduria: a novel, neurologically devastating inborn error of purine biosynthesis caused by mutation of ATIC. Am J Hum Genet 2004;74(6):1276–81.
[90] Asby DJ, Cuda F, Beyaert M, Houghton FD, Cagampang FR, Tavassoli A. AMPK activation via modulation of De Novo Purine biosynthesis with an inhibitor of ATIC Homodimerization. Chem Biol 2015;22(7):838–48.