Review

MOLECULAR METABOLISM

Untangling the interplay of genetic and metabolic influences on beta-cell function: Examples of potential therapeutic implications involving TCF7L2 and FFAR1*

Robert Wagner [1,3], Harald Staiger [1,2,3], Susanne Ullrich [1,2,3], Norbert Stefan [1,2,3], Andreas Fritsche [1,2,3], Hans-Ulrich Häring [1,2,3,*]

ABSTRACT

Deteriorating beta-cell function is a common feature of type 2 diabetes. In this review, we briefly address the regulation of beta-cell function, and discuss some of the main determinants of beta-cell failure. We will focus on the role of interactions between the genetic background and metabolic environment (insulin resistance, fuel supply and flux as well as metabolic signaling). We present data on the function of the strongest common diabetes risk variant, the single nucleotide polymorphism (SNP) rs7903146 in *TCF7L2*. As also mirrored by its interaction with glycemia on insulin secretion, this SNP in large part confers resistance against the incretin effect. Genetic influence on insulin secretion also interacts with free fatty acids, as evidenced by data on rs1573611 in *FFAR1*. Several medications marketed by now or currently under development for diabetes treatment engage these pathways, and therapeutic implications from these findings are soon to be expected.

Keywords Beta-cell function; Beta-cell failure; TCF7L2; FFAR1; Gene × environment interaction; Pharmacogenetics; Incretin resistance

1. INTRODUCTION

Diabetes is a heterogeneous disease with the obvious lowest common denominator of hyperglycemia as its main manifestation. The comprehensive disease phenotypes can be very different, especially when considering the dissimilarities of classical Type 1 and Type 2 diabetes. It is generally acknowledged today that one of the most common chronic diseases of the 21st century type 2 diabetes, is a mixture of pathogenetically diverse structures [1]. However, there is one common feature in all the pathological processes that yield abnormally elevated blood glucose: dysfunction of beta-cells resulting in the decline of insulin production and secretion. Even if most type 2 diabetes phenotypes are related to phenomena such as obesity and insulin resistance, restoration of the full potential of insulin secretion would lead to a remission of diabetes. In turn, as the relatively high rate of diabetes relapse after bariatric surgery [2] suggests, deteriorating beta-cell function would often prevail despite an improved metabolic milieu. Therefore, understanding the decline of beta-cells and finding ways to improve their function are essential in combating the disease. This review will examine examples for the impact of interactions between metabolic and genetic alterations in insulin secretion to envision a more efficient diabetes therapy on our way from conventional to personalized medicine. It will focus on genetic and metabolic interactions on incretin and free fatty acid (FFA) induced insulin secretion.

2. REGULATION OF INSULIN SECRETION

The regulation of insulin secretion in the beta-cell is primarily determined by plasma glucose concentration, which triggers the process generally known as glucose-stimulated insulin secretion (GSIS) [3]. However, other fuels such as FFAs and amino acids are also able to act as potent enhancers of insulin secretion. Beside fuels, specific hormones are capable of modulating insulin secretion in the beta-cell. The most important stimulators are incretins, namely glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide, formerly also known as gastric inhibitory polypeptide (GIP). They are secreted upon food stimuli by L- and K-cells of the gastrointestinal tract, respectively, contribute to as much as 70% of insulin secretion after a meal [4] and are widely used as medical treatment of diabetes.

The central event eliciting insulin secretion is the production of ATP which leads to the inhibition of ATP-sensitive inwardly-rectifying K^+_{ATP} channels, a consecutive depolarization of the plasma membrane with

[1]Department of Internal Medicine, Division of Endocrinology, Diabetology, Nephrology, Vascular Disease and Clinical Chemistry, University Hospital of the Eberhard Karls University, Tübingen, Germany [2]Institute for Diabetes Research and Metabolic Diseases of the Helmholz Centre Munich at the University of Tübingen (IDM), Tübingen, Germany [3]German Center for Diabetes Research (DZD), Neuherberg, Germany

*Correspondence to: Department of Internal Medicine, Division of Endocrinology, Diabetology, Nephrology, Vascular Disease and Clinical Chemistry, University Hospital of the Eberhard Karls University, Otfried-Müller-Str 10, 72076 Tübingen, Germany. Email: hans-ulrich.haering@med.uni-tuebingen.de (H.-U. Häring).

Received December 19, 2013 • Revision received January 7, 2014 • Accepted January 7, 2014 • Available online 22 January 2014

http://dx.doi.org/10.1016/j.molmet.2014.01.001

opening of voltage-dependent Ca^{2+} channels and Ca^{2+} influx into the beta-cell. However, in addition to glucose itself, insulin secretion is highly regulated by metabolites. While amino acids can also activate insulin secretion, incretins and fatty acids enhance GSIS. The potentiatory effects on insulin secretion are mediated through G-protein coupled receptors. Incretins stimulate GLP-1R and GIPR and act through cAMP-dependent signaling pathways involving the stimulation of PKA and EPAC2. This amplifying pathway strongly depends on glucose-induced increase of cytosolic Ca^{2+}. The mechanism by which fatty acids potentiate insulin secretion probably involves both metabolites and receptor activation [5]. The complex interplay of fuel metabolites and coupling factors of 3 intracellular metabolic networks, the (i) tricarboxylic-acid (Krebs/Szent-Györgyi) cycle, (ii) pyruvate cycle and (iii) glycerolipid/FFA cycle has been sophisticatedly modeled by Nolan and Prentki in 2008 [6]. In 2003, when the role of a cell-membrane receptor, the orphan G-protein coupled receptor 40 (GPR40) was established [7], it was renamed free fatty acid receptor 1 (FFAR1) and is now considered as an important mediator of FFA-induced insulin secretion [3]. This receptor, in contrast to incretin receptors which couple to adenylyl cyclase through Gs-proteins, is linked to Gq-protein-dependent stimulation of Phospholipase C and activates protein kinase D1 [8].

3. PATHOMECHANISMS OF DECREASED INSULIN SECRETION IN TYPE 2 DIABETES

3.1. Glucolipotoxicity

As we briefly reviewed above, glucose and FFAs are natural activators of insulin secretion. Paradoxically, both of these fuels seem to contribute to beta-cell failure under certain circumstances. Unfortunately, the precise steps of the development of glucolipotoxicity are not fully known and results of human studies on glucolipotoxicity are heterogeneous. Observational data showed that chronically elevated FFA predict a deterioration of beta-cell function in subjects with impaired glucose tolerance, while no such association was observed in subjects with normal glucose tolerance [9]. In an interventional study from our laboratory, a 24-h lipid infusion during a hyperglycemic clamp altered only insulin sensitivity, but did not change insulin secretion [10]. However, a lipid infusion over 4 days enhanced insulin secretion in subjects without a family history of diabetes, but reduced it in those who had a family history of diabetes [11]. Long-term consequences of elevated FFA levels were unequivocally shown only in animals. There is evidence that fatty acids, especially saturated fatty acids, lead to beta-cell apoptosis [12]. The activation and translocation of protein kinase C δ which stimulates the proapoptotic transcription factor FOXO1, was shown to be one of the key steps of FFA-induced beta-cells apoptosis [13,14]. The proposed complex pathomechanistic background of glucolipotoxicity has been excellently reviewed by Poitout and Robertson in 2008. They postulated that part of the genetic predisposition to diabetes might be related to the ability or inability of beta-cells to adequately respond to hyperglycemia and hyperlipidemia [15]. This notion fits to the main concept of type 2 diabetes etiology, namely that an ill-constellation of genetic predisposition and environmental factors such as fuel surplus lead to the metabolic derangements determining the disease.

3.2. Genetic background

Type 2 diabetes has long been recognized as a disease caused by the complex relationship of genetic predisposition and lifestyle factors. There is substantial evidence for a relevant genetic determination of beta-cell failure. In a family study examining diabetes-related quantitative traits, postprandial FFAs and insulin secretion showed the highest heritability, with 40–50% of the variance explained by genetic factors [16]. Furthermore, most genetic loci found to be associated with diabetes in hypothesis-free genome-wide association studies (GWAS) are thought to influence insulin secretion but not insulin sensitivity [17]. For some of the genes discovered by GWAS, such as *TCF7L2*, *CDKAL1*, *SLC30A8*, *MADD* and *ADCY5*, examination of quantitative glycemic traits revealed associations with insulin processing [18–20]. However, it was only possible for a handful of the discovered genes to experimentally detect precise modes of action. Cellular function still remains obscure in most cases. A further reason for the disappointment in GWAS was that their results could not hitherto be translated to a better prediction of diabetes [21]. The more than 60 discovered diabetes genes notwithstanding, only a fraction of the heritability ($\sim$15%) of type 2 diabetes can be actually statistically explained. This is primarily due to the low effect size of discovered diabetes related genetic variants of which the largest, the single nucleotide polymorphism (SNP) rs7903146 in *TCF7L2*, only amounts to an odds ratio (OR) of 1.37 per effect allele [22].

4. GENETIC AND METABOLIC INTERACTIONS ON INSULIN SECRETION

4.1. TCF7L2 and incretin action

By its discovery as the strongest genetic signal associated with type 2 diabetes, *TCF7L2* was catapulted into focus of interest in diabetes research. Previously known as a transcription factor of the WNT-signaling pathway, its link to diabetes was initially established tracing a linkage signal on chromosome 10q [23], and then replicated in a GWAS [24]. Data from oral glucose tolerance tests (OGTTs) soon revealed an effect on insulin secretion rather than insulin sensitivity [25,26]. Pursuing a hypothesis based on the role of WNT-signaling in the transcription of proglucagon which serves as precursor for GLP-1, we conducted a study to test the effect of SNPs in *TCF7L2* on incretin secretion and action. While confirming a robust effect of the risk allele of SNP rs7903146 on lowering insulin secretion, we also showed that the risk variant was not associated with altered incretin levels after oral glucose load, but with a lower incretin-induced insulin secretion. In this experiment, a GLP-1 infusion was started at minute 120 of a hyperglycemic clamp with a glucose target of 10 mmol/l [27]. Carriers of the risk allele had by 30 and 37% lower insulin levels in the first and second phases of GLP-1-induced insulin secretion, respectively [28]. This finding was corroborated by Lyssenko et al. who demonstrated that the SNP rs7903146 significantly deflected the regression line's slope between area under the curve (AUC) of insulin during OGTT and AUC insulin during intravenous glucose tolerance test in $\sim$400 participants of the Botnia cohort. The incretin effect, characterized by the relative difference of these AUCs, was even more strikingly lower when the analysis was restricted to participants with fasting hyperglycemia $>$ 5.4 mmol/l [29]. Additional support of the concept came from a study by Pilgaard et al. who demonstrated reduced insulin secretion in healthy *TCF7L2* risk carriers during a hyperglycemic clamp not only after GLP-1 infusion, but also after the administration of GIP [30]. A smaller study with OGTT and isoglycemic intravenous glucose infusions similarly suggested an impaired insulinotropic effect of incretins in *TCF7L2* risk allele carriers [31]. Only one published study that utilized an 8.5 mmol/l target glucose and a lower initial GLP-1 dose during the hyperglycemic clamp failed to detect an altered insulinotropic effect for *TCF7L2* risk allele carriers [32]. In summary, a substantial body of evidence has

accumulated from mechanistic studies suggesting that at least a major part, if not all, of the effect of *TCF7L2* is mediated by incretin resistance. Given that the incretin effect, at least in the case of GLP-1, gets physiologically more powerful with rising glucose [33], we hypothesized that insulin secretion in *TCF7L2* risk allele carriers would become more compromised when plasma glucose is elevated. To test this hypothesis, we examined the interaction of plasma glucose with *TCF7L2* genotypes in ~1600 subjects of the Tübingen Family Study. Indeed, AUC glucose interacted with the SNP rs7903146 in *TCF7L2* on several OGTT-derived insulin secretion parameters. A nominally significant interaction was also observed between the SNP and glycated hemoglobin (HbA1c) [34]. By linking this glucose-dependent secretion impairment in *TCF7L2* risk allele carriers and the known glucose-dependency of incretin-induced insulinotropism, we gained additional support for the gene's mode of action. That being said, precise details on *TCF7L2*'s effect on the molecular level are still obscure. Potential mechanisms have been discussed by us and others in reviews on incretins [35,36]. A recent paper of our group demonstrated that Nor-1, a novel glucose and incretin-dependent molecular regulator of insulin gene expression interacts with the TCF7L2 pathway, as also evidenced by a gene × gene interaction [37].

Nevertheless, even the direction of association between TCF7L2 expression and diabetes is elusive. Initial data showed that risk allele carriers have increased transcript levels and higher TCF7L2 mRNA expression associates with decreased insulin secretion [29]. In contrast, other studies suggested that lower TCF7L2 expression leads to decreased insulin secretion [38] and overexpression could support beta-cell regeneration [39]. The results of a recent study using transgenic mice with beta-cell specific dominant negative expression of TCF7L2 seem to be in agreement with these findings [40].

There is still more confusion in regard of TCF7L2's main site of action. Boj and associates reported that beta-cell specific deletion of TCF7L2 does not alter beta-cell function, but knock-out of TCF7L2 in the liver of adult mice reduces hepatic glucose production [41]. Differentiation of "acute" effects of TCF7L2 manipulation and ontogenetically "chronic" effects of the transcription factor in these experiments could perhaps lift some of these controversies. Emerging data on the role of alternative splicing of the TCF7L2 transcript [42] which is regulated by metabolic factors [43] will potentially contribute to a better understanding of the gene's function in the future.

4.2. Other genetic variants involved in incretin action

Beside *TCF7L2*, we identified other gene loci involved in impaired incretin action on insulin secretion using the hyperglycemic clamp technique. Genetic variation in *WFS1*, a diabetes risk gene identified by GWAS, impairs GLP-1-induced insulin secretion [44]. Furthermore, using the Metabochip, we identified three novel genetic loci (*TMEM114*; *CHST3* and *CTRB1/2*) with significant effects (30–40%) on GLP-1-stimulated insulin secretion during hyperglycemic clamps [45]. The SNP rs7202877 near *CTRB1/2* is a known diabetes risk locus.

4.3. TCF7L2 interacts with environmental factors

Data on *TCF7L2*-action demonstrate a genetically determined mechanism of action for glucotoxicity, thereby providing the first evidence for Poitout's aforementioned postulation. It is possible that genetic variation in *TCF7L2* relevantly contributes to the decline in beta-cell function, once the "first hit" through adverse lifestyle factors is committed. Epidemiological studies show that an interaction between fiber intake and genetic variation in *TCF7L2* influences the risk of type 2 diabetes [46–48] and the amount of weight loss during life style intervention [49,50]. Moreover, data from a large randomized controlled trial also suggested that metformin and lifestyle-intervention weakens the genetic risk conferred by *TCF7L2* [26]. Recent data from a large diet-intervention study demonstrated that high adherence to Mediterranean diet could protect rs7903146 risk allele carriers from elevated fasting glucose and even cardiovascular complications [51]. Glucose-raising medications are adverse environmental factors, whose effects can be magnified by genetic risk variants. This was impressively shown by an interaction between three *TCF7L2* risk variants and hydrochlorothiazide-intake [52]. The efficacy of sulfonylureas was also implicated in an interaction with *TCF7L2* risk variants [53–55]. This might derive from a lately discovered effector pathway of sulfonylureas, which is cAMP-dependent and shared with the incretin pathway [56].

4.4. Potential therapeutic implications of TCF7L2 risk genotypes

As summarized above, convincing evidence demonstrates that incretin resistance is the major mode of action for the strongest diabetes-related genetic variant. Involved pathways are potential substrates of gluco-toxicity. Although the variant's distinct effect on diabetes in large

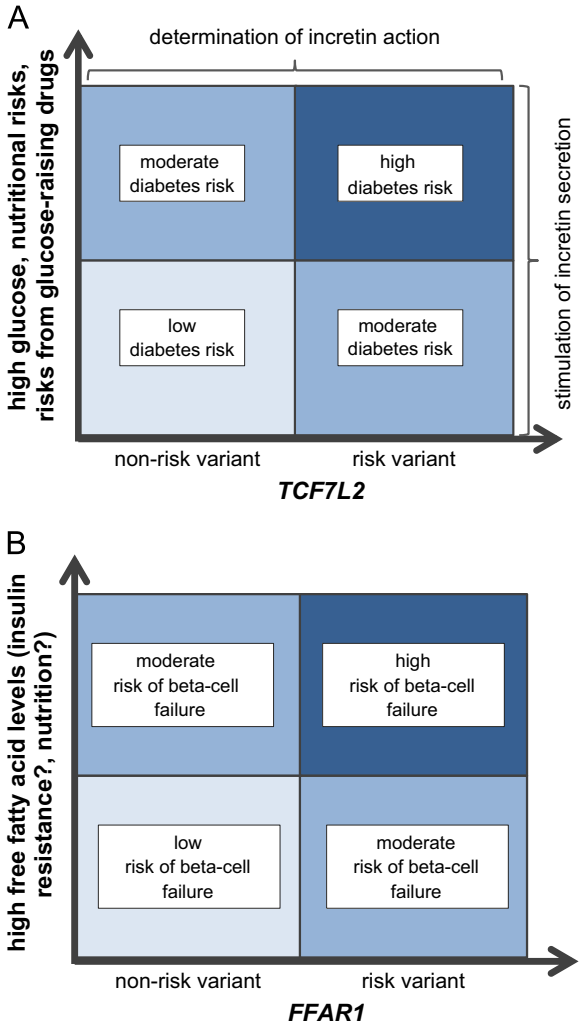

Figure. 1: Role of gene × environment interactions in the case of *TCF7L2* (A) and *FFAR1* (B, hypothesized) on diabetes and beta-cell failure.

meta-analyses was minor, its actual relevance probably exceeds the statistically shown odds ratio in specific adverse environmental contexts. Given the mounting use of medications which target increased incretin action (GLP-1 analogs and dipeptidyl-peptidase 4 (DPP4) inhibitors) further research is needed to test their pharmacogenetic interactions with *TCF7L2*. A proof-of-concept has been established for an interaction of the above mentioned SNP rs7202877 near CTRB1/2 with a decreased therapeutic efficacy of DPP4 inhibitors [45]. Early data from a phase IV study of a DPP4 inhibitor also suggest the presence of such an effect for SNP rs7903146 and a DPP4-inhibitor (personal communication). Reliable information is scant, but expert opinion estimates that 30% of patients are incretin non-responders [57]. We postulate that, at least in individuals with homozygous *TCF7L2* risk variants, a tailored approach to diabetes therapy right at the manifestation of the disease would lead to a longer compensation of beta-cell function. Alternative strategies could involve earlier administration of insulin or the use of novel drug regimens such as sodium-dependent glucose transporter 2 (SGLT-2) inhibitors.

4.5. Genetic variation in FFAR1, free fatty acids and insulin secretion

As we briefly summarized above, FFAs are Janus-faced players in the regulation of insulin secretion and one of their effector pathways involves FFAR1. Expression of FFAR1 was shown to be reduced in diabetes [58]. Mounting evidence indicates that FFAR1 mediates exclusively insulinotropic effects, and does not negatively influence insulin secretion [59,60]. We recently showed this in experiments employing an FFAR1 agonist and antagonist in the setting of INS-1E cells, human islets and mouse islets from control and FFAR1-knockout animals. While the natural FFAR1-ligand palmitate augmented insulin secretion, but also stimulated beta-cell apoptosis, the specific agonist increased insulin secretion without contributing to apoptosis. In addition, the FFAR1-agonist exhibited protection against apoptosis when applied together with palmitate. Concordant with this, use of an FFAR1-antagonist alone also led to increased apoptosis in INS1E-cells [61]. In humans, a reduced lipid-induced insulin secretion was demonstrated earlier in carriers of a loss-of-function mutation in *FFAR1* [62]. In our translational study, we showed in ~2100 subjects of the Tübingen Family Study that a variant with a minor allele frequency of 27% in a non-coding region near the *FFAR1* exon interacted with fasting FFA levels. The interaction between the SNP rs1573611 and fasting FFA was significantly associated with two independent insulin secretion parameters [61]. The result suggested that a common *FFAR1* variant could contribute to compromised insulin secretion in a high fat environment, thereby, providing additional proof-of-concept to Poitout's aforementioned postulation that part of the genetic predisposition to diabetes might be related to the inability of beta-cells to adequately increase insulin secretion in response to elevated fatty acid levels [15]. Moreover, differential FFA-induced insulin secretion for a variant in *FFAR1* could also implicate an altered therapeutic efficacy for a receptor agonist. Of note, several FFAR1 receptor agonists are under development, one of them is presently undergoing phase III clinical trials. Data on FFAR1 as a pharmacologic target, and its mode of action have recently been reviewed [63].

4.6. The missing heritability of diabetes genes

We presented evidence on *TCF7L2*'s role in incretin action. We also saw data supporting a robust gene x environment interaction for the gene's effect in modulation of insulin secretion which can at least partially be derived from the mode of action as an effector of a glucose-dependent insulin secretory pathway (Figure. 1a).

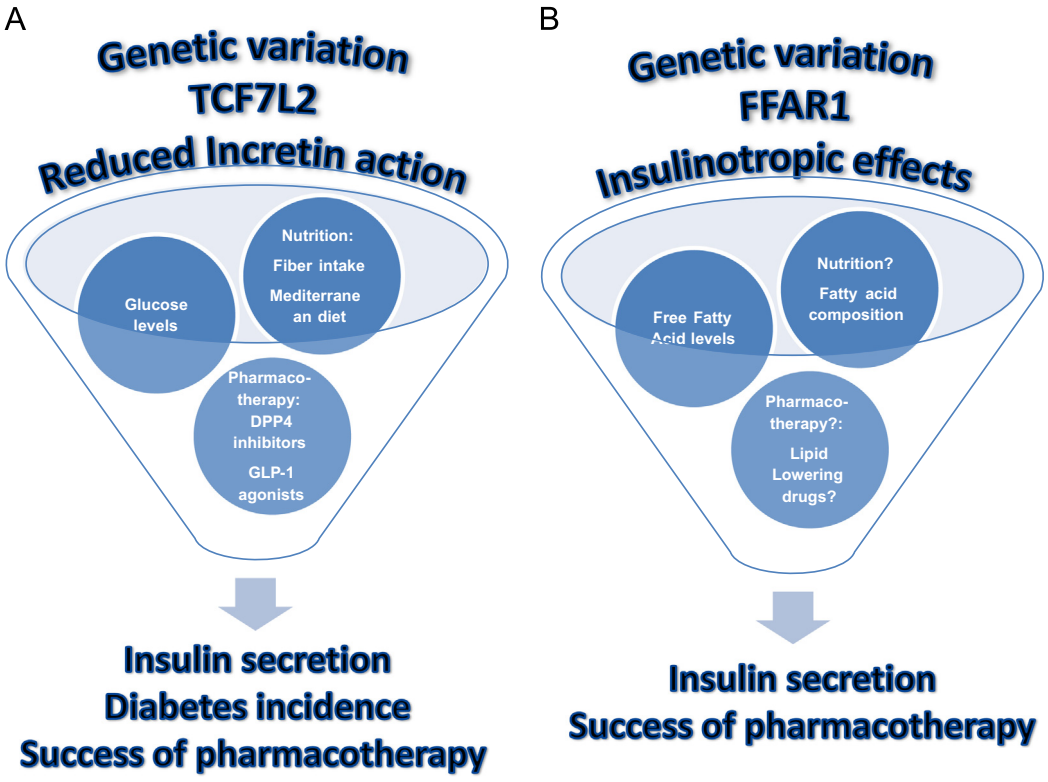

Figure. 2: Factors determining the influence of genetic variation (symbolized by a funnel) on insulin secretion and success of pharmacotherapy. (A) Known effects of genetic variation in *TCF7L2*, (B) Proposed effects of genetic variation in *FFAR1*.

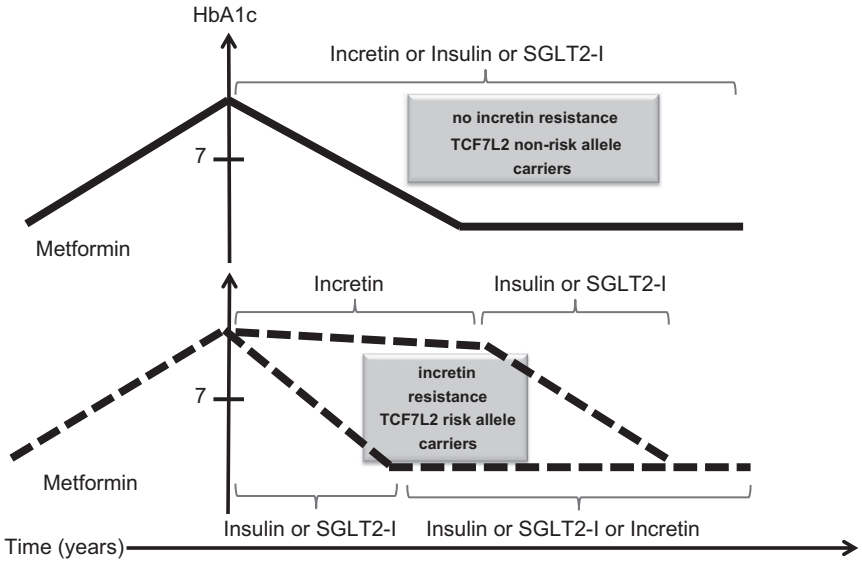

Figure. 3: Hypothetical scheme of the genetic variation in TCF7L2 on success of different pharmacotherapies. In TCF7L2 non-risk allele carriers the mode of therapy does not appear to influence the success of therapy (upper panel). Once glycemic control deteriorates, carriers of the T-allele of rs7903146 in *TCF7L2*, which causes incretin resistance, are less successful when adding an incretin-based therapy to metformin (lower panel). Primary addition of SGLT2 inhibitors or insulin to reduce glucose levels is likely to be more effective in those patients. After reduction of glucose levels, an incretin based therapy could be more effective because incretin resistance is more prominent during hyperglycemia. SGLT2-I: sodium-glucose co-transporter 2 inhibitors; solid line: carriers of *TCF7L2* wild-type (e.g. rs7903146 C-allele); dashed line: carriers of *TCF7L2* risk-variants (e.g. rs7903146 T-allele).

FFAs are insulinotropic substrates acting through FFAR1. We described a common genetic variant near *FFAR1*. In our models, the impact of this variant on insulin secretion became only evident when testing interactions with FFAs (Figure. 1b). Such genetic signals remain unrecognized by studies which do not account for gene × environment interactions.

Whether a substantial part of the "missing heritability" of diabetes, i.e. heritability not explained by previously discovered SNPs, is indeed masked by a complex network of gene × environment, and perhaps also gene × gene interactions is discussed controversially. Franks argues that the discovery of gene × environment interactions will not account for the missing heritability, because the used heritability estimates of type 2 diabetes are inherently clean of interacting factors [64]. Using statistical simulations of environmental interactions, Aschard and colleagues come to the conclusion that adding such interactions to a diabetes prediction model based on genetic risk would not relevantly increase predictive power [65]. In contrast, ~25% of the heritability of fasting insulin was explained by a gene × environment interaction between carbohydrate intake and the whole genome in a recent study. In the same study, ~40% of the heritability of the HOMA-B index was accounted for by an interaction of dietary *n*-6 polyunsaturated fatty acids with the genome [66]. The major problem with testing interaction effects is their "power hunger", i.e. much larger samples are required to detect them than with marginal effects. This difficulty probably also underlies the notoriously low replicability of interaction study results [67]. By attempting to depict the complex relationships of living organisms closer and more accurately, statistical models get more and more complicated. For example, a crosstalk between two biological pathways could be mirrored by a gene × gene interaction, which results in adding further variables and interaction terms to the model. Selecting the right models and combinations of variables without biological hypotheses gets, from a certain point on, computationally unmanageable. An additional key problem of gene × environment interaction testing is the low availability of precise environmental variables. This is particularly difficult with lifestyle or diet variables, but acquiring e.g. accurate measures of body fat distribution can also be prohibitively expensive on a larger scale. Homogenously phenotyped single-center populations probably have advantages in this regard. Several new approaches has been introduced to tackle the inherent problems of gene × environment interaction testing [67], and some studies using innovative methods to overcome the above mentioned difficulties have been published recently [68,69]. Nevertheless taking reasonable hypotheses from basic science, testing them in precisely phenotyped human cohorts and using a careful sense of proportion in judging the plausibility of results would still remain an important way of discovering new concepts.

5. CONCLUSION

Using two examples, namely genetic variation in *TCF7L2* and *FFAR1*, we have shown that interaction of genetic and metabolic parameters has an impact on beta-cell function. In both examples, this may influence the success of a pharmacotherapy: existing ones like DPP-4 inhibitors and GLP-1 agonists in the case of *TCF7L2* and upcoming ones like FFAR1-agonist in the case of *FFAR1*. There are several other interactive factors which influence the efficacy of pharmacotherapy in the two examples of this review which are shown in Figure. 2a and b. We propose a hypothetical example of a new therapeutic strategy which incorporates these data on *TCF7L2* in Figure. 3. Of course, future clinical studies should support these concepts.

Taken together, knowledge of the interactive factors and integrating this knowledge into therapeutic decisions will improve diabetes therapy in the future.

CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Tuomi T., Santoro N., Caprio S., et al. The many faces of diabetes: a disease with increasing heterogeneity. The Lancet (13), 2013, 62219-9. http://dx.doi.org/10.1016/S0140-6736(13)62219-9.

[2] Arterburn, D.E., Bogart, A., Sherwood, N.E., et al., 2013. A multisite study of long-term remission and relapse of type 2 diabetes mellitus following gastric bypass. Obesity Surgery 23:93–102, http://dx.doi.org/10.1007/s11695-012-0802-1.

[3] Prentki, M., Matschinsky, F.M., Madiraju, S.R.M., 2013. Metabolic signaling in fuel-induced insulin secretion. Cell Metabolism 18:162–185, http://dx.doi.org/10.1016/j.cmet.2013.05.018.

[4] Meier, J.J., Nauck, M.A., 2010. Is the diminished incretin effect in type 2 diabetes just an epi-phenomenon of impaired beta-cell function? Diabetes 59:1117–1125, http://dx.doi.org/10.2337/db09-1899.

[5] Henquin, J.-C., 2011. The dual control of insulin secretion by glucose involves triggering and amplifying pathways in β-cells. Diabetes Research and Clinical Practice 93 (uppl. 1):S27–S31, http://dx.doi.org/10.1016/S0168-8227(11)70010-9.

[6] Nolan, C.J., Prentki, M., 2008. The islet beta-cell: fuel responsive and vulnerable. Trends in Endocrinology & Metabolism 19:285–291, http://dx.doi.org/10.1016/j.tem.2008.07.006.

[7] Itoh, Y., Kawamata, Y., Harada, M., et al., 2003. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature 422:173–176 http://dx.doi.org/10.1038/nature01478.

[8] Ferdaoussi, M., Bergeron, V., Zarrouki, B., et al., 2012. G protein-coupled receptor (GPR)40-dependent potentiation of insulin secretion in mouse islets is mediated by protein kinase D1. Diabetologia 55:2682–2692, http://dx.doi.org/10.1007/s00125-012-2650-x.

[9] Stefan, N., Stumvoll, M., Bogardus, C., Tataranni, P.A., 2003. Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT. American Journal of Physiology – Endocrinology and Metabolism 284:E1156–E1161, http://dx.doi.org/10.1152/ajpendo.00427.2002.

[10] Stefan, N., Wahl, H.G., Fritsche, A., et al., 2001. Effect of the pattern of elevated free fatty acids on insulin sensitivity and insulin secretion in healthy humans. Hormone and Metabolic Research 33:432–438, http://dx.doi.org/10.1055/s-2001–16231.

[11] Kashyap, S., Belfort, R., Gastaldelli, A., et al., 2003. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop Type 2 diabetes. Diabetes 52:2461–2474, http://dx.doi.org/10.2337/diabetes.52.10.2461.

[12] Mandrup-Poulsen, T., 2001. Beta-cell apoptosis: stimuli and signaling. Diabetes 50:S58, http://dx.doi.org/10.2337/diabetes.50.2007.S58.

[13] Eitel, K., Staiger, H., Rieger, J., et al., 2003. Protein kinase C δ activation and translocation to the nucleus are required for fatty acid-induced apoptosis of insulin-secreting cells. Diabetes 52:991–997, http://dx.doi.org/10.2337/diabetes.52.4.991.

[14] Hennige, A.M., Ranta, F., Heinzelmann, I., et al., 2010. Overexpression of kinase-negative protein kinase Cdelta in pancreatic beta-cells protects mice from diet-induced glucose intolerance and beta-cell dysfunction. Diabetes 59:119–127, http://dx.doi.org/10.2337/db09-0512.

[15] Poitout, V., Robertson, R.P., 2008. Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocrine Reviews 29:351–366, http://dx.doi.org/10.1210/er.2007-0023.

[16] Almgren, P., Lehtovirta, M., Isomaa, B., et al., 2011. Heritability and familiality of type 2 diabetes and related quantitative traits in the Botnia Study. Diabetologia 54:2811–2819, http://dx.doi.org/10.1007/s00125-011-2267-5.

[17] Staiger, H., Machicao, F., Fritsche, A., 2009. Haring H-U, 2009. Pathomechanisms of type 2 diabetes genes. Endocrine Reviews 30:557–585.

[18] Wagner, R., Dudziak, K., Herzberg-Schäfer, S.A., et al., 2011. Glucose-raising genetic variants in MADD and ADCY5 impair conversion of proinsulin to insulin. PLoS ONE 6:e23639, http://dx.doi.org/10.1371/journal.pone.0023639.

[19] Dimas, A.S., Lagou, V., Barker, A., et al., 2013. Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. Diabetes , http://dx.doi.org/10.2337/db13-0949.

[20] Kirchhoff, K., Machicao, F., Haupt, A., et al., 2008. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 51:597–601.

[21] Schulze, M.B., Weikert, C., Pischon, T., et al., 2009. Use of multiple metabolic and genetic markers to improve the prediction of type 2 diabetes: the EPIC-Potsdam Study. Diabetes Care 32:2116–2119, http://dx.doi.org/10.2337/dc09-0197.

[22] Zeggini, E., Scott, L.J., Saxena, R., et al., 2008. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nature Genetics 40:638–645, http://dx.doi.org/10.1038/ng.120.

[23] Grant, S.F.A., Thorleifsson, G., Reynisdottir, I., et al., 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nature Genetics 38:320–323, http://dx.doi.org/10.1038/ng1732.

[24] Wellcome Trust Case Control Consortium, 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. Nature 447:661–678, http://dx.doi.org/10.1038/nature05911.

[25] Saxena, R., Gianniny, L., Burtt, N.P., et al., 2006. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes 55:2890–2895, http://dx.doi.org/10.2337/db06-0381.

[26] Florez, J.C., Jablonski, K.A., Bayley, N., et al., 2006. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. New England Journal of Medicine 355:241–250, http://dx.doi.org/10.1056/NEJMoa062418.

[27] Fritsche, A., Stefan, N., Hardt, E., et al., 2000. A novel hyperglycaemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. European Journal of Clinical Investigation 30:411–418.

[28] Schäfer, S.A., Tschritter, O., Machicao, F., et al., 2007. Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. Diabetologia 50:2443–2450, http://dx.doi.org/10.1007/s00125-007-0753-6.

[29] Lyssenko, V., Lupi, R., Marchetti, P., et al., 2007. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. Journal of Clinical Investigation 117:2155–2163, http://dx.doi.org/10.1172/JCI30706.

[30] Pilgaard, K., Jensen, C.B., Schou, J.H., et al., 2009. The T allele of rs7903146 TCF7L2 is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production in young healthy men. Diabetologia 52:1298–1307, http://dx.doi.org/10.1007/s00125-009-1307-x.

[31] Villareal, D.T., Robertson, H., Bell, G.I., et al., 2010. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. Diabetes 59:479–485, http://dx.doi.org/10.2337/db09-1169.

[32] Smushkin, G., Sathananthan, M., Sathananthan, A., et al., 2012. Diabetes-associated common genetic variation and its association with GLP-1 concentrations and response to exogenous GLP-1. Diabetes 61:1082–1089, http://dx.doi.org/10.2337/db11-1732.

[33] Kjems, L.L., Holst, J.J., Vølund, A., Madsbad, S., 2003. The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. Diabetes 52:380–386.

[34] Heni, M., Ketterer, C., Thamer, C., et al., 2010. Glycemia determines the effect of type 2 diabetes risk genes on insulin secretion. Diabetes 59:3247–3252, http://dx.doi.org/10.2337/db10-0674.

[35] Müssig, K., Staiger, H., Machicao, F., et al., 2010. Genetic variants affecting incretin sensitivity and incretin secretion. Diabetologia 53:2289–2297.

[36] Campbell, J.E., Drucker, D.J., 2013. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metabolism 17:819–837, http://dx.doi.org/10.1016/j.cmet.2013.04.008.

[37] Ordelheide, A.-M., Gerst, F., Rothfuss, O., et al., 2013. Nor-1, a novel incretin-responsive regulator of insulin genes and insulin secretion. Molecular Metabolism 2:243–255, http://dx.doi.org/10.1016/j.molmet.2013.06.003.

[38] Da Silva Xavier, G., Loder, M.K., McDonald, A., et al., 2009. TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells. Diabetes 58:894–905, http://dx.doi.org/10.2337/db08-1187.

[39] Shu, L., Zien, K., Gutjahr, G., et al., 2012. TCF7L2 promotes beta cell regeneration in human and mouse pancreas. Diabetologia 55:3296–3307.

[40] Takamoto I., Kubota N., Nakaya K., et al. TCF7L2 in mouse pancreatic beta cells plays a crucial role in glucose homeostasis by regulating beta cell mass. Diabetologia, 2013, 1–12. http://dx.doi.org/10.1007/s00125-013-3131-6.

[41] Boj, S.F., van Es, J.H., Huch, M., et al., 2012. Diabetes risk gene and wnt effector Tcf7l2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151:1595–1607, http://dx.doi.org/10.1016/j.cell.2012.10.053.

[42] Prokunina-Olsson, L., Welch, C., Hansson, O., et al., 2009. Tissue-specific alternative splicing of TCF7L2. Human Molecular Genetics 18:3795–3804 http://dx.doi.org/10.1093/hmg/ddp321.

[43] Kaminska, D., Kuulasmaa, T., Venesmaa, S., et al., 2012. Adipose tissue TCF7L2 splicing is regulated by weight loss and associates with glucose and fatty acid metabolism. Diabetes 61:2807–2813, http://dx.doi.org/10.2337/db12-0239.

[44] Schäfer, S.A., Müssig, K., Staiger, H., et al., 2009. A common genetic variant in WFS1 determines impaired glucagon-like peptide-1-induced insulin secretion. Diabetologia 52:1075–1082, http://dx.doi.org/10.1007/s00125-009-1344-5.

[45] 't Hart, L.M., Fritsche, A., Nijpels, G., et al., 2013. The CTRB1/2 locus affects diabetes susceptibility and treatment via the incretin pathway. Diabetes 62:3275–3281, http://dx.doi.org/10.2337/db13-0227.

[46] Fisher, E., Boeing, H., Fritsche, A., et al., 2009. Whole-grain consumption and transcription factor-7-like 2 (TCF7L2) rs7903146: gene-diet interaction in modulating type 2 diabetes risk. British Journal of Nutrition 101:478–481, http://dx.doi.org/10.1017/S0007114508020369.

[47] Cornelis, M.C., Qi, L., Kraft, P., Hu, F.B., 2009. TCF7L2, dietary carbohydrate, and risk of type 2 diabetes in US women. American Journal of Clinical Nutrition 89:1256–1262, http://dx.doi.org/10.3945/ajcn.2008.27058.

[48] Hindy, G., Sonestedt, E., Ericson, U., et al., 2012. Role of TCF7L2 risk variant and dietary fibre intake on incident type 2 diabetes. Diabetologia 55:2646–2654, http://dx.doi.org/10.1007/s00125-012-2634-x.

[49] Haupt, A., Thamer, C., Heni, M., et al., 2010. Gene variants of TCF7L2 influence weight loss and body composition during lifestyle intervention in a population at risk for type 2. Diabetes 59:747–750, http://dx.doi.org/10.2337/db09-1050.

[50] Heni, M., Herzberg-Schafer, S., Machicao, F., et al., 2012. Dietary fiber intake modulates the association between variants in TCF7L2 and weight loss during a lifestyle intervention. Diabetes Care 35:e24, http://dx.doi.org/10.2337/dc11-2012.

[51] Corella, D., Carrasco, P., Sorlí, J.V., et al., 2013. Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence a randomized controlled trial in a high-cardiovascular-risk population. Dia Care DC_130955 , http://dx.doi.org/10.2337/dc13-0955.

[52] Karnes, J.H., Gong, Y., Pacanowski, M.A., et al., 2013. Impact of TCF7L2 single nucleotide polymorphisms on hydrochlorothiazide-induced diabetes. Pharmacogenetics and Genomics 23:697–705, http://dx.doi.org/10.1097/FPC.0000000000000012.

[53] Pearson, E.R., Donnelly, L.A., Kimber, C., et al., 2007. Variation in TCF7L2 influences therapeutic response to sulfonylureas: a GoDARTs study. Diabetes 56:2178–2182, http://dx.doi.org/10.2337/db07-0440.

[54] Holstein, A., Hahn, M., Körner, A., et al., 2011. TCF7L2 and therapeutic response to sulfonylureas in patients with type 2 diabetes. BMC Medical Genetics 12:30.

[55] Javorský, M., Babjaková, E., Klimčáková, L., et al., 2013. Association between TCF7L2 genotype and glycemic control in diabetic patients treated with gliclazide. International Journal of Endocrinology 2013:374858, http://dx.doi.org/10.1155/2013/374858.

[56] Zhang, C.-L., Katoh, M., Shibasaki, T., et al., 2009. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. Science Signaling 325:607.

[57] Herzberg-Schäfer, S., Heni, M., Stefan, N., et al., 2012. Impairment of GLP1-induced insulin secretion: role of genetic background, insulin resistance and hyperglycaemia. Diabetes, Obesity and Metabolism 14:85–90, http://dx.doi.org/10.1111/j.1463-1326.2012.01648.x.

[58] Del Guerra, S., Bugliani, M., D'Aleo, V., et al., 2010. G-protein-coupled receptor 40 (GPR40) expression and its regulation in human pancreatic islets: the role of type 2 diabetes and fatty acids. Nutrition, Metabolism and Cardiovascular Diseases 20:22–25, http://dx.doi.org/10.1016/j.numecd.2009.02.008.

[59] Lan, H., Hoos, L.M., Liu, L., et al., 2008. Lack of FFAR1/GPR40 does not protect mice from high-fat diet–induced metabolic disease. Diabetes 57:2999–3006, http://dx.doi.org/10.2337/db08-0596.

[60] Wu, P., Yang, L., Shen, X., 2010. The relationship between GPR40 and lipotoxicity of the pancreatic β-cells as well as the effect of pioglitazone. Biochemical and Biophysical Research Communications 403:36–39, http://dx.doi.org/10.1016/j.bbrc.2010.10.105.

[61] Wagner, R., Kaiser, G., Gerst, F., et al., 2013. Reevaluation of fatty acid receptor 1 as a drug target for the stimulation of insulin secretion in humans. Diabetes 62:2106–2111, http://dx.doi.org/10.2337/db12-1249.

[62] Vettor, R., Granzotto, M., Stefani, D.D., et al., 2008. Loss-of-function mutation of the GPR40 gene associates with abnormal stimulated insulin secretion by acting on intracellular calcium mobilization. Journal of Clinical Endocrinology and Metabolism 93:3541–3550, http://dx.doi.org/10.1210/jc.2007-2680.

[63] Poitout, V., Lin, DC-H, 2013. Modulating GPR40: therapeutic promise and potential in diabetes. Drug Discovery Today 18:1301–1308, http://dx.doi.org/10.1016/j.drudis.2013.09.003.

[64] Franks, P.W., 2011. Gene × environment interactions in type 2 diabetes. Current Diabetes Reports 11:552–561, http://dx.doi.org/10.1007/s11892-011-0224-9.

[65] Aschard, H., Chen, J., Cornelis, M.C., et al., 2012. Inclusion of gene–gene and gene–environment interactions unlikely to dramatically improve risk prediction for complex diseases. American Journal of Human Genetics 90:962–972, http://dx.doi.org/10.1016/j.ajhg.2012.04.017.

[66] Zheng, J.-S., Arnett, D.K., Lee, Y.-C., et al., 2013. Genome-wide contribution of genotype by environment interaction to variation of diabetes-related traits. PLoS ONE 8:e77442, http://dx.doi.org/10.1371/journal.pone.0077442.

[67] Thomas, D., 2010. Gene–environment-wide association studies: emerging approaches. Nature Reviews Genetics 11:259–272, http://dx.doi.org/10.1038/nrg2764.

[68] Patel, C.J., Chen, R., Kodama, K., et al., 2013. Systematic identification of interaction effects between genome- and environment-wide associations in type 2 diabetes mellitus. Human Genetics 132:495–508, http://dx.doi.org/10.1007/s00439-012-1258-z.

[69] Franks P.W., Christophi C.A., Jablonski K.A., et al. Common variation at PPARGC1A/B and change in body composition and metabolic traits following preventive interventions: the Diabetes Prevention Program. Diabetologia, 2013, 1–6. http://dx.doi.org/10.1007/s00125-013-3133-4.

