Review

MOLECULAR METABOLISM

Biomarkers for type 2 diabetes

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ABSTRACT

Background: The prevalence and incidence of type 2 diabetes (T2D), representing >90% of all cases of diabetes, are increasing rapidly worldwide. Identification of individuals at high risk of developing diabetes is of great importance as early interventions might delay or even prevent full-blown disease. T2D is a complex disease caused by multiple genetic loci in interplay with lifestyle and environmental factors. Recently over 400 distinct association signals were published; these explain 18% of the risk of T2D.

Scope of review: In this review there is a major focus on risk factors and genetic and non-genetic biomarkers for the risk of T2D identified especially in large prospective population-based studies, and studies testing causality of the biomarkers for T2D in Mendelian randomization studies. Another focus is on understanding genome-phenome interplay in the classification of individuals with T2D into subgroups.

Major conclusions: Several recent large population-based studies and their meta-analyses have identified multiple potential genetic and non-genetic biomarkers for the risk of T2D. Combination of genetic variants and physiologically characterized pathways improves the classification of individuals with T2D into subgroups, and is also paving the way to a precision medicine approach, in T2D.

Keywords Type 2 diabetes; Biomarkers; Mendelian randomization; Genomics

1. INTRODUCTION

Type 2 diabetes (T2D) and its comorbidities have reached epidemic proportions. The prevalence and incidence of T2D, representing >90% of all cases of diabetes, are increasing rapidly throughout the world. The International Diabetes Federation has estimated that the number of people with diabetes is expected to rise from 425 million adults in 2017 to 629 million by 2045, and the proportion of people with T2D is increasing in most countries (https://www.idf.org/). Therefore, the identification of individuals at high risk of developing T2D is of great importance as early interventions might delay or even prevent full-blown disease.

Two major pathophysiological mechanisms characterize T2D, insulin resistance, especially in skeletal muscle and liver, and defective insulin secretion from the pancreas [1]. However, not all disease-causing pathways are completely understood, as T2D is a complex disorder resulting from an interplay between genes and environment. There is accumulating evidence that the risk of T2D is strongly influenced by genetic factors [2]. During the last 10 years the application of the genome-wide association studies (GWASs) in the genetics of complex diseases, including T2D, has led to remarkable discoveries and contributed significantly to population and complex-trait genetics, the biology of diseases, and translation towards new therapeutics [3]. Recently over 400 distinct association signals were reported, explaining 18% of T2D risk and offering insights into biological pathways causal for T2D [4]. Low-frequency variants contribute much less to T2D heritability than do common variants [5]. Insulin resistance is another important component determining the risk of T2D. Obesity is an insulin resistant state, and 'obesity epidemic' usually pays the way to 'diabetes epidemic'. Several lifestyle factors, including a lack of exercise and unhealthy diet, contribute to insulin resistance, and increase the risk of T2D. Relatively few genetic variants have been associated with insulin resistance. Functions of several genetic variants remain unknown [6].

In this review the focus is on risk factors and genetic and non-genetic biomarkers for T2D, identified especially in large prospective population-based studies, and studies testing the causality for the risk of T2D in Mendelian Randomization (MR) studies. Another focus is to understand genome-phenome interactions in the classification of individuals with T2D into subgroups.

2. NON-GENETIC RISK FACTORS FOR T2D

2.1. Biomarkers, lifestyle and environmental factors, dietary factors, medical history, and psychosocial factors

Previous studies have identified several risk factors for T2D, age, body mass index (BMI), waist circumference, sex, ethnicity, low physical activity, smoking, diet including low amount of fiber and high amount of saturated fat, ethnicity, family history of diabetes, history of gestational diabetes mellitus, elevated blood pressure, dyslipidemia, and different drug treatments (diuretics, unselected β-blockers, statins) [1]. Previous studies have often had limitations, including cross-sectional study design, a small sample size, and a sample not representative of the background population.

A recent review covered a total of 86 meta-analyses and Mendelian randomization studies for the risk factors of T2D, including biomarkers, lifestyle and environmental factors, dietary factors, medical history, and psychosocial factors. This review reported that 116 of 142

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associations were statistically significant at the level of $p < 0.05$, and 46 at the levels of $p < 10^{-6}$ [7]. The authors concluded that associations of alanine transaminase, uric acid, vitamin D, whole grains, healthy diet, sugar-sweetened beverages, sedentary lifestyle, preterm birth, metabolically healthy obesity, and conscientiousness had convincing evidence for the risk of T2D (sample size > 1,000 cases, $p < 10^{-6}$).

As an example of large prospective studies is our METabolic Syndrome In Men (METSIM) study including 10,197 men, aged from 45 to 73 years at entry, and randomly selected from the population register of the Kuopio town, Eastern Finland [8], and having a follow-up period of 4.6 years. In this study diabetes diagnosis was based on an oral glucose tolerance test (OGTT) or HbA1c according to the ADA criteria [9]. Obesity (BMI), and distribution of obesity (waist circumference, fat mass) are major risk factors for T2D according to several studies [7]. Recently we showed that short stature also increases the risk of T2D [10].

We have associated several laboratory measurements and biomarkers with the risk of T2D (Table 1). Among all the metabolites measured mannose had the strongest association in the METSIM study with T2D (HR 1.80, 95% CI, 1.32, 2.27) [11]. Mannose is an essential hexose required for glycoprotein synthesis, and it correlates closely with glucose [12], and inversely with insulin sensitivity and insulin secretion. However, the exact mechanisms of how mannose contributes to the risk of T2D remain unknown. We have also shown that fatty acids [13–15], proinsulin [16], inflammatory markers (glycoprotein acetyls, interleukin 1 receptor antagonist, hs-CRP) [17], ketone bodies (acetoacetate) [18], lipids, lipoproteins and apolipoproteins (total triglycerides, apolipoprotein/LDL cholesterol ratio) [19], glycerol [14], non-cholesterol sterols (desmosterol) [20], and amino acids (isoleucine, alanine) are associated with increased risk of T2D [21].

2.2. Metabolomics and novel circulating biomarkers

Metabolomics is a comprehensive characterization of metabolic changes connected to disease development and progression. High sensitivity and resolution of mass spectrometry achieved with liquid or gas chromatography allows the detection and quantification of thousands of metabolites. An alternative method to quantify metabolites is the high-throughput serum nuclear magnetic resonance platform, but the number of metabolites identified using this method is substantially lower compared with mass spectrometry [22].

By using high throughput technologies, metabolomics allows the identification and measurement of metabolites recognizable in a given biological sample. Identification of small biomolecules (metabolites) makes it possible to find early biomarkers for a disease of interest, including T2D and its comorbidities. A recent systematic review and meta-analysis covering the years from 2008 to 2017 included 14 studies and 4,592 individuals with T2D and 11,492 without T2D [23]. Their report noted a 1.89-, 1.63-, and 1.87-fold higher risk of T2D associated for leucine, alanine, and oleic acid, respectively, whereas lysophosphatidylcholine C18:0 and creatinine were associated with 20% and 37% decreased risk of T2D, respectively. Our 4.6-year follow-up study of the METSIM cohort included 5,181 participants having metabolomics data available for twenty amino acids at baseline. Five amino acids (tyrosine, alanine, isoleucine, aspartate and glutamate) were significantly associated with a decrease in insulin secretion and an increased risk of incident T2D after adjustment for confounding factors [24]. All essential amino acids, and especially branch-chain amino acids, stimulate insulin secretion and GLP-1 release [25]. The mechanisms of reduced insulin secretion of five amino acids in our study remains to be determined but could be explained, at least in part, by glucagon regulation [26,27].

Interestingly, a recent study demonstrated a causal relationship between the gut microbiome, short-chain fatty acids and metabolic diseases. The host-genetic-driven increase in gut production of the fecal short-chain fatty acid butyrate was significantly associated with improved insulin response after an OGTT, and another short-chain fatty propionate, was causally related to an increased risk of T2D in the MR. These data provide evidence of a causal effect of the gut microbiome on metabolic traits [28].

The metabolomics approach has limitations in the identification of metabolites for the risk of T2D. There is no consensus on how to standardize metabolomics results, making it difficult to compare the findings across different studies. Additionally, protocols and statistical approaches may differ, and instrumentation can yield varied sets of detectable metabolites [29]. Despite these potential limitations, studies applying metabolomics have the potential to identify a unique set of metabolites predictive of T2D.

Table 1 — Association of different biomarkers with the risk of type 2 diabetes in a 4.6-year follow-up of the METSIM cohort.

Metabolite	HR (95% CI)	p value	Reference
Mannose	1.80 (1.43–2.27)	5.3×10^{-7}	[11]
Dihomo-gamma-linoleic acid	1.53 (1.24–1.87)	5.1×10^{-5}	[13]
Fasting proinsulin	1.38 (1.33–1.43)	1.0×10^{-8}	[16]
Glycoprotein acetyls	1.37 (1.29–1.46)	1.0×10^{-8}	[17]
Acetoacetate	1.37 (1.07–1.80)	2.2×10^{-3}	[18]
Palmitoleic acid	1.35 (1.07–1.69)	1.0×10^{-4}	[15]
Total triglycerides	1.26 (1.11–1.44)	3.9×10^{-4}	[14]
Fasting fatty acids	1.19 (1.10–1.29)	3.0×10^{-5}	[14]
Desmosterol	1.19 (1.05–1.35)	5.0×10^{-3}	[20]
Glycerol	1.18 (1.12–1.24)	5.8×10^{-11}	[14]
Interleukin 1 receptor antagonist	1.18 (1.15–1.22)	1.0×10^{-4}	[17]
ApoB/LDL cholesterol ratio	1.12 (1.07–1.17)	1.0×10^{-4}	[19]
hs-CRP	1.07 (1.04–1.09)	1.0×10^{-4}	[17]
Isoleucine	1.10 (1.05–1.15)	3.3×10^{-5}	[21]
Alanine	1.02 (1.01–1.04)	6.7×10^{-5}	[21]

2.3. Circulating microRNAs

MicroRNAs (miRNAs) are short noncoding RNAs (21–23 nucleotides) that post-transcriptionally regulate gene expression. Multiple miRNAs are dysregulated in diabetes making them potential biomarkers for the risk of T2D [30]. However, conflicting results have been published on miRNAs as biomarkers in the risk of T2D, largely due to different study designs (cross-sectional vs. prospective), small sample size (often < 100), and miRNAs measured in the circulation [31–35]. Further validation studies are needed to identify miRNAs consistently predicting the progression from prediabetes to T2D.

3. GENETIC RISK SCORES

3.1. Cross-sectional studies

Genetic data can be used as a predictive measure of disease susceptibility by aggregating the effects of individual loci into a single genetic risk score (GRS). The first efforts to generate the GRSs for the risk of T2D were based on a small number of genetic variants, and therefore their predictive power remained of little value compared with clinical and laboratory risk factors [36]. We investigated the role of biochemical markers and T2D risk loci in the identification of previously undiagnosed diabetic subjects beyond the Finnish diabetes risk score in a cross-sectional study. The receiver operating characteristics area under the curve (ROC) for the identification of previously undiagnosed participants with T2D with the FINDRISC alone was 0.727, and 0.772

after adding total triglycerides, HDL cholesterol, adiponectin, and ALT in the model. Adding a total of 20 T2D genetic risk variants did not further improve the model [37].

In a systematic review including 34 papers from 30 studies published in 2000–2012, the investigators evaluated improvements in the performance of T2D risk prediction models after adding novel non-genetic or genetic biomarkers to traditional risk factors [38]. Eleven studies reported a modest (ranging from −0.004 to 0.1), but statistically significant change in the ROC curve. The authors concluded that novel circulating and genetic biomarkers did not substantially improve T2D risk prediction above and beyond traditional risk factors.

Currently 403 distinct association signals with T2D were found in the latest genome-wide association studies. Genetic predisposition to T2D risk is mainly explained by common variants of relatively small effect size [4]. These 403 signals captured close to 20% of overall risk of T2D, and the GRSs including ∼130,000 variants explained about 50% in analyses of the UK Biobank data [39]. The value of these new GRSs is currently not obvious for clinical practice but they show that in principle the effect of genetic variants on the risk of T2D is possible to determine.

3.2. Prospective studies

The GRSs have been used to investigate prospectively their joint effects on glycemic traits and the underlying mechanisms leading to hyperglycemia, although the number of genetic variants in these studies have been limited [40–42]. In general these studies showed associations of the GRSs with increased risk of T2D, and fasting glycemia. A Danish study recruiting 5,850 individuals showed that the GRS including 46 genetic variants was associated with a 6% increased risk of T2D, and a decrease in insulin secretion [41]. We generated the GRSs to evaluate changes in insulin secretion (GRS_{IS}), insulin sensitivity (GRS_{IS}), and incident T2D (GRS_{T2D}) in the prospective METSIM study [43]. GRS_{T2D} including 76 SNPs was significantly associated with an increased risk of incident T2D that was higher by twofold in the highest decile compared with the lowest decile. GRS_{IS} was significantly associated with an increase in fasting glucose, a decrease in insulin secretion, and an increased risk of incident T2D during follow-up. These findings emphasise an important role of impaired insulin secretion in the conversion to diabetes. By contrast, the GRS_{IR} did not significantly predict changes in glucose levels, or the conversion to diabetes [43]. A recent 10-year follow-up study in a general Japanese population showed that the GRS based on 84 T2D risk genetic variants predicted an increase in incident T2D, independently of conventional risk factors [44].

3.3. Limitations of the genetic risk scores

The GRSs have, however, limitations because a substantial part of individual predisposition to T2D comes from non-genetic factors (lifestyle, behavior, environment). Therefore, it remains unclear what the clinical utility of the GRSs is. For example, there are differences in ethnic backgrounds in study populations, and there is no consensus on what extent genetic risk simply recaptures information through BMI, family history and ethnic background.

4. MENDELIAN RANDOMIZATION STUDIES

Prospective large population-based studies and their meta-analyses show associations of the biomarkers with the risk for T2D, but they do not prove causality. Only the MR studies can claim causality by using common genetic variants to estimate the contribution of a risk factor to risk of a given disease outcome [45,46]. The basic principle of MR is that the genetic variants do not change over time and that the alleles are randomly allocated. These features of genetic variants help to avoid confounding in MR studies similarly as in randomized clinical trials. Thus, genetic variants are proxy measures for exposures (e.g. clinical traits, biomarkers), and they are considered to be free from confounding and reverse causation.

4.1. Anthropometric characteristics and lifestyle factors (Table 2)

Low birthweight has been associated with a high risk of T2D in epidemiological studies, but the causality of this association remained unclear. The study by Wang and collaborators included 3,627 individuals with and 12,974 without T2D of European ancestry from the Nurses' Health Study and the Health Professionals Follow-Up Study [47]. The GRS including five genetic variants. Low birthweight was associated with a 2.94-fold increased risk for T2D supporting the role of intrauterine exposures in the pathogenesis of T2D.

Several observational follow-up studies have indicated that obesity, estimated by BMI, is a very important risk factor for T2D [48]. Previously published MR studies on the causal role of BMI in the risk for T2D included a limited number of genetic variants. The genome-wide meta-analysis by Corbin and coworkers included 96 genetic variants and data from 12,171 cases with T2D and 56,862 controls of mainly European descent [49]. BMI was causally associated with a 26% increased risk for T2D. Similarly, waist-to-hip ratio adjusted for BMI was causally associated with a 38% increased risk for T2D, confirming previously published epidemiological studies showing that waist-to-hip ratio is an independent risk factor for T2D [50]. In observational studies T2D has been associated with an increased risk of hypertension, and vice versa. An MR study based on the UK Biobank data, including 11,855 individuals with hypertension and T2D as well as 318,664 controls, did not show that hypertension is causally associated with T2D [51]. Similarly, although the majority of studies including several large meta-analyses have indicated that coffee intake reduces the risk of diabetes [52], a MR study including 26,632 cases of diabetes and 171,200 controls did not show, however, a causal association between coffee intake and lower risk of T2D [53].

4.2. Laboratory measurements (Table 2)

Previous MR studies have reported conflicting results on the association of 25 (OH)-D on the risk of T2D, probably due to a too small sample size. Lu and collaborators included 58,000 cases and 370, 000 controls in their study, and reported a 14% decreased risk for T2D, providing support that higher vitamin D status is causally protective of T2D [54].

Several studies have indicated that statin treatment increases the risk of T2D [55]. Statins reduce LDL cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). Swerdlow and coworkers assesses whether this increase in risk is a consequence of inhibition of the *HMGCR* gene [56]. Data were available for up to 223,463 individuals from 43 genetic studies. Two genetic variants were investigated. The association of the rs12916-T allele with T2D was consistent. In 129,170 individuals belonging to randomized clinical trials, statins increased the risk of T2D (OR 1.12, 95% CI 1.06–1.18 in all trials; 1.11, 95% CI 1.03–1.20) in placebo or standard care controlled trials, and 1.12, 95% CI 1.04–1.22 in intensive-dose vs moderate dose trials. The investigators concluded that the risk of T2D attributable to statin treatment is at least partially explained by HMGCR inhibition [56].

Previous studies have suggested that higher concentrations of amino-terminal pro–B-type natriuretic peptide (NT-proBNP) lowers the risk of T2D [57]. A MR meta-analysis included 7,508 cases and 8,572

Table 2 — Mendelian randomization studies on clinical characteristics, behavioral traits and biomarkers and type 2 diabetes.

Exposure	Genetic intrument	Cases/controls	Causal effect size OR (95% CI)	Reference
Low birth weight	GRS	3,627/12,974	2.94 (1.70–5.16)	[47]
Body mass index	GRS	12,171/56,862	1.26 (1.17–1.34)	[49]
Waist/hip ratio	GRS	34,840/149,821	1.82 (1.38–2.42)	[50]
Hypertension	GRS	11,855/318,664	Not significant	[51]
Coffee consumption	GRS	26,632/171,200	Not significant	[53]
Adiponectin	GRS	15,960/64,731	Not significant	[61]
CRP	GRS	6,698/15,872	Not significant	[62]
Vitamin D	GRS	58,000/370,000	0.86 (0.77–0.97)	[54]
IL-1RA	GRS	18,715/61,692	Not significant	[63]
Urate	GRS	26,488/83,964	Not significant	[64]
HDL cholesterol	GRS	2,587/45,040	Not significant	[65]
Total triglycerides	GRS	5,637/6,860	Not significant	[66]
LDL cholesterol	2 variants	14,976/74,395	1.12 (1.06–1.18)	[56]
Natriuretic peptide	1 variant	7,508/8,572	0.82 (0.74–0.90)	[58]
Fetuin	GRS	34,550/66,266	Not significant	[67]
Branch chain amino acids	GRS	47,877/267,694	1.44 (1.26–1.65)	[60]

GRS, Genetic Risk Score; CI, confidence intervals; CRP, C-reactive protein; IL-1RA, Interleukin receptor 1-A antagonist; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

controls from 11 case–control studies, and supported a causal relationship between rs198389 within the NT-proBNP locus and a lower rate of incident T2D (OR = 0.94 per C allele, 95% CI 0.91–0.97). These results provide evidence for a potential causal role of the BNP system in the etiology of T2D [58]. Further studies are needed to investigate the mechanisms underlying this association.

High circulating levels of branched chain amino acids (BCAAs) have been associated with insulin resistance and T2D in previous observational studies [59]. In a GWAS of BCAAs the strongest signal was for leucine, encoding an activator of the mitochondrial branched-chain alpha-ketoacid dehydrogenase (BCKD) responsible for the rate-limiting step in BCAA catabolism [60]. They demonstrated that a genetically predicted difference of 1 SD in amino acid level was associated with an odds ratio for T2D of 1.44 (95% CI 1.26–1.65, $p = 9.5 \times 10^{-8}$) for isoleucine, 1.85 (95% CI 1.41–2.42, $p = 7.3 \times 10^{-6}$) for leucine, and 1.54 (95% CI 1.28–1.84, $p = 4.2 \times 10^{-6}$) for valine. The authors concluded that their findings are consistent with a causal role of BCAAs in the etiology of T2D. However, the mechanisms how amino acids increase the risk of T2D remain unclear. MR studies on adiponectin [61], CRP [62], IL1RA [63], urate [64], HDL cholesterol [65], total triglycerides [66], and Fetuin-A [67] did not demonstrate causal associations with the risk of T2D (Table 2).

4.3. Metabolomics

A Metabolomics approach has been applied to diabetes in several population-based studies in recent years, summarized in [68]. Metabolomics profiling was previously performed typically in a small subset of large populations, and the number of metabolites was limited. In recent studies MR analysis has been combined in metabolomics in order to claim causality of the metabolites found to be associated with the risk of diabetes.

Nowak and collaborators investigated the effects of insulin resistance and insulin secretion on fatty acid levels [69]. The original cohort included 910 elderly men (ULSAM cohort). Insulin sensitivity was determined with gold standard measurement, the hyperinsulinemic euglycemic clamp, and beta-cell function with a Disposition Index during an oral glucose tolerance test. A total of 192 metabolites were measured using untargeted plasma metabolomics by liquid chromatography/mass spectrometry. MR analysis was based on two separate cohorts (PIVUS and TwinGene, n = 2,613) followed by replication in three independent studies profiled on different metabolomics platforms (KORA/TwinsUK, n = 7,824; CHARGE consortium, n = 8,961; and Finnish consortium, n = 8,330). In the observational part of the study the authors reported that bile acid, glycerophospholipid and caffeine metabolism were associated with insulin resistance, and fatty acids biosynthesis markers with impaired insulin secretion. In MR analysis the authors discovered and replicated causal effects of insulin resistance on lower levels of monosaturated fatty acids, palmitoleic acid and oleic acid. Beta-cell function did not have causal effects on any metabolites measured. The limitation of this study is a relatively small size of the ULSAM cohort, and the limited number of metabolites measured.

Liu and collaborators developed a MR approach based on genetic risk scores for metabolite levels utilizing a pathway-based sensitivity analysis to control for nonspecific effects [70]. They focused on 124 metabolites in 2,564 participants, and tested causal effects of each metabolite with glucose and T2D and vice versa. The authors concluded that elevated plasma triglycerides might be partially responsible for the risk of T2D, which is disagreement with previous reports (Table 2). They also claimed that genetic predisposition to T2D associates with increased levels of alanine, decreased levels of phosphatidylcholine alkyl-acyl C42:5, and phosphatidylcholine alkyl-acyl C44:4 [70]. Compared to previously published MR studies their sample size was limited, and therefore the findings need to be replicated in other population-based studies.

Merino and collaborators identified the metabolite profile of individuals with normal fasting glucose who progressed to T2D among 1150 Framingham Heart Study Offspring cohort participants [71]. In adjusted Cox proportional hazard models, the T2D risk per 1 SD increases in glycine and phenylalanine were 0.65 (95% CI 0.54, 0.78) and 1.35 (95% CI 1.11, 1.65), respectively. These results are in agreement with our results from a randomly selected population-based METSIM study [24].

5. MECHANISMS INCREASING THE RISK OF TYPE 2 DIABETES

The main pathophysiological characteristics of T2D are impaired insulin secretion and insulin resistance in muscle and liver. T2D is often preceded by a long period of prediabetes, characterized by insulin resistance and elevation of fasting (impaired fasting glucose) or 2 h glucose (impaired glucose tolerance) in an OGTT [72]. Impairment in

pancreatic beta-cell function occurs early in the natural history of T2D, and diabetes is diagnosed when the pancreas is no longer able to increase insulin secretion to compensate for insulin resistance in peripheral insulin-sensitive tissues. Additional disturbances affecting insulin secretion in T2D are incretin hormone deficiency/resistance in the gastrointestinal tract [73], and hyperglucagonemia [74].

5.1. Insulin secretion

Most of the common genetic variants known to be associated with the risk of T2D affect insulin processing and insulin secretion and only a few affect insulin sensitivity [6]. This supports the notion that the main mechanism for the conversion to T2D is impaired beta-cell function. In spite of that there are no major efforts to find biomarkers reflecting changes in insulin secretion, as a mediator for conversion to diabetes. Insulin resistance has been more on the focus of interest, although insulin sensitivity is more determined by lifestyle (exercise, diet, obesity) and environmental factors than is beta-cell function.

5.2. Insulin resistance

Insulin resistance, a condition in which cells fail to respond normally to insulin, plays an important role in the development of glucose intolerance and T2D. Insulin resistance is tissue specific. Skeletal muscle is a major tissue responsible for glucose uptake in the insulin stimulated state resulting in increased glycogen synthesis. The liver plays an important role in maintaining normal glucose levels by regulating gluconeogenesis and glycogenolysis. Normally, insulin suppresses liver glucose production and inhibits the genes encoding gluconeogenesis. In the fat cell insulin prevents accelerated lipolysis [74].

Insulin resistance has also been in the focus in different non-genetic risk scores aiming to identify individuals at high risk of T2D. The Finnish Diabetes Risk Score is composed of eight easily available parameters (age, BMI, waist circumference, hypertension, physical activity, diet, history of hyperglycemia, and family history of diabetes) [75], and it predicts not only T2D, but also cardiovascular disease and mortality [76]. In the METSIM study, including 8,749 non-diabetic participants, the FINDRISC was significantly associated with decreases in insulin secretion and insulin sensitivity ($p < 0.0001$), and with a 4.14-fold increased risk of incident T2D [77]. These results were unexpected given the fact that practically all questions deal with clinical characteristics related to insulin resistance, but on the other hand are in line with studies emphasizing the role of insulin secretion in the conversion to diabetes.

5.3. Heterogeneity of T2D

T2D is a heterogeneous disease for which disease-causing pathways are incompletely understood. Although impaired insulin secretion and insulin resistance are recognized as two major mechanisms in the pathophysiology of T2D, many mechanisms are still unknown. Unlike other biomarkers, genetic markers do not change with disease progression.

Dimas and collaborators examined the association of 37 established T2D susceptibility loci and indices of proinsulin processing, insulin secretion, and insulin sensitivity in 58,614 nondiabetic subjects [6]. Cluster analysis classified the risk loci into five major categories on the basis of their association with glycemic phenotypes. The first cluster was characterized by the effects of the risk alleles of *PPARG*, *KLF14*, *IRS1*, *GCKR* on insulin sensitivity, the second cluster by the effects of the risk alleles of *MTNR1B* and *GCK* on decreased insulin secretion and fasting hyperglycemia, the third cluster by the effects of the risk alleles of *ARAP1* on insulin processing, the fourth cluster by the effects of the risk alleles of *TCF7L2*, *SLC30A8*, *HHEX/IDE*, *CDKAL1*, *CDKN2A/2B* on insulin processing and secretion without a change in fasting glucose levels, and the fifth cluster including 20 risk loci with no clear-cut associations with glycemic traits [6]. Therefore, the cluster analysis summarized for the first time diverse mechanisms whereby T2D diabetes risk variants impact disease predisposition.

We applied Bayesian nonnegative matrix factorization clustering to identify new pathways driven by 94 T2D genetic variants based on recent GWASs, and 47 diabetes-related traits. We identified five robust clusters of T2D loci and traits [78]. Two of them were related to reduced insulin secretion (beta-cell cluster with high proinsulin levels, proinsulin cluster with low proinsulin levels), and three of them were related to insulin resistance, characterized by obesity (high BMI and waist circumference), "lipodystrophy-like" fat distribution (low BMI, low adiponectin, low HDL cholesterol, high triglycerides), and liver/lipid cluster (low triglycerides). Increased genetic risk scores in these clusters were associated with distinct clinical outcomes, including increased blood pressure, coronary artery disease, and stroke [78]. This approach, a combination of genetic variants and physiologically characterized pathways, demonstrated that cluster analysis is a powerful method in the classification of individuals with T2D into subgroups. Our approach is different from that of Ahlqvist and collaborators [79] where individuals were clustered using six clinical and laboratory parameters, and not on gene variants. Phenotypic data changes with disease progression which is a drawback of this approach. The limitation of our study is that we used a relatively small number of genetic variants given the fact that currently >400 genetic variants have been confirmed to be associated with the risk of T2D [4].

6. FUTURE OF THE BIOMARKER STUDIES

6.1. Precise measurements of insulin secretion and insulin resistance

Previous studies aiming to identify biomarkers for the risk of T2D have weaknesses. Often the diagnosis of diabetes has not been based on the measurement of fasting glucose, 2-hour glucose or HbA1c levels causing underestimation of incident T2D. Secondly, even in the case that new biomarkers have been identified for T2D, it has remained unclear what are the mechanisms leading to the conversion to T2D. Especially important is to focus on impaired beta-cell function because it is the most important mechanism leading to diabetes. Measuring beta-cell function is also less prone to lifestyle and environmental effects compared to studies on insulin sensitivity.

In most of the studies HOMA-IR or HOMA-IS, measures of insulin resistance and insulin secretion, have been used. The limitation of these methods is that fasting measurements do not allow the estimation of glucose-stimulated insulin response. Furthermore, the measurement of beta-cell function should be adjusted for prevailing insulin sensitivity to avoid bias. Measuring insulin sensitivity and beta-cell function reliably is possible if glucose and insulin levels have been measured at least three different time points in an OGTT.

We measured insulin and glucose at 0, 30 and 120 min in the METSIM study which allows the calculation of first phase insulin secretion (insulin AUC, from 0 to 30 min/divided by corresponding glucose AUC, InsAUC30/GluAUC30), and insulin sensitivity using the Matsuda Insulin Sensitivity Index, based on glucose and insulin levels at 0, 30 and 120 min [80]. We found that our measure of beta-cell function had the highest correlation with a gold standard of insulin secretion (insulin secretion during the first 10 min in an intravenous glucose tolerance test) compared to ten other measures of insulin secretion tested (HOMA-beta, Insulinogenic index, InsAUC120/GluAUC120, InsAUC120/GluAUC120, First-phase Stumvoll index, Second-phase Stumvoll index, fasting insulin, insulin at 30 min of an OGTT, insulin at 120 min of an

OGTT, insulin AUC during an OGTT). Similarly, the Matsuda index had the highest correlation among five other indices (1/fasting insulin, 1/HOMA-IR, QUICKI, Matsuda ISI, MCR Stumvoll, ISI Stumvoll) with the M value from the hyperinsulinemic euglycemic clamp [81]. Based on these indices we calculated the Disposition Index (Matsuda ISI x InsAUC30/GluAUC30) which is a measure of beta-cell function adjusted for prevailing insulin sensitivity, and suitable for large population-based studies. Mathematical modeling to determine beta-cell function need multiple measurements in an OGTT, and is not feasible for large scale population-based studies [82,83].

6.2. Towards a precision medicine approach in type 2 diabetes

The precision medicine approach reflects the expectation that a deeper understanding of the genome and phenome improves our diagnostic and prognostic capabilities, and allows the tailoring of the treatment to the individual characteristics of each patient [84]. Application of precision medicine is easier in monogenic diabetes because there are discrete subgroups defined by molecular genetics, in contrast to T2D [85]. T2D is polygenic in which lifestyle and environmental factors play an important role, in addition to genetic predisposition. In monogenic diabetes the first step is to identify a single pathogenic genetic variant which helps to tailor the treatment according to diagnosis. In T2D over 400 genetic variants have been found [4], and each of them has only a moderate or small effect on the risk of T2D. These genetic variants influence multiple processes in tissues and cells, including beta-cells (islet development, islet senescence, islet function), adipocytes, skeletal muscle, liver, and other tissues. Importantly, lifestyle and environmental factors modify the natural course of T2D.

Combination of genetic variants and physiologically characterized pathways improves the classification of individuals with T2D into subgroups which could potentially have different treatments. Recent developments especially in genomics offer a solid ground to improve our understanding of subgroups of T2D. Two previous studies have successfully applied cluster analysis to subdivide individuals with T2D into subgroups characterized by impaired beta-cell function and insulin resistance [6,78]. It is likely that including in the modelling the most recent genomics and extensive phenotyping results we can further improve classification of T2D using $>$400 genetic variants associated with T2D, and more detailed phenotypes now available in the biomarker spectrum.

What are the clinical benefits of understanding the heterogeneity of T2D? With respect to monogenic diabetes subtypes, including MODY, transient and permanent neonatal diabetes, precise diabetes medicine is already now successfully applied [85], but T2D is more complex given the fact that this disease is caused by an interplay of multiple common variants with lifestyle and environmental factors. Therefore, it is not likely that new developments in the understanding of the heterogeneity of T2D will change the treatment options in near future because currently available drugs already cover the major pathophysiological disturbances is T2D, impaired insulin secretion and insulin action. However, efforts should be continued to obtain a better understanding of the major pathophysiological mechanisms and processes in T2D to improve treatment capabilities for patients with T2D.

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CONFLICT OF INTEREST

None.

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