Aus dem Deutschen Diabetes-Zentrum, Leibniz-Zentrum für Diabetesforschung
an der Heinrich-Heine-Universität Düsseldorf

Direktor: Univ. Prof. Dr. Michael Roden

Diabetes subphenotypes and their impact on precision medicine in type 1 and type 2 diabetes

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Oana Patricia Zaharia

(2020)

Als Inauguraldissertation gedruckt mit Genehmigung der Medizinischen Fakultät der Heinrich-Heine-Universität

gez.:

Dekan: Univ. Prof. Dr. Nikolaj Klöcker

Erstgutachter: Univ. Prof. Dr. Michael Roden

Zweitgutachter: PD. Dr. med. Ralf Westenfeld

Drittgutachter: Prof. Dr. med. Dirk Müller-Wieland

Widmung

Meinen Eltern

Teile dieser Arbeit wurden veröffentlicht:

Zaharia OP, Bobrov P, Strassburger K, Bodis K, Karusheva Y, Scholz M, Markgraf DF, Burkart V, Schloot NC, Mussig K, Szendroedi J, Roden M: Metabolic characteristics of recently diagnosed adult-onset autoimmune diabetes mellitus. *J Clin Endocrinol Metab* 2018; 103:429-437

Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, Bodis K, Markgraf DF, Burkart V, Mussig K, Hwang JH, Asplund O, Groop L, Ahlqvist E, Seissler J, Nawroth P, Kopf S, Schmid SM, Stumvoll M, Pfeiffer AFH, Kabisch S, Tselmin S, Haring HU, Ziegler D, Kuss O, Szendroedi J, Roden M: Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *Lancet Diabetes Endocrinol* 2019; 7:684-694

Summary

Currently, the main forms of diabetes mellitus, type 1 and type 2 diabetes, are defined by basic pathophysiological differences, which do not address the wide range of clinical presentations of diabetes. This broad clinical and metabolic heterogeneity along with the diverse pathophysiological alterations may also require distinct strategies for prevention and treatment. Therefore, recent studies aimed at identifying simple clinical tools to allow for a new classification of patients with diabetes and facilitate a more precise diagnosis and tailored treatment.

The aim of this thesis was to detect subphenotypes of patients with type 1 and type 2 diabetes with distinct metabolic features based on different pathophysiological mechanisms and to examine possible differences in the risk of or presence of comorbidities and complications. The studies were based on the prospective observational German Diabetes Study (GDS), which includes patients with recent-onset diabetes mellitus. The first study examined the features of autoimmune diabetes and its formerly proposed subgroup, “latent autoimmune diabetes of the adult (LADA)”, and revealed new subphenotypes with distinct metabolic profiles. The second study applied a clustering algorithm based on clinical data on patients with diabetes at diagnosis and after five years.

These studies show that patients with diabetes can be allocated to specific subphenotypes (clusters), which exhibit distinct metabolic alterations and different risk patterns at the time of diagnosis for the development of diabetes-related comorbidities and complications. Specifically, these studies showed that persons with autoimmune diabetes may also present with features of insulin resistance, whereas those without autoimmune diabetes but severe insulin resistance are at higher risk of diabetes-related nephropathy and non-alcoholic fatty liver disease.

These results will improve the understanding of disease heterogeneity and the identification of specific groups with distinct risk and progression of diabetes. Thereby, these studies introduce the concept of precision medicine to diabetes mellitus, which aims at tailored prevention and treatment of groups of individuals with specific genetic, molecular or metabolic features.

Zusammenfassung

Gegenwärtig sind die Hauptformen von Diabetes mellitus, Typ-1- und Typ-2-Diabetes, durch grundlegende pathophysiologische Unterschiede definiert, die es nicht ermöglichen, die große Vielfalt der klinischen Manifestationen von Diabetes zu adressieren. Diese hohe klinische und metabolische Heterogenität sowie die vielfältigen pathophysiologischen Veränderungen erfordern jedoch unterschiedliche Vorbeugungs- und Behandlungsstrategien. Aktuelle Studien versuchten deshalb, einfache klinische Instrumente zu identifizieren, die eine Neuklassifizierung von Patienten mit Diabetes erlaubt, um eine genauere Diagnose und maßgeschneiderte Behandlung zu ermöglichen. Ziel dieser Arbeit war es, Subphänotypen von Patienten mit Typ 1- und Typ-2-Diabetes mit distinkten Stoffwechselmerkmalen basierend auf unterschiedlichen patho-physiologischen Mechanismen zu identifizieren und mögliche Unterschiede im Risiko oder der Prävalenz von Komorbiditäten und Komplikationen zu untersuchen. Die Studien stützten sich auf die prospektive Beobachtungsstudie Deutsche Diabetes-Studie (GDS), die Patienten mit neu-manifestiertem Diabetes mellitus einschließt. Die erste Studie untersuchte die Eigenschaften des Autoimmundiabetes und seiner früher vorgeschlagenen Untergruppe „latenter Autoimmundiabetes bei Erwachsenen (LADA)“ und ergab neue Subphänotypen mit unterschiedlichen Stoffwechselprofilen. In der zweiten Studie wurde ein Clustering-Algorithmus, der auf einfachen klinischen Daten basiert, auf Patienten mit Diabetes zum Zeitpunkt der Diagnose und nach fünf Jahren angewendet.

Diese Studien zeigen, dass Patienten mit Diabetes bestimmten Subphänotypen (Clustern) zugeordnet werden können. Diese Subphänotypen weisen unterschiedliche metabolische Veränderungen zum Zeitpunkt der Diagnose und unterschiedliche Risikomuster für die Entwicklung von diabetes-assoziierte Komorbiditäten und Komplikationen auf. Insbesondere können Patienten mit Autoimmundiabetes auch Merkmale einer Insulinresistenz aufweisen, wohingegen Patienten ohne Autoimmundiabetes, aber mit schwerer Insulinresistenz ein höheres Risiko für diabetische Nephropathie und nichtalkoholische Fettlebererkrankung aufweisen können.

Diese Ergebnisse werden das Verständnis der Heterogenität des Diabetes und die Identifizierung von Gruppen mit spezifischem Risiko der Diabetesprogression verbessern. Damit führen diese Studien das Konzept der Präzisionsmedizin bei Diabetes mellitus ein, welches auf die maßgeschneiderte Prävention und Behandlung von Personengruppen mit spezifischen genetischen, molekularen oder metabolischen Merkmalen abzielt.

List of abbreviations

ADA	American Diabetes Association
APRI	Aspartate Aminotransferase to Platelet Ratio Index
BMI	Body Mass Index
CAN	Cardiovascular Autonomous Neuropathy
CART	Cardiovascular Autonomic Reflex Tests
DSPN	Diabetic Sensorimotor Polyneuropathy
eGFR	Estimated Glomerular Filtration Rate
FFA	Free Fatty Acids
FLI	Fatty Liver Index
GAD	Glutamic-Acid-Decarboxylase
GDS	German Diabetes Study
HbA1c	Glycated Hemoglobin
HCC	Hepatocellular Carcinoma
HDL	High-Density Lipoprotein
HOMA-B	Homeostatic Model Assessment of Beta-Cell Function
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HRV	Heart Rate Variability
hsCRP	High Sensitivity C Reactive Protein
ICA	Islet Cell Autoantibodies
IDDM	Insulin Dependent Diabetes Mellitus
IVGTT	Intravenous Glucose Tolerance Test
LADA	Latent Autoimmune Diabetes of the Adult
LDL	Low-Density Lipoprotein
MARD	Mild Age-Related Diabetes
MOD	Mild Obesity-Related Diabetes
MODY	Maturity-Onset Diabetes of the Young
MRS	Magnetic Resonance Spectroscopy
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NCV	Nerve Conduction Velocity
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
SAID	Severe Autoimmune Diabetes
SIDD	Severe Insulin Deficient Diabetes
SIRD	Severe Insulin Resistant Diabetes
SNAP	Sensory Nerve Action Potentials
WHO	World Health Organisation

Index of contents

1. Introduction

1.1. History of diabetes classification

Historically, the diagnosis of diabetes was based on clinical appearance, signs and symptoms, rather than the underlying pathophysiological mechanism (1). Medical writings of ancient civilizations describing an ailment of polyuria and painless emaciation are attributed to diabetes. The term 'mellitus' was added in the 18th century to differentiate from diabetes 'insipidus' (1). This contrast in nomenclature was reflecting the biochemical differences of glucose content in diabetes mellitus as opposed to the diluted urine in diabetes insipidus. As the cause of diabetes was not known at that point, the disease was generally attributed to kidney dysfunction.

A set of experiments initiated in the late 18th century deepened the understanding of the basic metabolic principles of human physiology and had far-reaching consequences for medicine and for diabetes in particular. The French physicians, Bouchardat and Lancereaux, were the first to separate 'diabete gras' (fat diabetes) from 'diabete maigre' (lean diabetes), laying the foundation for the classification in different types of diabetes (2). It was in the 19th century when the function of glands and hormones was more accurately identified and the cause of diabetes was linked to pancreatic dysfunction. However, most publications still considered diabetes only an accompanying phenomenon of other diseases, such as infections(3), while Naunyn was the only to suggest 'true diabetes' to be an independent entity in 1906 (4). Going forward different classifications of diabetes were discussed.

At the beginning of the 20th century, Sir Harold Himsworth (5) followed up the work of Wilhelm Falta in Vienna, who had attempted to correlate glucose tolerance with insulin sensitivity (6). As a result, a standardized insulin–glucose tolerance test was devised, which became the forerunner of modern glucose–insulin clamp techniques and was used to distinguish between "insulin-sensitive" and "insulin-insensitive" types of diabetes. "Insulin-sensitive" patients did not differ from metabolically healthy controls in their sensitivity to insulin and were susceptible to develop ketoacidosis, whereas "insulin-insensitive" patients did not. From this it was inferred that "insulin-sensitive" patients lacked the capacity to produce insulin while, on the other hand, "insulin-insensitive" patients could not respond to insulin. This observation established the scientific basis for the subsequent distinction between type 1 and type 2 diabetes, and the subsequent hallmark of insulin resistance for type 2 diabetes.

In 1917, Boston scientist Elliott Joslin established himself as one of the world's leading diabetes experts by his textbook on “The Treatment of Diabetes Mellitus” (7), which reported that a fasting diet combined with regular exercise could significantly reduce the risk of death in diabetes patients. In 1949, Priscilla White, a collaborator of Joslin and founding member of the Joslin Clinic, introduced the White Classification of Diabetic Pregnancies (8). This pioneering concept classifies pregnant patients with diabetes according to their level of risk and tailors their treatment protocol accordingly. However, the term ‘gestational diabetes’ was introduced in 1964 by O’Sullivan (1).

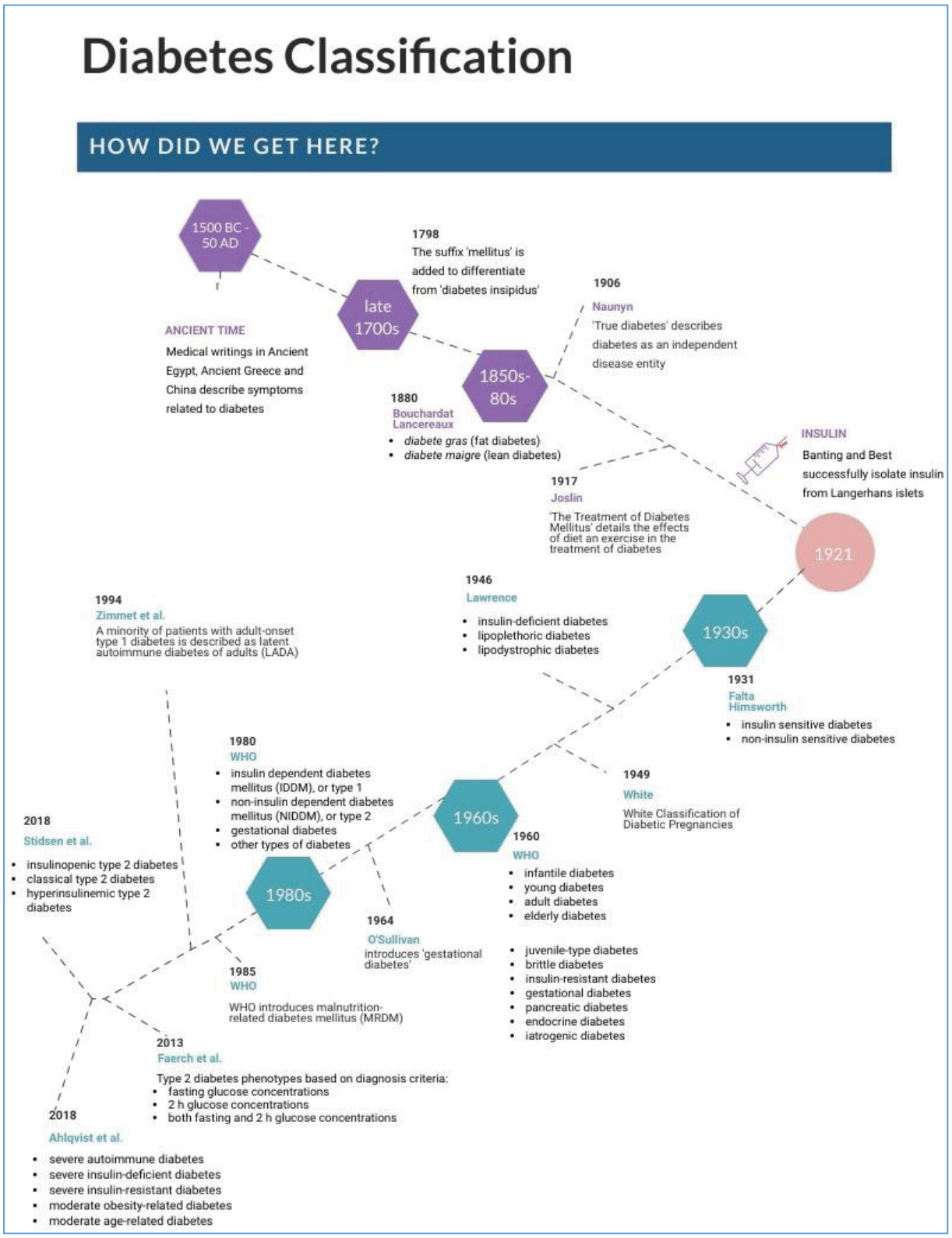
Robert Lawrence, founder of the British Diabetic Association, provided one of the earliest descriptions and detailed study of the rare condition now known as lipodystrophic diabetes in 1946 (9). He categorized diabetes in insulin-deficient, lipoplethoric and lipodystrophic. While the first two groups mimic previous descriptions of ‘diabete gras’, ‘asthenic diabetes’ or ‘juvenile-onset diabetes’ or ‘diabete maigre’, ‘sthenic diabetes’ or ‘maturity-onset diabetes’, respectively, the third group seemed to have a different, previously neglected, metabolic background.

The World Health Organization (WHO) published its first classification system for diabetes in 1965 using four categories based on age of diagnosis: infantile or childhood (with onset between the ages of 0–14); young (with onset between the ages of 15–24 years); adult (with onset between the ages of 25–64 years); and elderly (with onset at the age of 65 years or older). In addition to classifying diabetes by age, WHO recognized other forms of diabetes: juvenile-type; brittle; insulin-resistant; gestational; pancreatic; endocrine and iatrogenic (10). In 1979, the National Diabetes Data Group produced a consensus document standardizing the nomenclature and definitions for diabetes mellitus. This document was endorsed one year later by WHO, who published its first widely accepted and globally adopted classification of diabetes in 1980 and an updated version of this in 1985. These classifications included two major classes of diabetes: insulin dependent diabetes mellitus (IDDM), or type 1; and non-insulin dependent diabetes mellitus (NIDDM), or type 2. The 1985 report omitted the terms “type 1” and “type 2”, but retained the classes IDDM and NIDDM, and introduced a class of malnutrition-related diabetes mellitus. Both the 1980 and 1985 reports included two other classes of diabetes: “other types” and “gestational diabetes mellitus” (10).

In 1997, an international expert committee released a report with new recommendations for the classification and diagnosis of diabetes mellitus (11). The use of classification systems and standardized diagnostic criteria facilitates a common language among

patients, physicians, other health care professionals and scientists. A timeline of the diabetes classification is presented in Fig. 1.

Figure 1: Timeline of diabetes classification



1.2. Current diabetes classification

Diabetes mellitus is a prevalent disease characterized by chronic hyperglycemia. According to ADA guidelines (12) there are four diagnostic criteria for diabetes mellitus: fasting plasma glucose, 2-hour plasma glucose in 75 g oral glucose tolerance test (OGTT), HbA1c and random plasma glucose with reference values shown in Table 1.

Table 1: Criteria for diagnosis of diabetes according to the American Diabetes Association

Diagnostic criteria	**Diabetes mellitus**
HbA1c*#	≥ 6.5 % (48 mmol/mol)
or	
Fasting plasma glucose#**	≥126 mg/dl (7.0 mmol/l)
or	
2-h plasma glucose during an OGTT*#**	≥ 200 mg/dl (11.1 mmol/l)
or	
Random plasma glucose	≥ 200 mg/dl (11.1 mmol/l) and classical symptoms of hyperglycemia

*Table 1 legends: * The test should be performed in a laboratory using a method that is certified according to the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial assay (DCCT); ** No caloric intake for at least 8 hours; *** The test should be performed using a glucose load equivalent of 75 g anhydrous glucose dissolved in water; # In the absence of hyperglycemia symptoms, the test results should be confirmed by repeated measurement. OGTT, oral glucose tolerance test; h, hours; HbA1c, glycated hemoglobin A1c.*

Based on pathophysiological and clinical criteria, the American Diabetes Association (ADA) classifies diabetes into the following general categories (12):

- *Type 1 diabetes* which is considered to be due to autoimmune beta-cell destruction, usually leading to absolute insulin deficiency
- *Type 2 diabetes* which is considered to be due to a progressive loss of beta-cell insulin secretion frequently in the context of insulin resistance

- *Gestational diabetes mellitus* characterized by diagnosis in the second or third trimester of pregnancy with no overt diabetes prior to gestation and which enters remission after delivery
- *Specific types of diabetes due to other causes*, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas, and drug- or chemical-induced diabetes

In contrast, the WHO does not consider HbA1c a suitable diagnostic test for diabetes. The following Table 2 summarizes the 2006 WHO recommendations (10) for the diagnostic criteria for diabetes. It should be noted, however, that the cut off levels for diagnosing diabetes do not differ between the guidelines.

Table 2: Criteria for diagnosis of diabetes according to the World Health Organization

Diagnostic criteria	**Diabetes mellitus**
Fasting plasma glucose	≥126 mg/dl (7.0 mmol/l)
or	
2-h plasma glucose during an OGTT*#	≥ 200 mg/dl (11.1 mmol/l)

*Table 2 legends: * The test should be performed using a glucose load equivalent of 75 g anhydrous glucose dissolved in water; # If 2–h plasma glucose is not measured, status is uncertain as diabetes or impaired glucose tolerance cannot be excluded.*

The WHO acknowledges the limitations with the data from which the diagnostic criteria for diabetes are derived. However, the current criteria distinguish a group with significantly increased premature mortality and increased risk of microvascular and cardiovascular complications.

Similarly, the European guidelines (13) refer to both ADA and WHO criteria. Although the WHO and ADA diagnostic criteria are clear, there are practical considerations when choosing a method to diagnose diabetes. Limitations with HbA1c have to be considered, such as interference as a result of hemoglobin variants, anemia, and availability. Overall, European guidelines (13) recommend that diagnosis of diabetes is based on HbA1c or

fasting plasma glucose, and on OGTT if still in doubt. Repeated testing is advisable to confirm the diagnosis. In patients with cardiovascular disease, the recommended methods are essentially the same: glycemic control testing with HbA1c and/or fasting plasma glucose first, and if inconclusive, an OGTT, which is the employed specifically as means of diagnosing impaired glucose tolerance.

Furthermore, guidelines consistently refer to type 1 and type 2 diabetes as the main forms of diabetes, distinct from gestational diabetes, and other specific forms due to genetic, pancreatic surgery or other causes. Type 1 and type 2 diabetes are therefore the most prevalent forms of diabetes, but both may be heterogeneous diseases themselves with considerable variation in clinical presentation and disease progression. A precise diabetes classification is important for determining the adequate treatment with primary focus on lowering high blood glucose levels and preventing diabetes-specific complications. The following section will focus on the main clinical and metabolic traits described for each of the main diabetes types: type 1 and type 2 diabetes. The exclusion of other types of diabetes from the following analyses is reinforced by the already clearly established etiology of the specific types of diabetes, which require unique treatment or elimination of the underlying cause.

1.3. Clinical and metabolic traits of new-onset type 1 diabetes

Type 1 diabetes is characterized by deficiency of insulin due to destruction of pancreatic beta-cells, progressing to absolute insulin deficiency (14). This form, previously called IDDM or “juvenile-onset diabetes,” accounts for 5–10% of diabetes and is due to cellular-mediated autoimmune destruction of the pancreatic beta-cells.

Type 1 diabetes is characterized by the presence of one or more beta-cell directed autoantibodies (15). Autoimmune markers include cytoplasmic islet cell autoantibodies (ICA) and autoantibodies to glutamic-acid-decarboxylase (GAD or GADA), insulin, the tyrosine phosphatases IA-2 and IA-2b (IAA), and zinc transporter protein ZnT8. Auto-antibodies targeting pancreatic beta-cells are the diagnostic markers of type 1 diabetes (15), although they are not detectable in all patients and decrease with age. The disease has strong HLA associations, with linkage to the DQA and DQB genes which can be either predisposing or protective (16). Compared with other ethnicities type 1 diabetes is more common in Caucasian individuals (17). Studies have shown that Caucasian individuals were more likely to have multiple positive autoantibodies when compared with other ethnicities (18). The higher incidence of islet autoimmunity observed in

Caucasian individuals could be due to the different distribution of type 1 diabetes-associated HLA genotypes, with increased frequency of susceptibility and decreased frequency of protective types when compared with other ethnicities (19). People who have auto-antibodies to pancreatic beta-cell proteins are likely to develop either acute-onset or slowly progressive insulin dependence.

Already in 1977, Irvine (20) suggested subtypes of type 1 diabetes depending on their symptom severity and clinical outcome. The Japan Diabetes Society divides type 1 diabetes into three categories depending on the manner of onset and progression, namely fulminant, acute-onset, or slowly progressive type 1 diabetes (21). Among the patients who develop ketosis or diabetic ketoacidosis within 3 months after the onset of hyperglycemic symptoms and require insulin treatment continuously after the diagnosis of diabetes, those with anti-islet autoantibodies are considered to have acute-onset autoimmune type 1 diabetes mellitus. In contrast, those whose endogenous insulin secretion is depleted (preserved fasting serum C-peptide) without measurable beta-cell autoantibodies are diagnosed with acute-onset type 1 diabetes mellitus (20; 21).

It is likely that the proposed subgroups, even though described in different populations and presented by different nomenclature, represent overarching groups with similar features and can therefore be generalized.

The fundamental model of type 1 diabetes natural disease progression is based on a 1986 article by George Eisenbarth (22). This paper suggested 6 stages of developing type 1 diabetes starting with genetic susceptibility, which possibly triggered by a precipitating event leads to overt immunologic abnormalities. This is followed by a progressive loss of insulin release, which can for a short period of time still be counteracted through lifestyle changes. Eventually the compensatory mechanisms are overloaded, the insulin release can no longer fulfil the metabolic needs of the organism, glucose accumulates in the blood stream and triggers overt diabetes. Ultimately there will be no detectable C-peptide levels, indicative of beta-cell depletion. Although the model still holds to a large extent, there are subtleties that suggest it is not as simple as originally proposed. It is clear that the rate of decline of beta-cell function and the disease process evolves at different rates in each patient.

Some forms of type 1 diabetes present with permanent insulinopenia and are prone to ketoacidosis, but have no evidence of beta-cell autoimmunity. Although only a minority of patients with type 1 diabetes, the incidence appears to be higher in individuals of African or Asian ancestry, who display episodic ketoacidosis and exhibit varying degrees

of insulin deficiency between episodes (19). This form of diabetes is strongly inherited and is not HLA associated (19). The requirement for insulin replacement in affected patients may be intermittent, distinguishing them from the typical clinical presentation of type 1 diabetes.

Typically, type 1 diabetes occurs in younger individuals (23) presenting with polyuria, polydipsia and weight loss. However, type 1 diabetes may occur at any age, sometimes with slow progression. Patients with type 1 diabetes may present with ketoacidosis as the first manifestation of the disease. The rate of beta-cell destruction is quite variable, being rapid in some individuals and slow in others.

Type 1 diabetes is caused by autoimmune destruction of beta-cells, leading to absolute insulin deficiency during its natural history. For this reason, type 1 diabetes has been traditionally associated, with a leaner phenotype and absence of other cardiovascular risk factors upon diagnosis. However, obesity is often recognized in individuals with type 1 diabetes already at diagnosis, owing partly to its rising incidence in the general population. Not only is obesity compatible with a type 1 diabetes diagnosis, but it is also potentially a risk factor for its development. Furthermore, the cardiovascular risk of type 1 diabetes, as often underestimated, leads to numerous untreated patients, which in turn increases the risk of developing severe cardiac events up to 90-fold in certain groups (24).

Although impaired insulin action along with inadequate beta-cell function and insulin secretion are the primordial markers, patients with type 1 diabetes can also exhibit insulin resistance (25). In particular, insulin resistance has been observed in patients with poorly controlled and long standing type 1 diabetes (26-28). This has previously been attributed to glucotoxicity mechanisms (29) and more recent studies revealed a complex nature of insulin resistance in type 1 diabetes, a condition that has been termed “double diabetes” (30). Double-diabetes was a term coined to describe individuals with type 1 diabetes showing clinical features compatible with type 2 diabetes (31). It has been variably used in literature, to describe both individuals with obesity and other insulin resistance characteristics since diagnosis and those who have gained weight during follow-up, becoming obese over time. Predominant metabolic dysregulation and less dominant autoimmune aberration is seen in these patients, even though they are autoantibody positive. The definition of an intermediate subtype between both types of diabetes has been difficult and suggested a broad grey zone reflecting a continuum of diabetes rather than distinct phenotypes. Genetic factors have been also proposed to

contribute to insulin resistance as suggested by associations with several genes linked to high risk of type 2 diabetes (32). Whether insulin sensitivity is principally impaired in certain subgroups of newly-diagnosed adult type 1 diabetes patients, remains unknown.

1.3.1. The so-called ‘latent autoimmune diabetes of adults’: a controversial subtype

The presence of a distinct subtype, termed “latent autoimmune diabetes” has been introduced by Zimmet et al. (33) and is controversially discussed since then (34; 35). Some patients with adult-onset diabetes mellitus present with positive diabetes-associated antibodies, mainly antibodies against GAD and ICA (36-38). Autoimmunity typically defines type 1 diabetes, yet there has been an attempted separation of a subgroup as latent autoimmune diabetes of the adult (LADA) (39; 40), 1.5 diabetes (34) or non-insulin requiring autoimmune diabetes (NIRAD) (41), based on the following criteria: positivity for diabetes-associated autoantibodies, age at diagnosis older than 30 years and no insulin therapy during the first 6–12 months after diagnosis. According to current estimates, up to 10% of all patients with type 2 diabetes meet the criteria of LADA (40). European and American guidelines no longer define LADA as an independent diabetes type, but classify these patients as having type 1 diabetes (14; 42).

Autoantibody positivity in patients with diabetes is associated with younger age at onset, less secretion of insulin, and faster progression to insulin dependency than for antibody-negative patients (43). Of note, the metabolic phenotype might be determined by the antibody titer. LADA patients with high GAD antibody titer are phenotypically closer to type 1 diabetes showing lower BMI while those with low GAD antibody titer rather resemble patients with type 2 diabetes (44). There is no distinct appearance for patients with LADA, reflected by the wide heterogeneity in the clinical presentation at disease onset. These patients may present with some clinical characteristics of type 2 diabetes (45) and are preferentially treated with oral glucose-lowering medication or incretins, as insulin is not required during the first years upon diagnosis (46). Nevertheless, there is a paucity of data with regard to metabolic characteristics of this specific subgroup of patients with autoimmune diabetes.

1.4. Clinical and metabolic traits of new-onset type 2 diabetes

Type 2 diabetes is characterized by a combination of insulin resistance and beta-cell failure, in association with obesity and sedentary lifestyle. Type 2 diabetes, previously referred to as “noninsulin-dependent diabetes” or “adult-onset diabetes,” accounts for 90–95% of all diabetes. Insulin resistance and an impaired first-phase insulin secretion causing postprandial hyperglycemia characterize the early stage of type 2 diabetes. This is followed by a deteriorating second-phase insulin response and persistent hyperglycemia in the fasting state. This diabetes form encompasses individuals who have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance.

Most, but not all, patients with type 2 diabetes are overweight or obese. Excess weight is associated with insulin resistance. Patients who are not obese or overweight by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (47). Based on previous studies, it is safe to assume that at least part of the differences in insulin sensitivity observed between the prediabetic and diabetic individuals can be attributed differences in obesity and mainly abdominal fat (48). Differences in insulin sensitivity between groups were largely explained by differences in overall and particularly abdominal visceral obesity indicative of a close link between fat depots in the abdomen and glucose regulation in the fasting state, potentially mediated by adipokines (49). Furthermore, with increasing obesity in the young there is a trend towards a decreasing age of onset. The epidemic of lack of exercise, of obesity together with dietary factors is bound to produce an insulin-resistant phenotype even in the young, that may accelerate the development of renal pathology and cardiovascular disease (50).

Various mechanisms have been proposed and discussed to cause type 2 diabetes. Insulin resistance and impaired insulin secretion remain the core defects in type 2 diabetes, but other pathophysiological abnormalities contribute to the dysregulation of glucose metabolism. Impaired insulin secretion in type 2 diabetes is caused by pancreatic beta-cell dysfunction due to lipotoxicity, glucotoxicity and resistance to incretins. Beta-cell dysfunction occurs following increased FFA levels, obesity, insulin resistance, and inflammation (51). Initially the beta-cells compensate by increasing the release of insulin; however, over time this compensatory mechanism fails and leads to reduction in beta-cell mass. The loss of beta-cell mass occurs from cellular

degranulation, resulting in an increase in glucagon from α-cells and decreased insulin secretion. The reduced plasma insulin levels trigger an increase in glucose levels. Glucose-sensitive tissues, including skeletal muscle and adipocytes, are unable to accommodate the hyperglycemic milieu. Increased fat accumulation in adipocytes also leads to an increased release of proinflammatory cytokines and increased lipolysis. A further release of FFAs stimulates the liver to increase glucose production. Persistent glucose release preserves the hyperglycemic environment sustaining the vicious cycle. Ultimately, insulin resistance is the result of impaired insulin receptor signaling. Causes of the insulin resistance include genetic abnormalities, ectopic lipid accumulation, mitochondrial dysfunction, inflammation and endoplasmic reticulum stress (52). Environmental and lifestyle factors such as obesity, an unhealthy diet and physical inactivity and genetic factors contribute to the multiple pathophysiological disturbances that are responsible for impaired glucose homeostasis in type 2 diabetes. Furthermore, studies suggest that type 2 diabetes can be subtly modulated by common variants acting through any one of several distinct mechanisms by the wide range of therapeutic interventions that have the proven capacity to ameliorate, to some degree at least, the diabetic state such as calorie restriction, exercise, and metabolic surgery (53).

Peripheral organs such as the liver, muscle and kidney become insulin resistant, leading to reduced glucose uptake from blood, excessive renal glucose reabsorption and increased gluconeogenesis, all of which contribute to hyperglycemia. Individuals with type 2 diabetes are at high risk for both microvascular complications such as retinopathy, nephropathy and neuropathy and macrovascular complications such as cardiovascular disease, associated with hyperglycemia and insulin resistance. The severity and duration of hyperglycemia determine the risk of microvascular complications. Macrovascular complications are frequently augmented by dyslipidemia, hypertension, hyperglycemia and inflammation. There remains, however, a paucity of data regarding the prevalence or susceptibility to diabetes complications in specific subgroups of type 2 diabetes patients.

The multiple pathogenic disturbances present in type 2 diabetes are currently not reflected in the therapeutic guidelines, which propose a first-line treatment with metformin in patients with type 2 diabetes (54), independent of the underlying metabolic dysfunction. Until recently, most management algorithms proposed stepwise treatment escalation upon failure with oral blood glucose-lowering drugs leading to insulin treatment (55). Patients treated with metformin monotherapy tend to progressively

deteriorate in their glycemic control over time, requiring further therapeutic steps to improve the sustainability of glycemic control (56). There are also reports suggesting that early intensive glycemic control could lead to diabetes remission (57). Given the plethora of medications, the challenge lies in matching the right drug with the right patient at the right time to obtain the best clinical outcome (58).

Nevertheless, the complex metabolic interplay dictates that multiple antidiabetic agents, used in combination, will be required to maintain normoglycemia, especially in patients who present with severe dysglycemia at diagnosis (59). The treatment must not only be effective and safe but also improve the quality of life. Several novel medications are in development, but the greatest need is for agents that enhance insulin sensitivity, halt the progressive pancreatic beta-cell failure and prevent or reverse the micro- and macrovascular complications (60).

1.5. New classification of type 2 diabetes

Due to the large heterogeneity in the clinical presentation as well as the complex and diverse pathophysiological disease mechanism, it does not seem to be accurate to classify patients with type 2 diabetes as one sole entity, as they present with different metabolic features and require distinct therapeutic and prevention strategies. Therefore, recent studies have attempted to restructure the classification of patients with diabetes and identify novel clinical tools in order to facilitate a more precise clinical diagnosis and tailored therapy. There are currently many research groups advocating for individualized therapeutic approach and customized therapy that have identified specific patient subgroups that require special consideration (61-63). Treatment should ideally target the underlying pathophysiological defect, and identification of subphenotypes enables the clinician to choose the most relevant treatment.

1.5.1. Cluster analysis

A recent Swedish cohort study challenged the current paradigm of classifying diabetes patients by allocating adult-onset diabetes into five clusters on the basis of different pathophysiological and genetic profiles (12; 64). This analysis was based on an unbiased cluster allocation using common variables such as autoimmunity, age at diagnosis, body mass index (BMI), glycemic control, surrogates of beta-cell function, and insulin resistance using simple homeostasis model estimates (HOMA-B and HOMA-IR) (65). The k-means clustering via nearest centroid approach (64), assigned

each patient to a predefined cluster: severe insulin-resistant (SIRD), severe insulin-deficient diabetes (SIDD), moderate age-related diabetes (MARD) or moderate obesity-related (MOD). Severe autoimmune diabetes (SAID) encompassed patients with positive GAD antibodies (64). The centroides were determined from sex-specific mean values derived from population-based data. Table 3 summarizes the main anthropometric and clinical characteristics of the clusters as described in the Swedish population by Ahlqvist et al. (64).

Table 3. Cluster classification

	Cluster Name	**N (%)**	**Anthropometric and metabolic characteristics**	**Specific susceptibility to complications**
1	Severe autoimmune diabetes (SAID)	6	young age, low BMI, poor metabolic control, insulin deficiency, GAD autoimmunity	
2	Severe insulin-deficient diabetes (SIDD)	18	young age, low BMI, poor metabolic control, insulin deficiency, no GAD autoimmunity	Retinopathy
3	Severe insulin-resistant diabetes (SIRD)	15	high BMI, overt insulin resistance	Nephropathy Indirect signs of susceptibility to NAFLD (increased transaminases)
4	Moderate obesity-related diabetes (MOD)	22	younger age, obesity, not insulin resistant	
5	Moderate age-related diabetes (MARD)	39	older age, modest metabolic alterations	

Table 3 legends: BMI, body mass index, GAD, glutamic-acid-decarboxylase, NAFLD, non-alcoholic fatty liver disease.

While there is already some evidence for an association between cluster assignment and risk for nephropathy and cardiovascular diseases (64), risk stratification for diabetic neuropathy and non-alcoholic fatty liver disease (NAFLD) using appropriate measurements has not been addressed so far. NAFLD is frequently present at or even before the onset of diabetes and emerged not only as a major risk factor of end-stage liver disease, but also as a predictor of cardiovascular disease (66; 67). Of note, the risk for liver-related mortality increases exponentially with increase in fibrosis stage (68).

This innovative approach aimed at developing stratified customized treatment in line with the concept of precision medicine (63; 69). Such stratification may have important implications not only for the diagnosis and management of diabetes but also for predicting diabetes-related comorbidities and the risk for diabetes complications. Therefore, we aim to address further implications of risk stratification via clustering reaching towards less investigated areas of diabetes complications and its application in the German population.

1.5.2. Stratification based on glycemic control, insulin sensitivity and insulin secretion

Other research groups have attempted different further classifications of diabetes. Based on the ADA diagnostic criteria of diabetes (Table 1), a British study subdivided patients with type 2 diabetes into subgroups (70). Subsequently patients with type 2 diabetes were subdivided into 3 groups: F-DM phenotype for patients fulfilling the fasting plasma glucose criterion, 2h-DM phenotype for patients with plasma glucose 2hours after OGTT above 200 mg/dl, and F-2h-DM phenotype for patients fulfilling both criteria simultaneously. The study further assessed metabolic features in this groups and identified differences in insulin sensitivity and beta-cell function. Diabetic individuals with isolated elevated 2-hour glucose concentrations had a physiological level of absolute early insulin release, but significantly lower insulin sensitivity in both the fasting and glucose-stimulated state. These results suggest that progression from prediabetes to type 2 diabetes is characterized by development of insulin resistance and a lack of ability to sufficiently compensate by increasing insulin secretion.

In contrast to the 2h-DM phenotype, F-DM was characterized by significantly reduced beta-cell function and also had a significant reduction in insulin sensitivity. Longitudinal

studies (71) showed that a reduction in the insulin secretory capacity can precede the development of peripheral insulin resistance in individuals with isolated fasting hyperglycemia. As expected, the F-2h-DM phenotype had the most overt metabolic impairment, yet the authors suggest that the progression to the F-2h-DM phenotype is merely an advancement of either of the other phenotypes left untreated. However, this classification does not account for the underlying pathophysiological defects leading to diabetes, but only reflects the severity of the disease, depending on how far diabetes has progressed in an individual.

Using the HbA1c definition of diabetes (12) does not allow further stratification and does not account for the diversity found within the diabetes subgroups captured by the glucose criteria. In general, patients with HbA1c-diagnosed diabetes had insulin resistance and beta-cell dysfunction in the same low range as patients diagnosed by F-DM and F-2h-DM, suggesting that the cut-off point for HbA1c of 6.5% identifies individuals in direct need for pharmacological therapies to correct for beta-cell dysfunction.

Further studies attempted to assess patients stratified by insulin sensitivity and beta-cell function, as hallmarks of diabetes. The insulin secretion and sensitivity are part of a dependent continuum, but the extremes will differ greatly. A Danish Study (63) used the homeostatic assessment model to subphenotype patients with type 2 diabetes into insulinopenic (high insulin sensitivity and low beta cell function), classical (low insulin sensitivity and low beta cell function), or hyperinsulinemic (low insulin sensitivity and high beta cell function) type 2 diabetes. Insulinopenic type 2 diabetes patients had a nearly normal waist circumference and a lower prevalence of cardiovascular disease, compared to the two other subphenotypes. In contrast, hyperinsulinemic type 2 diabetes patients had more abdominal obesity and a higher prevalence of cardiovascular disease. This study further highlights the need of better risk stratification of patients with type 2 diabetes with regard to cardiovascular disease.

1.6. Precision medicine in diabetes

Precision medicine is a new direction in diabetes therapy which considers a stepwise approach, incorporating and building the evidence from clinical studies in order to achieve quantifiable, implementable outcomes based on disease etiology, pathophysiological traits and risk scores (72). Making the correct diagnosis and using the correct treatment from diagnosis can be challenging, but it is crucial to prevent long-

term morbidity and mortality. While many studies advocate for individualized medicine, the enormous impact of environment and lifestyle factors make it almost impossible to carry out personalized management. However, the stratification of diabetes subphenotypes may allow for stratified management of specific groups with similar metabolic features and common risk for the development of diabetes-related complications.

Although the traditional classification into type 1 and type 2 diabetes has proven useful in differentiating distinct pathophysiological mechanisms with clear therapeutic implications, it remains insufficient in explaining the wide variety of clinical manifestations of diabetes (73). Furthermore, algorithms of prediction and prevention of diabetes complications, the rate of beta-cell failure, the proper methods of weight management, or medication suitability remain scarce. Precision medicine is the concept that specific treatments can be targeted to groups of individuals with specific genetic, cellular, or molecular features, is a key aspect of modern healthcare, and its use is rapidly expanding also in the field of diabetes (74). While at first, the application of precision medicine has been demonstrated in monogenic diabetes, which were in many cases misdiagnosed as either type 1 or type 2 diabetes (75), current research attempts to expand its applicability to all types of diabetes. Therefore, also the polygenic subtypes, now bundled together under the broad umbrella of type 2 diabetes can profit from precision medicine.

New technological developments coupled with improved computational power, increased statistical sophistication and drug manufacturing advances introduced diagnostic and therapeutic arrays with comprehensive understanding of the physiopathology behind type 2 diabetes (73). Thus, it was suggested that patterns of human genetics variation can also distinguish subgroups of patients and reveal new treatment targets.

So far, however, the clinical application of genetics in diabetes remains limited to rare, monogenic subtypes. Models for the management of type 2 diabetes embrace distinctive subtypes of type 2 diabetes, each of which has the potential to be mapped to a specific remedial therapy or intervention. In reality, effective strategies in this area must address the multifactorial etiology of type 2 diabetes and a continuous spectrum of predisposition mediated by multiple pathways.

One approach describing of the pathophysiological architecture of type 2 diabetes predisposition focuses on the intermediary processes contributing to type 2 diabetes risk

(76). The most obvious of these include obesity, fat distribution, islet development and function, and insulin sensitivity. Each of these processes is itself multifactorial, influenced by genetic and non-genetic factors, and effects both diabetes risk and the phenotype of any diabetes that results. Several recent studies have added empirical support for this approach, developing process-specific risk scores for assessments of the risk for diabetes complications such as diabetic nephropathy and cardiovascular disease (77).

Many models capture the range of diabetes subtypes as a continuum consistent with the genetic architecture of diabetes and real-world clinical observation (42; 64). Overall, the majority of studies suggest that type 2 diabetes, is the consequence not of a major defect, but in the confluence of suboptimal performance of different metabolic processes contributing in parallel.

This model provides a framework for understanding the mechanistic basis of heterogeneity and its clinical consequences. It also allows for the existence of cases at the extremes of the distribution for which a targeted intervention might be particularly effective. Genetic risk scores that capture each of these processes may help to tease apart heterogeneity in phenotype, progression, and therapeutic response. They may allow identification, within the overall population, of subsets of selected individuals in whom the profile of genetic predisposition is dominated by defects in a single pathway, facilitating personalized, mechanism-specific interventions.

It is yet not fully understood why some patients show rapid progression of microvascular and/or macrovascular complications or require aggressive escalation of therapy. The assessment of risk factors and deleterious patterns provides heterogeneous results when studied in patients with type 2 diabetes, as not all patients with diabetes are affected equally. Altogether, these results highlight the heterogeneity of type 2 diabetes and could account for differences in disease progression and response to glucose-lowering treatment, as seen in clinical practice. However, these findings also suggest that we might not yet have a reliable way to move forward towards a precision medicine approach for treatment of type 2 diabetes. An approach only based on commonly available clinical variables could prove insufficiently precise.

2. Aims

The aim of this work was to identify subgroups of patients with type 1 and type 2 diabetes with specific metabolic features and thereby contribute to an updated classification of these patients based on the different mechanisms involved in disease progression. The multicentric prospective GDS follows patients with diabetes from the first year after their diagnosis, permitting the monitoring of the natural course of disease, with a focus on comorbidities and complications (78). The GDS represents an ideal setting to analyze the link between clinical and metabolic characteristics by comprehensive phenotyping. In order to facilitate precision medicine in diabetes, my research is targeting a better understanding of disease heterogeneity and its impact on potential treatments for specific subtypes.

To this end, the first study focuses on patients with autoimmune diabetes - type 1 diabetes and the subgroup formerly named LADA, while addressing subphenotypes with distinct metabolic profiles within these patients.

The second study encompasses the application of a soft clustering algorithm based on clinical data on patients with non-autoimmune diabetes of the GDS. We aimed to examine whether measurement of insulin sensitivity and secretion by gold standard methods resembles the proposed diabetes clusters. We hypothesized that different insulin sensitivity based “subphenotypes” differently correlate with NAFLD and neuropathy therefore requiring specific risk factor management.

3. Study population and methods

GDS is an ongoing prospective observational study that examines patients within the first 12 months of diabetes diagnosis employing comprehensive metabolic phenotyping. Patients aged 18-69 years are followed for 20 years including systematical clinical examinations at 5 year-intervals and annual telephone interviews in between. Informed consent is obtained from all volunteers prior to inclusion into the trial. The study is conducted according to the Declaration of Helsinki. The study is registered at ClinicalTrial.gov (registration no: NCT01055093), and the study design is approved by the ethics board of Heinrich Heine University Düsseldorf (reference number 4508).

The overarching aims of the GDS are: (i) to identify subphenotypes of diabetes mellitus, (ii) to detect predictors of diabetes-associated comorbidities, (iii) to aid with the development of tailored preventive and therapeutic strategies, (iv) to recognize novel risk factors of disease progression. The study design (78) offers the unique possibility to reassess patients after 5 years disease duration using the same setting as for baseline.

Diabetes diagnosis is based on ADA criteria (12) as summarized in Table 2. Patients with diabetes of other causes, such as pancreoprive or gestational diabetes are not included in the study. The main inclusion criterion is diagnosis of diabetes according to current guidelines (12). Further inclusion and exclusion criteria apply. In brief, the study excludes patients with poor glycemic control (HbA1c > 9.0 %), hyperlipidemia (triglycerides and low-density lipoproteins ≥ double upper reference limit), severe heart failure (New York Heart Association class ≥ II), severe or acute renal disease (serum creatinine ≥ 1.6 mg/dl), severe or acute liver disease (liver transaminases ≥ double upper reference limit), known peripheral artery disease stage IV, known severe or acute psychiatric disorders, active malignant diseases, previous venous thromboembolic events, anemia (hemoglobin < 11 mg/dl) or recent blood donation, acute infection (leukocytes > 20% upper reference limit), immunosuppressive therapy (including in the context of active autoimmune diseases). Furthermore, the study excludes patients who have participated in an interventional clinical study within the preceding 3 months.

Patients undergo comprehensive metabolic phenotyping using non-invasive or minimally invasive techniques in order to assess glucose metabolism, insulin sensitivity, beta-cell function, and anthropometric and clinical parameters. Blood chemistry is analyzed in a centralized unit for routine laboratory parameters (78). Adipose-tissue insulin resistance index is calculated from fasting concentrations of insulin and free fatty

acids (FFA) (79). Further indices of liver health such as the fatty liver index (FLI) and AST (aspartate aminotransferase) to Platelet Ratio Index (APRI) are computed from routine laboratory parameters (80). Antibodies against GAD and ICA are determined by a radioligand assay and by indirect immunofluorescence, respectively (78; 81). Cutoff levels for GAD are 2 units/ml and 40 JDF-units for ICA (82).

Further, the glucagon stimulation test is performed by measuring fasting blood glucose, fasting insulin and C-peptide levels (83). At 0 min, a bolus of 1 mg glucagon (GlucaGen; Novo Nordisk, Mainz, Germany) was injected intravenously and a second blood sample was obtained after 6 minutes for measurements of insulin and C-peptide (78). The difference between C-peptide and insulin concentrations between 0 min and 6 min were determined to assess glucagon-stimulated C-peptide and insulin secretion capacity (84). In order to assess insulin sensitivity, the gold standard method of a hyperinsulinemic-euglycemic clamp test is employed. This modified Botnia clamp consists of an intravenous glucose tolerance test (IVGTT) followed by a hyperinsulinemic-eugycemic clamp test with frequent measurements of blood glucose, C-peptide and insulin (78). The IVGTT is started by administrating a 30% glucose infusion bolus (1 mg/kg body weight) followed by timed blood sampling for 60 minutes. A priming insulin dose is then applied (10 mU*kg (body weight)$^{-1}$* min^{-1} for 10 minutes) continued by constant infusion of 1.5 mU*kg (body weight)$^{-1}$*min^{-1} (Insuman Rapid; Sanofi; Frankfurt, Germany). Blood glucose concentration is maintained at 90 mg/dl by a variable 20% glucose infusion. Total C-peptide secretion is determined from the incremental area under the curve for C-peptide levels during the 1-hour IVGTT and whole body insulin sensitivity is assessed from whole body mean glucose infusion rates (M-value) with glucose space correction (85).

Patients with no contraindications against magnetic resonance investigation techniques (e.g. pacemaker, magnet-sensitive implants) undergo magnetic resonance spectroscopy (MRS) measurements on a 3-T MR scanner (Achieva X-series, Philips Healthcare). Single voxel stimulated echo acquisition mode ^{1}H-MRS is performed for quantitative assessment of HCL. Both water suppressed and non-suppressed ^{1}H-MRS are taken in the identical voxel within the homogeneous part of liver tissue, avoiding major vessels and gallbladder, with a volume of interest of 25x25x25 mm^{3}. Hepatic γATP and Pi concentrations are determined by ^{31}P-MRS using 3D image selected in vivo spectroscopy with proton decoupling (number of signal averages=128) within a volume of interest of 60x60x60 mm^{3}. All liver spectra are subsequently processed using jMRUI

software (12). HCL content (%) is then calculated by the methylene peak at 1.3 ppm in water-suppressed MRS, relative to the sum of the methylene and water peaks at 4.7 ppm in water non-suppressed MRS (86).

In order to assess diabetic neuropathy, the GDS employs electrophysiological testing, quantitative sensory testing, and clinical neuropathy score surveys (87; 88). Peroneal, median, and ulnar motor nerve conduction velocity (NCV) is measured in the peroneal nerve, sural, median, and ulnar sensory NCV, and sensory nerve action potentials (SNAPs) are determined in the sural nerve at a skin temperature of 33–34°C using surface electrodes (Nicolet VikingQuest, Natus Medical, San Carlos, CA, USA). Vibration perception thresholds (Vibrameter, Somedic, Stockholm, Sweden) and thermal detection thresholds (TSA-II NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel) are measured (87). The neurological examination is quantified using the Neuropathy Disability Score (88). Neuropathic symptoms are assessed using the Neuropathy Symptom Score. Diabetic sensorimotor polyneuropathy (DSPN) is defined according to modified Toronto Consensus criteria (89). Assessment of heart rate variability (HRV) is performed during the hyperinsulinemic-euglycemic clamp (90). In brief, R-R intervals are recorded in the supine position using a digital Spider View Holter recorder with seven electrodes to record three-channel electrocardiogram. HRV is computed from the Holter monitor recordings with the SyneScope V.3.00 analysis system (MicroPort CRM, Munich, Germany). Cardiovascular autonomic reflex tests (CARTs) including heart rate changes in response to a Valsalva maneuver (Valsalva ratio) and orthostatic posture (maximum-to-minimum (max/min) 30:15 ratio) are performed using a VariaCardio TF5 system (MIE Medical Research, Leeds, UK). The presence of two or three abnormal results (two for borderline, three for definite) among the seven autonomic cardiovascular indices (including the five standard CARTs and other time and frequency domain indices of HRV) is considered as a criterion for cardiac autonomic neuropathy (CAN) diagnosis (91).

Taken together, these methods offer a comprehensive overview of patients' metabolic phenotype and characterize even the early stages of diabetes-associated diseases. The cohort studied is among the largest prospective European cohorts of recent onset diabetes and most likely the largest with comprehensive phenotyping of both metabolism and comorbidities.

4. Publications

4.1. Metabolic characteristics of recently diagnosed adult-onset autoimmune diabetes mellitus

Zaharia OP, Bobrov P, Strassburger K, Bodis K, Karusheva Y, Scholz M, Markgraf DF, Burkart V, Schloot NC, Mussig K, Szendroedi J, Roden M: Metabolic Characteristics of Recently Diagnosed Adult-Onset Autoimmune Diabetes Mellitus. *J Clin Endocrinol Metab* 2018;103:429-437

Metabolic Characteristics of Recently Diagnosed Adult-Onset Autoimmune Diabetes Mellitus

Oana P. Zaharia,[1,2] Pavel Bobrov,[2,3] Klaus Strassburger,[2,3] Kálmán Bódis,[1,2] Yanislava Karusheva,[1,2] Michaela Scholz,[1,2] Daniel F. Markgraf,[1,2] Volker Burkart,[1,2] Nanette C. Schloot,[1] Karsten Müssig,[1,2,4] Julia Szendroedi,[1,2,4] and Michael Roden,[1,2,4] for the GDS Group

[1]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf D-40225, Germany; [2]German Center for Diabetes Research (DZD e.V.), Munich-Neuherberg 85764, Germany; [3]Institute for Biometrics and Epidemiology, German Diabetes Center, Düsseldorf D-40225, Germany; and [4]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf D-40225, Germany

Context and Objective: Among patients diagnosed with type 2 diabetes, autoimmune diabetes often remains undetected. Metabolic features of these patients are insufficiently characterized at present.

Design, Setting, and Patients: This study compared age- and sex-matched adult (aged 41 to 62 years) humans with recent-onset diabetes: patients positive for antibodies against glutamic acid decarboxylase (GAD) and/or cytoplasmic islet-cell antigen with an insulin-free period of >6 months [antibody positive/insulin negative (ab+/ins−); previously termed *latent autoimmune diabetes of adults*], type 1 diabetes [antibody positive/insulin positive (ab+/ins+)], and type 2 diabetes [antibody negative/insulin negative (ab−/ins−)], as well as glucose-tolerant humans (controls) of the German Diabetes Study (n = 41/group). β-Cell function was assessed from glucagon tests and intravenous glucose tolerance tests (IVGTTs), and insulin sensitivity was determined from hyperinsulinemic-euglycemic clamps.

Results: Of the ab+/ins− patients, 33 (81%) were initially diagnosed as having type 2 diabetes. In ab+/ins−, body mass index (BMI) was higher than in ab+/ins+ (27.8 ± 5.3 kg/m^2 vs 25.0 ± 3.5 kg/m^2, $P < 0.05$), lower than in ab−/ins− (31.9 ± 5.8 kg/m^2, $P < 0.05$), and similar to controls (29.4 ± 6.6 kg/m^2). In ab+/ins−, GAD antibody titers correlated negatively with BMI ($r = -0.40$, $P < 0.05$) and with C-peptide secretion in glucagon stimulation tests ($r = -0.33$, $P < 0.05$). β-Cell function from IVGTT was 228% higher in ab+/ins− than in ab+/ins+ but 35% lower than in ab−/ins− and 61% lower than in controls (all $P < 0.05$). Insulin sensitivity in ab+/ins− was comparable to ab+/ins+ and controls but 41% higher than in ab−/ins− ($P < 0.05$) after adjustment for BMI and fasting blood glucose or hemoglobin A1c.

Conclusion: Even shortly after diagnosis, ab+/ins− patients feature partly preserved β-cell function and chronic hyperglycemia, which possibly contributes to the observed impairment of whole-body insulin sensitivity. (***J Clin Endocrinol Metab*** **103: 429–437, 2018)**

Some patients with adult-onset diabetes mellitus present with positive diabetes-associated antibodies, mainly antibodies against glutamic acid decarboxylase (GAD) and cytoplasmic islet-cell antigen (ICA) (1–3). Autoimmunity typically defines type 1 diabetes, yet there has been an attempt to identify a subgroup as latent autoimmune

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 1 August 2017. Accepted 1 December 2017.
First Published Online 6 December 2017

Abbreviations: ab−/ins−, antibody negative/insulin negative; ab+/ins−, antibody positive/insulin negative; ab+/ins+, antibody positive/insulin positive; BMI, body mass index; GAD, glutamic acid decarboxylase; GDS, German Diabetes Study; HbA1c, hemoglobin A1c; hsCRP, high-sensitivity C-reactive protein; ICA, islet-cell antigen; IVGTT, intravenous glucose tolerance test; JDRF, Juvenile Diabetes Research Foundation; LADA, latent autoimmune diabetes of the adult; LDL, low-density lipoprotein.

diabetes of the adult (LADA) (4, 5), 1.5 diabetes (6), or noninsulin-requiring autoimmune diabetes (7), based on the following criteria: positivity for diabetes-associated autoantibodies, age at diagnosis older than 30 years, and no insulin therapy during the first 6 to 12 months after diagnosis. According to current estimates, up to 10% of all patients with type 2 diabetes meet the criteria of LADA (5). These patients may present with some clinical characteristics of type 2 diabetes (8) and are preferentially treated with oral glucose-lowering medication or incretins, as insulin is not required during the first years after diagnosis (9). Of note, European guidelines do not define LADA as an independent diabetes type but classify these patients as having type 1 diabetes (10, 11).

Autoantibody positivity in patients with diabetes is associated with younger age at onset, less secretion of insulin, and faster progression to insulin dependency than for antibody-negative patients (12). Of note, the metabolic phenotype might be determined by the antibody titer in that patients with a high GAD antibody titer are phenotypically closer to type 1 diabetes with lower body mass index (BMI), whereas those with a low GAD antibody titer resemble patients with type 2 diabetes (13).

Regarding β-cell function in patients with LADA, some but not all studies show an accelerated decline in insulin secretion compared with type 2 diabetes (14, 15). However, only few data exist on β-cell function and insulin sensitivity in patients with adult-onset autoimmune diabetes compared with type 2 diabetes and controls. Moreover, diabetes-related comorbidities of these patients have been scarcely reported in the literature (16). Some patients appear to develop micro- and macrovascular complications despite improved glycemic control (17) and independently of features of the so-called metabolic syndrome (7).

This study aimed to characterize patients with adult-onset autoimmune diabetes by differentiating between those on oral glucose-lowering medication [antibody positive/insulin negative (ab+/ins−); so-called LADA], those on insulin treatment immediately after diabetes diagnosis [antibody positive/insulin positive (ab+/ins+); type 1 diabetes], and those with type 2 diabetes (ab−/ins−). Insulin secretion and sensitivity were quantified by employing gold-standard methods (18) in patients and healthy controls of the prospective observational German Diabetes Study (GDS), which allows examining patients with short known diabetes duration of <1 year and monitoring disease progression.

Subjects and methods

Volunteers

This nested case-control study comprised 41 ab+/ins− patients, 41 ab+/ins+ patients, 41 ab−/ins− patients, and 41 glucose-tolerant healthy humans. Of all patients tested positive for GAD antibodies and/or ICA, the ab+/ins− patients were selected by applying the following criteria: age >30 years at diagnosis and absence of insulin treatment during the first 6 months after diabetes diagnosis (19). Then, volunteers of the GDS with type 1 diabetes, those with type 2 diabetes, and healthy humans were subsequently pairwise matched for age and sex to these patients. Diabetes-related symptoms and their duration before the diagnosis, family history of diabetes, and medication as well as manifestation of diabetes-related sequelae were recorded. All participants were recruited from the GDS, which follows volunteers with recently diagnosed diabetes (known disease duration <12 months) and glucose-tolerant humans, aged 18 to 69 years. The design of the study was approved by the ethics committee of Heinrich-Heine University of Düsseldorf (reference number 4508), registered at Clinicaltrials.gov (identifier number: NCT01055093), and is performed according to the Declaration of Helsinki as reported previously (18). Briefly, diagnosis of diabetes is based on American Diabetes Assoication criteria (19). Exclusion criteria comprised the following: specific types of diabetes due to other causes; pregnancy; acute or severe chronic heart, hepatic, renal, or psychiatric diseases; and immunosuppressive treatment. Acute inflammatory syndromes were excluded by high-sensitivity C-reactive protein (hsCRP) >1 mg/dL (20) and impaired kidney function by an estimated glomerular filtration rate <60 mL/min/1.73 m^2.

Glucagon stimulation test

The test was performed as described previously (20). Fasting blood glucose, fasting insulin, and C-peptide levels were determined. At 0 minutes, a bolus of 1 mg glucagon (GlucaGen; Novo Nordisk, Mainz, Germany) was injected intravenously, and a second blood sample was obtained after 6 minutes for measurements of insulin and C-peptide (18). The difference between C-peptide and insulin concentrations between 0 and 6 minutes was determined to assess glucagon-stimulated C-peptide and insulin secretion capacity (ΔC-peptide, Δinsulin) (21).

Modified Botnia clamp test

This test consists of an intravenous glucose tolerance test (IVGTT) followed by a hyperinsulinemic-euglycemic clamp test with frequent measurements of blood glucose, C-peptide, and insulin (18). The IVGTT was started by administrating a 30% glucose infusion bolus (1 mg/kg body weight) followed by timed blood sampling for 60 minutes. A priming insulin dose was then applied [10 mU · kg (body weight)$^{-1}$ · min^{-1} for 10 minutes], continued by a constant infusion of 1.5 mU · kg (body weight)$^{-1}$ · min^{-1} (Insuman Rapid; Sanofi, Frankfurt, Germany). Blood glucose concentration was maintained at 90 mg/dL by a variable 20% glucose infusion. The total clamp duration was 180 minutes. The mean steady-state duration (between 130 and 180 minutes) was 27 ± 1 minutes for ab+/ins−, 26 ± 1 minutes for ab−/ins−, 25 ± 1 minutes for ab+/ins+, and 26 ± 1 minutes for controls (all $P > 0.05$), with a mean blood glucose level during the steady state of 90 ± 1 mg/dL for patients with ab+/ins−, 91 ± 1 mg/dL for ab−/ins−, 90 ± 1 mg/dL for ab+/ins+, and 91 ± 1 mg/dL for controls (all $P > 0.05$).

Total C-peptide secretion was determined from the incremental area under the curve for C-peptide levels during the

1-hour IVGTT, and whole-body insulin sensitivity was assessed from whole-body mean glucose infusion rates (M-value) with glucose space correction.

Laboratory analyses

Antibodies against GAD and ICA were measured as described (22). In brief, autoantibodies to full-length GAD and ICA were determined by a radioligand assay and by indirect immunofluorescence, respectively (23, 24). Cutoff levels were 0.9 U/mL for GAD and 5 Juvenile Diabetes Research Foundation (JDRF) units for ICA. Patients were classified as positive for antibodies against GAD or ICA with levels >0.9 U/mL for GAD or >20 JDRF units for ICA. All other parameters were analyzed as described previously (18).

Statistics

Data are presented as mean (standard deviation) or median (first quartile, third quartile) for continuous variables and percentages for categorical variables. Skewed data were log-transformed before analysis (M-value, total C-peptide secretion, hsCRP, triglycerides). To compare groups, covariance pattern models were applied, which account for the matched-pair design by estimating the covariance matrix of the residuals within matched pairs. Similar procedures were used to analyze binary data. To account for multiple group comparisons, the Tukey-Kramer correction was applied. Associations between parameters have been evaluated using Spearman and adjusted (partialized) Spearman correlation coefficients (r) and corresponding P values. Regression analyses adjusted for age, sex, and BMI were performed to exclude these as confounding factors. Additional analyses were adjusted for fasting blood glucose and hemoglobin A1c (HbA1c). P values <5% were considered to indicate significant differences or correlations. Statistical analyses were performed with SAS (version 9.3; SAS Institute, Cary, NC).

Results

Anthropometric and clinical characteristics of the study population

Anthropometric and clinical data are shown in Table 1. Patients with ab+/ins+ were slightly younger than participants of the other groups. BMI was on average 10% higher in ab+/ins− patients than in ab+/ins+ patients, 22% lower than in ab−/ins− patients, and intermediary in controls. The prevalence of a family history of diabetes was similar between all groups of patients with diabetes. hsCRP and HbA1c did not differ between the diabetes groups but were lower in the control group (all $P < 0.05$). Most patients with diabetes had excellent glycemic control with HbA1c <7% (53 mmol/mol) according to current guidelines (11) [30 (73%) of ab+/ins−, 30 (73%) of ab+/ins+, and 33 (81%) of ab−/ins− patients].

Per definition, only patients with LADA and type 1 diabetes had positive diabetes-associated autoantibodies (ab+). Compared with ab+/ins+ patients, ab+/ins−

Table 1. Characteristics of the Study Population

Characteristic	ab+/ins−	ab+/ins+	ab−/ins−	Controls
Total No. (male/female)	41 (25/16)	41 (25/16)	41 (25/16)	41 (25/16)
Age, y	50.8 ± 9.4[a]	48.0 ± 7.0[b,c,d]	50.8 ± 9.3[a]	51.1 ± 9.7[a]
BMI, kg/m^2	27.8 ± 5.3[a,b]	25.0 ± 3.5[b,c,d]	31.9 ± 5.8[a,d]	29.4 ± 6.6[a]
WHR	0.93 ± 0.09	0.90 ± 0.10	0.96 ± 0.07[c]	0.92 ± 0.08[b]
Known diabetes duration, mo	6.9 ± 3.4	6.1 ± 2.6	5.9 ± 3.1	—
Duration of diabetes-related symptoms prior to diagnosis, mo	3.6 ± 4.7	2.4 ± 4.2	4.0 ± 5.6	—
Family history of type 1 diabetes, n (%)	2 (5)	1 (2)	1 (2)	—
Family history of type 2 diabetes, n (%)	12 (29)	12 (29)	21 (51)	—
hsCRP, mg/dL	0.18 (0.09, 0.34)[c]	0.13 (0.05, 0.22)[c]	0.37 (0.18, 0.57)[c]	0.13 (0.07, 0.23)[a,b,d]
HbA1c, % (mmol/mol)	6.6 ± 1.1 (49 ± 12)[c]	7.0 ± 1.5 (52 ± 16)[c]	6.3 ± 0.7 (45 ± 8)[c]	5.3 ± 0.3 (34 ± 3)[a,b,d]
eGFR, mL/min/1.73 m^2	91.3 ± 14.9	89.5 ± 12.4	85.4 ± 14.8	91.8 ± 13.1
Total cholesterol, mg/dL	193.0 ± 33.2[a]	206.7 ± 40.7[d]	202.0 ± 53.3	204.5 ± 32.2
LDL cholesterol, mg/dL	120.3 ± 34.0[a]	127.0 ± 39.3[d]	125.3 ± 36.5	134.3 ± 33.6
HDL cholesterol, mg/dL	53.0 ± 16.7[a,c]	63.7 ± 19.7[b,d]	45.3 ± 12.9[a]	60.3 ± 16.5[d]
Triglycerides, mg/dL	128.3 ± 89.7	92.4 ± 49.5	146.3 ± 77.4[c]	110.3 ± 52.8[b]
Lipid-lowering therapy, n (%)	4 (10)	1 (2)	6 (15)	0 (0)
Antihypertensive therapy, n (%)	16 (39)	5 (12)[b]	20 (49)[a,c]	11 (27)[b]
GAD, U/mL	2.2 (0.9, 34.0)[a]	38.9 (12.6, 92.4)[d]	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)
ICA >20 JDRF, n (%)	30 (73)	41 (100)	0 (0)	0 (0)

Anthropometric and clinical data of the study population are shown as n (%), mean ± standard deviation, or median (first quartile, third quartile). Positive GAD antibodies and/or cytoplasmic ICAs were used as markers for diagnosing autoimmune diabetes (ab+).

Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; WHR, waist-to-hip ratio.

[a] $P \leq 0.05$ vs type 1 diabetes.

[b] $P \leq 0.05$ vs type 2 diabetes.

[c] $P \leq 0.05$ vs controls.

[d] $P \leq 0.05$ vs LADA.

patients had 95% lower GAD titers [2.2 (0.9, 34.0) U/mL vs 38.9 (12.6, 92.4) U/mL, $P < 0.05$]. ICA titers >20 JDRF units were found in 30 (73%) ab+/ins− and in 41 (100%) ab+/ins+ patients. BMI negatively correlated with GAD titers in ab+/ins− patients ($r = -0.47$, $P < 0.05$).

Metabolic syndrome components and diabetes-related comorbidities

When analyzing further cardiovascular risk factors comprising the so-called metabolic syndrome (25), we observed that there was no difference in waist-to-hip ratio between ab+/ins− patients and patients of the other groups. The lipid profile as presented in Table 1 shows similarities in total, low-density lipoprotein (LDL), and high-density lipoprotein cholesterol and triglyceride levels between ab+/ins− and ab−/ins−. Of the ab+/ins− patients, 29 (71%) had fasting triglyceride levels <150 mg/dL (26), and 14 (34%) achieved LDL levels <100 mg/dL as recommended in the European Society of Cardiology guidelines (11). Hypertension was present in 22 (54%) of the patients with ab+/ins−, 14 (34%) of the patients with ab+/ins+, 29 (71%) of the patients with ab−/ins−, and 21 (51%) of the controls. No differences were evident between ab+/ins− patients and patients of the other groups with regard to antihypertensive treatment.

Regarding diabetes-related comorbidities, some patients had micro- and macrovascular complications already within the first year after diagnosis: two cases of diabetic retinopathy, one case of diabetic neuropathy, and two cases of myocardial infarction in patients with ab+/ins− (12%); one case of peripheral artery disease and one case of stroke in patients with ab−/ins− (5%); one case of peripheral artery disease in glucose-tolerant controls (2%); and no diagnosed complications in patients with ab+/ins+ (0%).

Insulin sensitivity and β-cell function

Whole-body insulin sensitivity in ab+/ins− patients was comparable to that of ab+/ins+ patients but 41% higher than in ab−/ins− patients and 16% lower than in controls (Fig. 1A). After further adjustments for fasting blood glucose and HbA1c, only differences between ab+/ins− patients and ab−/ins− patients remained ($P < 0.05$).

With regard to β-cell function, total C-peptide secretion during IVGTT (Fig. 1B) was 228% higher in ab+/ins− patients compared with ab+/ins+ patients, 35% lower compared with ab−/ins− patients, and 61% lower compared with controls (all $P < 0.05$). These differences remained after adjustment for age, sex, and BMI.

Similarly, ΔC-peptide levels following glucagon stimulation (Fig. 1C) were 180% higher in ab+/ins− patients compared with ab+/ins+ patients, 31% lower than in ab−/ins− patients, and 53% lower than in controls (all $P < 0.05$). Furthermore, in patients with ab+/ins−, GAD antibody titers inversely correlated with ΔC-peptide ($r = -0.33$, $P < 0.05$) (Fig. 2). Correspondingly, Δinsulin in ab+/ins− patients was 144% higher compared with ab+/ins+ patients, 62% lower than in ab−/ins− patients, and 138% lower than in controls (all $P < 0.05$) (Fig. 1D). Further analyses with adjustments for fasting glucose confirmed higher insulin secretory response in ab+/ins− compared with ab+/ins+ and controls and similarity between ab+/ins− and ab−/ins−.

Discussion

The ab+/ins− patients, reflecting the so-called LADA, as defined by positive diabetes-associated antibodies, age >30 years at diagnosis, and no insulin treatment during the first 6 months upon diabetes diagnosis, had higher insulin sensitivity than matched patients with type 2 diabetes and presented with better β-cell function parameters than patients with type 1 diabetes, independent of BMI. In the ab+/ins− patients, GAD antibody titers were found to inversely correlate with both C-peptide release after glucagon stimulation and BMI. Of note, the high prevalence of diabetes-related complications is noteworthy, despite the lower prevalence of components of the metabolic syndrome in these newly diagnosed ab+/ins− patients (27).

Although ab+/ins− patients have a lower body weight and younger age at onset of diabetes than patients with type 2 diabetes (28), previous studies have attributed differences in metabolic parameters to BMI (14). Consequently, we adjusted our analyses to account for this confounding factor. Also, similar to the study by Hawa *et al.* (28), we found an inverse association between BMI and the GAD autoantibody titer, indicating that ab+/ins− patients with lower β-cell–directed immunoreactivity are closer to the typical phenotype of type 2 diabetes. Previous studies revealed contradictory data on the relationship between BMI and GAD autoantibodies, either in favor (29) or against (30) a role of obesity as an accelerator of insulin resistance, thereby driving the immune response and promoting β-cell apoptosis. Although the cross-sectional design does not allow us to establish causality, the current study detected an interaction between BMI, GAD autoantibody titers, and β-cell function. Adjusting for ΔC-peptide abolished the correlation between BMI and GAD autoantibodies, suggesting that this correlation is mediated by impaired β-cell function. In line, a direct relationship between BMI and ΔC-peptide

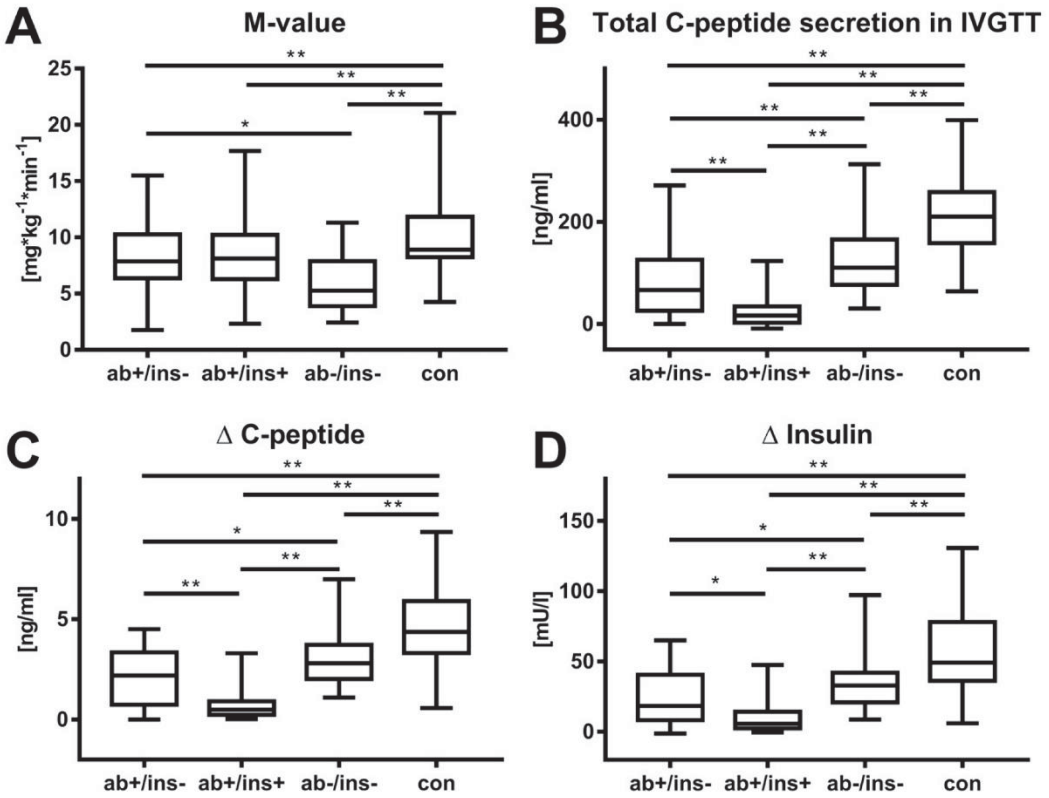


Figure 1. Metabolic characteristics of patients with newly diagnosed LADA (ab+/ins−), patients with type 1 diabetes (ab+/ins+), patients with type 2 diabetes (ab−/ins−), and controls (con) showing (A) insulin sensitivity (M-value), (B) total C-peptide secretion during IVGTT, and (C) C-peptide and (D) insulin secretion after glucagon stimulation. Box plots show medians and whiskers from minimum to maximum. *P* values refer to comparison of data adjusted for age, sex, and BMI. Data were ln-transformed where applicable. $*P \leq 0.05$, $**P \leq 0.05$ after correction for multiple testing.

has been reported (31). Because of its prospective design (18), the source study of this analysis, the GDS, should be able to address at least some aspects of the role of obesity for diabetes-related autoimmunity and β-cell function in the future.

Previous studies implied that the onset of disease in patients with adult-onset autoimmune diabetes can be insidious, similar to that observed in patients with type 2 diabetes, which promotes a postponed medical attendance and possibly a delayed initiation of adequate treatment (5). Nevertheless, we showed that latency of diagnosis following the first appearance of diabetes-related symptoms was not different between ab+/ins− and ab−/ins− patients, and neither were family history of diabetes nor HbA1c levels. Therefore, the clinical appearance does not show clear criteria to distinguish latent autoimmune diabetes from type 2 diabetes.

The methods of achieving glycemic control have been viewed with controversy in ab+/ins− patients because there is no consensus on therapeutic strategies (32). Previous studies have reported insufficient glycemic control in ab+/ins− patients, endorsing immediate insulin treatment (9). In contrast, in our cohort, HbA1c levels in ab+/ins− patients were comparable to those of ab−/ins− patients and well within the guideline-imposed limits for glycemic control (11). Furthermore, there was no difference in those patients incorrectly attributed to type 2 diabetes compared with those attributed to so-called LADA. Although guidelines (11) advocate for distinguishing patients presenting with diabetes-associated antibodies from type 2 diabetes, in our cohort, most ab+/ins− patients were initially diagnosed as having type 2 diabetes. Even without insulin treatment 6 months from disease onset, 73% of the ab+/ins− patients achieve excellent glycemic control when treated with biguanides, sulphonylureas, and/or dipeptidylpeptidase-4 inhibitor (10, 33).

Previous studies on ab+/ins− patients have described pronounced dyslipidemia (34), which is regarded as an

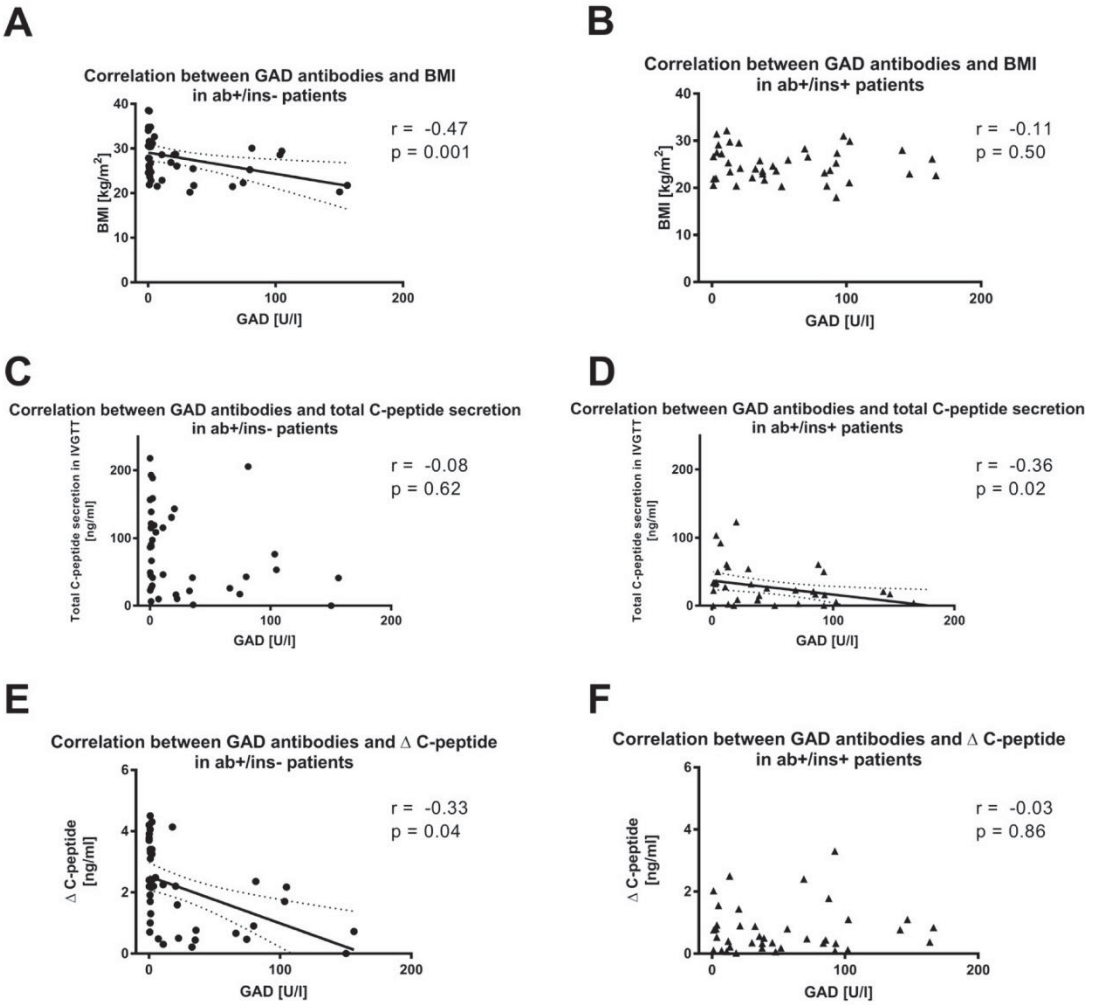


Figure 2. Correlation of GAD antibodies with BMI (A, B), with β-cell function expressed as total C-peptide secretion during the IVGTT (C, D), and with C-peptide secretion upon glucagon stimulation (E, F) in ab+/ins− patients (circles) and ab+/ins+ patients (triangles). (A, E) BMI and glucagon-stimulated C-peptide secretion inversely correlated with GAD antibody titers in ab+/ins− patients. (D) Glucose-stimulated total C-peptide secretion inversely correlated with GAD antibody titers in ab+/ins+ patients. Individual data are presented as scatter plots with linear regression including 95% confidence intervals and with partial Pearson correlations coefficients (*r*) and corresponding *P* values.

important atherogenic risk factor. This, coupled with reduced insulin sensitivity and hypertension, can contribute to an increased cardiovascular risk (35), increasing the hazard for vascular events. Our findings are in line with a Spanish study, which showed only satisfactory lipid control in ab+/ins− patients even after several years of disease duration (36). Similarly, ab+/ins− patients of our study had a heterogeneous lipid profile, with lower LDL levels than ab+/ins+ patients independent of age, BMI, and waist-to-hip ratio, and presumably benefited from the lipid-lowering therapy but at the same time had lower high-density lipoprotein levels than patients with type 2 diabetes. Our results warrant further research into the factors influencing the lipid profile of ab+/ins− patients, as we showed the presence of micro- and macrovascular complications in 12% of these patients already within the first year after diagnosis. However, this study was not powered to compare the prevalence of diabetes-related complications between the groups.

Up to now, ab+/ins− patients have been scarcely investigated with regard to insulin sensitivity. There is compelling evidence that insulin resistance can be present in both type 2 diabetes and type 1 diabetes (37), although

to a lesser degree in the latter. Our results suggest that a higher BMI is not the main contender in determining insulin resistance in adult-onset autoimmune diabetes. Even though ab+/ins− patients had higher whole-body insulin sensitivity than their type 2 diabetes counterparts of similar age, they also had a lower insulin sensitivity than age- and BMI-matched controls. The effect of elevated fasting glucose and glycemic control on the group comparison analyses indicates that hyperglycemia may at least contribute to the reduced insulin sensitivity in ab+ patients. Given the short known disease duration of these patients, these findings suggest an important role of glucose toxicity even in the early course of disease. There are contradicting data on describing LADA as a distinct disease entity with a unique metabolism, as it encompasses features that are present in both type 1 and type 2 diabetes. Interestingly, our ab+/ins− patients showed both better insulin sensitivity and preserved β-cell function, distinct from patients with type 2 diabetes, independent of age, sex, or BMI.

The preserved residual capacity of C-peptide stimulation in ab+/ins− patients reflects a decelerated loss of β-cell functionality compared with type 1 diabetes. Nonetheless, patients with autoimmune diabetes who have a reduced C-peptide secretion will be prone to a shorter interval of insulin-free therapy than patients with type 2 diabetes (38). Despite the presence of β-cell-directed autoimmunity, confirmed by the presence of diabetes-associated autoantibodies, there seems to be only a mild autoimmune reactivity. The differences between ab+/ins− patients and controls were preserved after adjustments for fasting blood glucose with correction for multiple analysis, thereby ruling out a relevant role of the fasting blood glucose level as a confounding factor for β-cell function.

The strength of our study design relies on the well-characterized metabolism of ab+/ins− patients in comparison with matched patients with type 1 and type 2 diabetes and healthy controls. Patients included in the study had similar disease duration and had undergone gold-standard methods following standard operating procedures to evaluate β-cell function and insulin sensitivity. The specific metabolic features of ab+/ins− patients should raise clinical awareness. These patients will not benefit from insulin substitution based on established dosing algorithms for type 1 diabetes but will require more individualized treatment, taking into account their lower insulin sensitivity. Furthermore, in the presence of diabetes-related autoimmunity, patients may also need intensive lifestyle modification and monitoring of both physical fitness and body fat mass to improve their insulin resistance. Although the 8.3% prevalence of ab+/ins− in patients with type 2 diabetes of the GDC cohort lies in the range of previously described studies, the relatively small number of subjects is a limitation of the study.

In conclusion, adult patients with recently diagnosed autoimmune diabetes have higher insulin sensitivity than patients with type 2 diabetes, but specifically, patients with autoimmune diabetes without initial insulin treatment (previously termed LADA) have a partly preserved β-cell function at the onset of diabetes. Compared with healthy humans, these ab+/ins− patients show impaired whole-body insulin sensitivity possibly due to chronic hyperglycemia.

Acknowledgments

Financial Support: The GDS was initiated and financed by the DDZ–German Diabetes Center, which is funded by the German Federal Ministry of Health (Berlin, Germany) and the Ministry of Innovation, Science, Research and Technology of the state North Rhine-Westphalia (Düsseldorf, Germany) and from the German Federal Ministry of Education and Research (BMBF) grant to the German Center for Diabetes Research (DZD e.V.) and the Schmutzler Stiftung, Germany.

Clinical Trial Information: ClinicalTrials.gov no. NCT01055093 (registered 25 January 2010).

Author Contributions: O.P.Z. wrote the manuscript and researched data. K.B., Y.K., D.F.M., and M.S. researched data. P.B. and K.S. performed the statistical analyses. J.S., K.M., V.B., N.C.S., and M.R. researched data, contributed to the discussion, and reviewed/edited the manuscript. All authors critically reviewed the manuscript. M.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

Correspondence and Reprint Requests: Michael Roden, MD, Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, c/o Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany. E-mail: michael.roden@ddz.uni-duesseldorf.de.

Disclosure Summary: N.C.S. is employed by Lilly Deutschland GmbH and a visiting scientist at the Institute for Clinical Diabetology of the German Diabetes Center. The other authors have nothing to disclose.

References

1. Stenström G, Gottsäter A, Bakhtadze E, Berger B, Sundkvist G. Latent autoimmune diabetes in adults: definition, prevalence, beta-cell function, and treatment. *Diabetes*. 2005;**54**(Suppl 2):S68–S72.
2. Laugesen E, Østergaard JA, Leslie RD; Danish Diabetes Academy Workshop and Workshop Speakers. Latent autoimmune diabetes of the adult: current knowledge and uncertainty. *Diabet Med*. 2015;**32**(7):843–852.
3. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC. Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia*. 2005;**48**(11):2206–2212.

4. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;**383**(9911):69–82.
5. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: a disease with increasing heterogeneity. *Lancet*. 2014;**383**(9922):1084–1094.
6. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM. Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? *Diabetes*. 2005;**54**(Suppl 2): S62–S67.
7. Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C, Capizzi M, Arpi ML, Bazzigaluppi E, Dotta F, Bosi E; Non Insulin Requiring Autoimmune Diabetes Study Group. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. *Diabetes Care*. 2007;**30**(4):932–938.
8. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes*. 1993;**42**(2):359–362.
9. Kumar A, de Leiva A. Latent autoimmune diabetes in adults (LADA) in Asian and European populations. *Diabetes Metab Res Rev*. 2017;**33**(5):e2890.
10. Leslie RD, Palmer J, Schloot NC, Lernmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. *Diabetologia*. 2015;**59**(1):13–20.
11. Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, Marre M, Marx N, Mellbin L, Ostergren J, Patrono C, Seferovic P, Uva MS, Taskinen MR, Tendera M, Tuomilehto J, Valensi P, Zamorano JL, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, De Backer G, Sirnes PA, Ezquerra EA, Avogaro A, Badimon L, Baranova E, Baumgartner H, Betteridge J, Ceriello A, Fagard R, Funck-Brentano C, Gulba DC, Hasdai D, Hoes AW, Kjekshus JK, Knuuti J, Kolh P, Lev E, Mueller C, Neyses L, Nilsson PM, Perk J, Ponikowski P, Reiner Z, Sattar N, Schächinger V, Scheen A, Schirmer H, Strömberg A, Sudzhaeva S, Tamargo JL, Viigimaa M, Vlachopoulos C, Xuereb RG. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on Diabetes, Pre-Diabetes, and Cardiovascular Diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur Heart J*. 2013;**34**(39):3035–3087.
12. Blaslov K, Bulum T, Knežević-Ćuća J, Duvnjak L. Relationship between autoantibodies combination, metabolic syndrome components and diabetic complications in autoimmune diabetes in adults. *Endocrine*. 2014;**48**(2):551–556.
13. Mollo A, Hernandez M, Marsal JR, Esquerda A, Rius F, Blanco-Vaca F, Verdaguer J, Pozzilli P, de Leiva A, Mauricio D; Action LADA 8. Latent autoimmune diabetes in adults is perched between type 1 and type 2: evidence from adults in one region of Spain. *Diabetes Metab Res Rev*. 2013;**29**(6):446–451.
14. Juhl CB, Bradley U, Holst JJ, Leslie RD, Yderstraede KB, Hunter S; Action LADA Consortium. Similar weight-adjusted insulin secretion and insulin sensitivity in short-duration late autoimmune diabetes of adulthood (LADA) and type 2 diabetes: Action LADA 8. *Diabet Med*. 2014;**31**(8):941–945.
15. Hernandez M, Mollo A, Marsal JR, Esquerda A, Capel I, Puig-Domingo M, Pozzilli P, de Leiva A, Mauricio D; Action LADA Consortium. Insulin secretion in patients with latent autoimmune diabetes (LADA): half way between type 1 and type 2 diabetes: Action LADA 9. *BMC Endocr Disord*. 2015;**15**(1):1.
16. Isomaa B, Almgren P, Henricsson M, Taskinen MR, Tuomi T, Groop L, Sarelin L. Chronic complications in patients with slowly progressing autoimmune type 1 diabetes (LADA). *Diabetes Care*. 1999;**22**(8):1347–1353.
17. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. *Nat Rev Dis Primers*. 2017;**3**:17016.
18. Szendroedi J, Saxena A, Weber KS, Strassburger K, Herder C, Burkart V, Nowotny B, Icks A, Kuss O, Ziegler D, Al-Hasani H, Müssig K, Roden M; GDS Group. Cohort profile: the German Diabetes Study (GDS). *Cardiovasc Diabetol*. 2016; **15**(1):59.
19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;**35**(Suppl 1):S64–S71.
20. Weber KS, Nowotny B, Strassburger K, Pacini G, Müssig K, Szendroedi J, Herder C, Roden M; GDS Group. The role of markers of low-grade inflammation for the early time course of glycemic control, glucose disappearance rate, and β-cell function in recently diagnosed type 1 and type 2 diabetes. *Diabetes Care*. 2015;**38**(9): 1758–1767.
21. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H; Type 1 Diabetes Trial Net Research Group, European C-Peptide Trial Study Group. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care*. 2008;**31**(10): 1966–1971.
22. Lohmann T, Kellner K, Verlohren HJ, Krug J, Steindorf J, Scherbaum WA, Seissler J. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia*. 2001;**44**(8):1005–1010.
23. Kaas A, Pfleger C, Hansen L, Buschard K, Schloot NC, Roep BO, Mortensen HB; Hvidøre Study Group on Childhood Diabetes. Association of adiponectin, interleukin (IL)-1ra, inducible protein 10, IL-6 and number of islet autoantibodies with progression patterns of type 1 diabetes the first year after diagnosis. *Clin Exp Immunol*. 2010;**161**(3):444–452.
24. Mortensen HB, Swift PG, Holl RW, Hougaard P, Hansen L, Bjoerndalen H, de Beaufort CE, Knip M; Hvidoere Study Group on Childhood Diabetes. Multinational study in children and adolescents with newly diagnosed type 1 diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis. *Pediatr Diabetes*. 2009;**11**(4):218–226.
25. Kahn R, Buse J, Ferrannini E, Stern M; American Diabetes Association, European Association for the Study of Diabetes. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*. 2005;**28**(9): 2289–2304.
26. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, Goldberg AC, Howard WJ, Jacobson MS, Kris-Etherton PM, Lennie TA, Levi M, Mazzone T, Pennathur S; American Heart Association Clinical Lipidology, Thrombosis, and Prevention Committee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*. 2011;**123**(20):2292–2333.
27. Lee SH, Kwon HS, Yoo SJ, Ahn YB, Yoon KH, Cha BY, Lee KW, Son HY. Identifying latent autoimmune diabetes in adults in Korea: the role of C-peptide and metabolic syndrome. *Diabetes Res Clin Pract*. 2009;**83**(2):e62–e65.
28. Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R, Mauricio D, De Leiva A, Yderstraede K, Beck-Neilsen H, Tuomilehto J, Sarti C, Thivolet C, Hadden D, Hunter S, Schernthaner G, Scherbaum WA, Williams R, Brophy S, Pozzilli P, Leslie RD; Action LADA Consortium. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. *Diabetes Care*. 2012;**36**(4):908–913.

29. Wilkin TJ. The convergence of type 1 and type 2 diabetes in childhood: the accelerator hypothesis. *Pediatr Diabetes*. 2011; **13**(4):334–339.
30. Cedillo M, Libman IM, Arena VC, Zhou L, Trucco M, Ize-Ludlow D, Pietropaolo M, Becker DJ. Obesity, islet cell autoimmunity, and cardiovascular risk factors in youth at onset of type 1 autoimmune diabetes. *J Clin Endocrinol Metab*. 2015;**100**(1): E82–E86.
31. Chung JO, Cho DH, Chung DJ, Chung MY. Associations among body mass index, insulin resistance, and pancreatic β-cell function in Korean patients with new-onset type 2 diabetes. *Korean J Intern Med (Korean Assoc Intern Med)*. 2012;**27**(1):66–71.
32. Pipi E, Marketou M, Tsirogianni A. Distinct clinical and laboratory characteristics of latent autoimmune diabetes in adults in relation to type 1 and type 2 diabetes mellitus. *World J Diabetes*. 2014;**5**(4): 505–510.
33. Brophy S, Brunt H, Davies H, Mannan S, Williams R. Interventions for latent autoimmune diabetes (LADA) in adults. *Cochrane Database Syst Rev*. 2007;(3):CD006165.
34. Li X, Zhou Z, Huang G, Su H, Yan X, Yang L. Metabolic syndrome in adult-onset latent autoimmune diabetes. *Metab Syndr Relat Disord*. 2005;**3**(2):174–180.
35. Marini MA, Fiorentino TV, Succurro E, Pedace E, Andreozzi F, Sciacqua A, Perticone F, Sesti G. Association between hemoglobin glycation index with insulin resistance and carotid atherosclerosis in non-diabetic individuals. *PLoS One*. 2017;**12**(4):e0175547.
36. Arranz Martín A, Lecumberri Pascual E, Brito Sanfiel MA, Andía Melero V, Nattero Chavez L, Sánchez López I, Cánovas Molina G, Arrieta Blanco F, González Perez Del Villar N; Grupo de Diabetes de la Sociedad de Endocrinología, Nutrición y Diabetes de Madrid (SENDIMAD). Clinical and metabolic profile of patients with latent autoimmune diabetes in adults in specialized care in Madrid. *Endocrinol Diabetes Nutr*. 2017;**64**(1):34–39.
37. Kaul K, Apostolopoulou M, Roden M. Insulin resistance in type 1 diabetes mellitus. *Metabolism*. 2015;**64**(12):1629–1639.
38. Nambam B, Aggarwal S, Jain A. Latent autoimmune diabetes in adults: a distinct but heterogeneous clinical entity. *World J Diabetes*. 2010;**1**(4):111–115.

4.2. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study

Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, Bodis K, Markgraf DF, Burkart V, Mussig K, Hwang JH, Asplund O, Groop L, Ahlqvist E, Seissler J, Nawroth P, Kopf S, Schmid SM, Stumvoll M, Pfeiffer AFH, Kabisch S, Tselmin S, Haring HU, Ziegler D, Kuss O, Szendroedi J, Roden M: Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *Lancet Diabetes Endocrinol* 2019; 7(9):684-694

Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study

*Oana P Zaharia, Klaus Strassburger, Alexander Strom, Gidon J Bönhof, Yanislava Karusheva, Sofia Antoniou, Kálmán Bódis, Daniel F Markgraf, Volker Burkart, Karsten Müssig, Jong-Hee Hwang, Olof Asplund, Leif Groop, Emma Ahlqvist, Jochen Seissler, Peter Nawroth, Stefan Kopf, Sebastian M Schmid, Michael Stumvoll, Andreas F H Pfeiffer, Stefan Kabisch, Sergey Tselmin, Hans U Häring, Dan Ziegler, Oliver Kuss, Julia Szendroedi, Michael Roden, for the German Diabetes Study Group**

Summary

Background Cluster analyses have proposed different diabetes phenotypes using age, BMI, glycaemia, homoeostasis model estimates, and islet autoantibodies. We tested whether comprehensive phenotyping validates and further characterises these clusters at diagnosis and whether relevant diabetes-related complications differ among these clusters, during 5-years of follow-up.

Methods Patients with newly diagnosed type 1 or type 2 diabetes in the German Diabetes Study underwent comprehensive phenotyping and assessment of laboratory variables. Insulin sensitivity was assessed using hyperinsulinaemic-euglycaemic clamps, hepatocellular lipid content using magnetic resonance spectroscopy, hepatic fibrosis using non-invasive scores, and peripheral and autonomic neuropathy using functional and clinical criteria. Patients were reassessed after 5 years. The German Diabetes Study is registered with ClinicalTrials.gov, number NCT01055093, and is ongoing.

Findings 1105 patients were classified at baseline into five clusters, with 386 (35%) assigned to mild age-related diabetes (MARD), 323 (29%) to mild obesity-related diabetes (MOD), 247 (22%) to severe autoimmune diabetes (SAID), 121 (11%) to severe insulin-resistant diabetes (SIRD), and 28 (3%) to severe insulin-deficient diabetes (SIDD). At 5-year follow-up, 367 patients were reassessed, 128 (35%) with MARD, 106 (29%) with MOD, 88 (24%) with SAID, 35 (10%) with SIRD, and ten (3%) with SIDD. Whole-body insulin sensitivity was lowest in patients with SIRD at baseline (mean 4·3 mg/kg per min [SD 2·0]) compared with those with SAID (8·4 mg/kg per min [3·2]; p<0·0001), MARD (7·5 mg/kg per min [2·5]; p<0·0001), MOD (6·6 mg/kg per min [2·6]; p=0·0011), and SIDD (5·5 mg/kg per min [2·4]; p=0·0035). The fasting adipose-tissue insulin resistance index at baseline was highest in patients with SIRD (median 15·6 [IQR 9·3–20·9]) and MOD (11·6 [7·4–17·9]) compared with those with MARD (6·0 [3·9–10·3]; both p<0·0001) and SAID (6·0 [3·0–9·5]; both p<0·0001). In patients with newly diagnosed diabetes, hepatocellular lipid content was highest at baseline in patients assigned to the SIRD cluster (median 19% [IQR 11–22]) compared with all other clusters (7% [2–15] for MOD, p=0·00052; 5% [2–11] for MARD, p<0·0001; 2% [0–13] for SIDD, p=0·0083; and 1% [0–3] for SAID, p<0·0001), even after adjustments for baseline medication. Accordingly, hepatic fibrosis at 5-year follow-up was more prevalent in patients with SIRD (n=7 [26%]) than in patients with SAID (n=5 [7%], p=0·0011), MARD (n=12 [12%], p=0·012), MOD (n=13 [15%], p=0·050), and SIDD (n=0 [0%], p value not available). Confirmed diabetic sensorimotor polyneuropathy was more prevalent at baseline in patients with SIDD (n=9 [36%]) compared with patients with SAID (n=10 [5%], p<0·0001), MARD (n=39 [15%], p=0·00066), MOD (n=26 [11%], p<0·0001), and SIRD (n=10 [17%], p<0·0001).

Interpretation Cluster analysis can characterise cohorts with different degrees of whole-body and adipose-tissue insulin resistance. Specific diabetes clusters show different prevalence of diabetes complications at early stages of non-alcoholic fatty liver disease and diabetic neuropathy. These findings could help improve targeted prevention and treatment and enable precision medicine for diabetes and its comorbidities.

Funding German Diabetes Center, German Federal Ministry of Health, Ministry of Culture and Science of the state of North Rhine-Westphalia, German Federal Ministry of Education and Research, German Diabetes Association, German Center for Diabetes Research, Research Network SFB 1116 of the German Research Foundation, and Schmutzler Stiftung.

Introduction

Findings of a Swedish cohort study published in 2018 have challenged the current paradigm of classifying patients with adult-onset diabetes mellitus;[1] patients were allocated into five clusters based on different pathophysiological and genetic profiles.[2] This analysis comprised an unbiased cluster allocation[1] using common variables such as autoimmunity, age at diagnosis, BMI,

Lancet Diabetes Endocrinol 2019

Published Online
July 22, 2019
http://dx.doi.org/10.1016/S2213-8587(19)30187-1

See Online/Comment
http://dx.doi.org/10.1016/S2213-8587(19)30218-9

*Members listed in the appendix

Institute for Clinical Diabetology (O P Zaharia MD, A Strom PhD, G J Bönhof MD, Y Karusheva MD, S Antoniou MD, K Bódis MD, D F Markgraf PhD, V Burkart PhD, K Müssig MD, J-H Hwang PhD, D Ziegler MD, J Szendroedi MD, M Roden MD), **and Institute for Biometrics and Epidemiology** (K Strassburger PhD, O Kuss PhD), **German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany; German Center for Diabetes Research, Munich, Germany** (J Seissler MD, P Nawroth MD, S Kopf MD, S M Schmid MD, M Stumvoll MD, A F H Pfeiffer MD, S Kabisch MD, S Tselmin MD, H U Häring MD, O P Zaharia, K Strassburger, A Strom, G J Bönhof, Y Karusheva, S Antoniou, K Bódis, D F Markgraf, V Burkart, K Müssig, J-H Hwang, D Ziegler, O Kuss, J Szendroedi, M Roden); **Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany** (S Antoniou, K Bódis, D Ziegler, J Szendroedi, M Roden); **Genomics, Diabetes and Endocrinology, Department of Clinical Sciences, Lund University, Skåne University Hospital, Malmö, Sweden** (O Asplund MSc, L Groop MD, E Ahlqvist MD); **Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig Maximilians Universität, and Clinical Cooperation Group Diabetes, Ludwig Maximilians Universität München, and Helmholtz Zentrum**

München, Munich, Germany (J Seissler); Department of Internal Medicine in Endocrinology and Metabolism, University Hospital Heidelberg, Heidelberg, Germany (P Nawroth, S Kopf); Department of Medicine 1 – Endocrinology and Diabetology, University of Lübeck, Lübeck, Germany (S M Schmid); Department of Medicine, Endocrinology and Nephrology, University of Leipzig, Leipzig, Germany (M Stumvoll); Department of Endocrinology, Diabetes and Nutrition, Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany (A F H Pfeiffer, S Kabisch); Department of Clinical Nutrition, German Institute of Human Nutrition, Potsdam-Rehbrücke, Berlin, Germany (A F H Pfeiffer, S Kabisch); Department of Medicine III, University Hospital Carl Gustav Carus, Dresden, Germany (S Tselmin); Department of Internal Medicine, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry and Institute of Diabetes Research and Metabolic Diseases, University Hospital Tübingen, Tübingen, Germany (H U Häring); and Institute of Medical Statistics, Düsseldorf University Hospital and Medical Faculty, Heinrich Heine University Düsseldorf, Germany (O Kuss)

Correspondence to: Dr Michael Roden, Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, 40225 Düsseldorf, Germany
michael.roden@ddz.de

See Online for appendix

Research in context

Evidence before this study

A study of Swedish and Finnish cohorts published in 2018 challenged the paradigm of classifying patients with diabetes. In that study, patients with adult-onset diabetes were categorised into five clusters based on autoimmunity, age at diagnosis, BMI, glycaemic control, and homoeostasis model assessment of β-cell function and surrogates of insulin resistance. Patients suggested to be insulin-resistant were at higher risk of diabetic nephropathy and—to some extent—cardiovascular diseases.

Added value of this study

In our study, we did comprehensive phenotyping of an independent cohort of patients at the time of diagnosis, with reassessment after 5 years. Whole-body and adipose-tissue insulin resistance distinguished clusters of patients with type 2 diabetes. Cluster analysis identified patients at incipient stages of diabetic neuropathy and those at increased risk for progression of non-alcoholic fatty liver disease (NAFLD) within 5 years after diagnosis. Measurement of more than one islet-directed antibody augmented the detection of patients with autoimmune diabetes. Cluster allocation changed during the course of disease in individuals at the periphery of a cluster.

Implications of all the available evidence

Distinct diabetes clusters can show specific risk patterns of diabetes-related complications. Present evidence advocates for further studies on these clusters to validate their role for targeted prevention and treatment of diabetic neuropathy and NAFLD in clusters at highest risk and their contribution to precision medicine in diabetes.

glycaemic control, and homoeostasis model estimates of β-cell function (HOMA-B) and insulin resistance (HOMA-IR).[3] This innovative approach was aimed at developing stratified treatment strategies, in line with the idea of precision medicine,[4,5] and has important implications for the diagnosis and management of diabetes and for predicting diabetes-related comorbidities. Although some evidence already exists for an association between cluster assignment and risk for nephropathy and cardiovascular diseases,[1] risk stratification for diabetic neuropathy and non-alcoholic fatty liver disease (NAFLD) using appropriate measurements has not been addressed so far.

Diabetic neuropathy is a prevalent disabling disorder with a wide pattern of symptoms, a cause that is subject to much debate, and a broad spectrum of risk factors.[6,7] However, little is known about the clinical and metabolic features predicting the development or progression of diabetic neuropathy. Similarly, NAFLD is frequently associated with diabetes and has emerged as a major risk factor for end-stage liver disease and is a predictor of cardiovascular disease.[8,9] Of note, risk for liver-related mortality grows exponentially with an increase in fibrosis stage.[10]

The prospective multicentre German Diabetes Study monitors the natural course of disease, from the first year after diagnosis, with a focus on comorbidities and complications, using comprehensive phenotyping.[11] Here, we aimed to examine whether measurements of insulin sensitivity and secretion by gold standard methods endorse the diabetes clusters proposed in the Swedish study.[1] We postulated that specific cluster-based subphenotypes differently correlate with diabetes-related complications, therefore requiring targeted risk factor management.

Methods

Study population

We included in our study patients newly diagnosed with type 1 or type 2 diabetes who were participants in the prospective German Diabetes Study.[11] Study group members and study centres are listed in the appendix (p 11). Patients had a known disease duration of less than 12 months, were aged 18–69 years, and underwent comprehensive phenotyping. Diagnosis of diabetes was based on American Diabetes Association criteria.[2] Specific exclusion criteria were diabetes of other causes (ie, monogenic diabetes syndromes, diseases of the exocrine pancreas, and gestational diabetes), pregnancy, and acute or severe chronic heart, hepatic, renal, or psychiatric diseases.

The German Diabetes Study is approved by the ethics boards of Heinrich Heine University, Düsseldorf, Germany (ref 4508), and of associated centres. The study was done according to the Declaration of Helsinki. All participants provided written informed consent.

Procedures

Routine laboratory variables were analysed in a centralised laboratory (German Diabetes Center, Düsseldorf, Germany), as previously described.[11] Patients underwent an identical protocol for blood sampling, following standard operating procedures, and diabetes-related autoantibodies were measured systematically in every study participant. In brief, glutamic acid decarboxylase antibodies were measured by a radioligand assay[11] (cutoff 2 U/mL),[12] islet-cell autoantibodies were measured by indirect immunofluorescence (cutoff 20 JDF [Juvenile Diabetes Foundation] units), and insulin autoantibodies were measured by radioimmunoassay (cutoff 0·4 U/mL).[12] The estimated glomerular filtration rate (eGFR) was calculated based on creatinine and cystatin C[13] and used to define kidney function as normal (stage 1, eGFR >90 mL/min per 1·73 m²), mildly impaired (stage 2, eGFR 60–90 mL/min per 1·73 m²), or moderately impaired (stage 3, eGFR <60 mL/min per 1·73 m²). Urinary albumin levels of 20–200 mg/L defined microalbuminuria and levels greater than 200 mg/L defined

macroalbuminuria.[14] The adipose-tissue insulin resistance index was calculated from fasting concentrations of insulin and free fatty acids.[15] The fatty liver index, NAFLD fibrosis score, and aspartate aminotransferase-to-platelet ratio index were calculated from routine laboratory variables.[16]

To measure insulin secretion and sensitivity, we did the modified Botnia clamp test, which consists of an intravenous glucose tolerance test followed by a hyperinsulinaemic-euglycaemic clamp test, with repeated measurements of blood glucose, C-peptide, and insulin concentrations, as previously described.[11] Total C-peptide secretion was ascertained from the incremental area under the curve (AUC) during the intravenous glucose tolerance test. Whole-body insulin sensitivity (M value) was assessed from mean glucose infusion rates during the steady state.[17] To assess hepatocellular lipid content, we did proton magnetic resonance spectroscopy with a stimulated echo acquisition mode (STEAM) sequence in a 3 T scanner (Achieva X-series, Philips Healthcare, Best, Netherlands), as described previously.[18]

To measure peripheral nerve function, we did electrophysiological testing, quantitative sensory testing, and clinical neuropathy score surveys, as described.[19] Motor nerve conduction velocity in the peroneal nerve, sensory nerve conduction velocity, sensory nerve action potentials in the sural nerve, and vibration and thermal detection thresholds were measured as described.[19] The neurological examination was done using the Neuropathy Disability Score, and neuropathic symptoms were assessed with the Neuropathy Symptom Score.[20] Diabetic sensorimotor polyneuropathy was defined according to modified Toronto Consensus criteria.[21]

To measure autonomic nerve function, we did cardiovascular autonomic reflex tests, including heart rate changes after the Valsalva manoeuvre (Valsalva ratio) and orthostatic posture (maximum-to-minimum 30:15 ratio), with findings recorded using a VariaCardio TF5 system (MIE Medical Research, Leeds, UK). The presence of three abnormal results among seven autonomic cardiovascular indices—ie, coefficient of variation, low-frequency and mid-frequency power spectrum at rest, mean circular resultant, postural change in systolic blood pressure, maximum-to-minimum 30:15 ratio, and Valsalva ratio—confirmed the diagnosis of cardiac autonomic neuropathy.[22,23]

To examine eye conditions in patients we did fundus photography. Images were assessed by trained ophthalmologists. Retinopathy was diagnosed in accordance with international guidelines.[24]

Statistical analysis

Patients with complete datasets at baseline and 5-year follow-up with respect to age, BMI, glycaemia, homoeostasis model estimates calculated using C-peptide values (HOMA-IR and HOMA-B), and glutamic acid decarboxylase antibodies were included in the analysis. At baseline, we applied the sex-specific classification rules published by Ahlqvist and colleagues[1] using the nearest centroid approach, so every patient was assigned to a predefined cluster—ie, mild age-related diabetes (MARD), mild obesity-related diabetes (MOD), severe insulin-resistant diabetes (SIRD), or severe insulin-deficient diabetes (SIDD). Patients with positive glutamic acid decarboxylase antibodies were allocated to the severe autoimmune diabetes (SAID) cluster.[1] The classification algorithm was applied again at 5-year follow-up to assess cluster migration patterns.

Data are presented as mean (SD), median (IQR), or proportion (%). Further logistic regression analyses were done to assess the predictive power of clustering and diabetes-related complications. Data are given as unadjusted AUCs and corresponding 95% CIs. Skewed data were log-transformed before analysis. To account for multiple group comparisons, Tukey-Kramer correction was applied. We judged p values less than 0·05 significant. Statistical analyses were done with SAS version 9.4 (SAS Institute, Cary, NC, USA). Figures were drawn using GraphPadPrism version 7.03 (GraphPad Software, San Diego, CA, USA).

The German Diabetes Study is registered at ClinicalTrials.gov, number NCT01055093, and is ongoing.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

1105 patients with newly diagnosed diabetes in the German Diabetes Study cohort were included in our study at baseline. Anthropometric and clinical data for these patients are shown in table 1, stratified by cluster. 247 (22%) of 1105 patients were assigned to the SAID cluster. Patients with SAID had positive glutamic acid decarboxylase antibodies and were more likely to be of a younger age, have relatively low BMI, have poor glycaemic control, and have overt insulin deficiency compared with patients allocated to other clusters. Furthermore, patients with SAID seemed to have a more favourable lipid profile compared with those allocated to other clusters. Among patients with SAID, 158 (67%) received insulin on diagnosis (appendix p 2). 28 (3%) of 1105 patients were assigned to the SIDD cluster and showed similarities with patients with SAID, but none had glutamic acid decarboxylase antibodies. Among patients with SIDD, 12 (44%) were treated with insulin on diagnosis (appendix p 2). 121 (11%) of 1105 patients were assigned to the SIRD cluster. Patients with SIRD were characterised by high BMI and whole-body and adipose-tissue insulin resistance compared with patients allocated to other clusters. 323 (29%) of 1105 patients were assigned to the MOD

	SAID (n=247)	SIDD (n=28)	SIRD (n=121)	MOD (n=323)	MARD (n=386)
Female	100 (40%)	5 (18%)	39 (32%)	148 (46%)	100 (26%)
Male	147 (60%)	23 (82%)	82 (68%)	175 (54%)	286 (74%)
Age (years)	37·7 (27·7–50·5)	43·8 (33·8–51·0)	58·6 (52·9–64·1)	45·7 (39·3–51·7)	58·8 (53·0–64·2)
BMI (kg/m²)	26·5 (5·4)	27·0 (3·7)	34·2 (4·5)	34·7 (6·4)	27·4 (3·4)
Waist-to-hip ratio	0·90 (0·09)	0·94 (0·06)	1·00 (0·08)	0·96 (0·09)	0·95 (0·08)
HOMA-B	56·1 (36·8–82·1)	39·3 (25·2–47·3)	172·7 (147·7–209·9)	96·7 (73·7–128·3)	86·0 (64·6–109·8)
HOMA-IR	1·1 (0·7–1·7)	1·7 (1·2–2·9)	3·9 (3·2–5·1)	2·7 (2·0–3·4)	1·9 (1·3–2·4)
Fasting blood glucose (mg/dL)	126 (34)	185 (58)	111 (29)	128 (29)	120 (25)
HbA_{1c} (%)	6·4% (0·9)	8·7% (1·3)	6·2% (0·7)	6·5% (0·9)	6·3% (0·7)
HbA_{1c} (mmol/mol)	46 (10)	72 (14)	44 (8)	48 (10)	45 (8)
hsCRP (mg/dL)	0·11 (0·06–0·27)	0·21 (0·08–0·42)	0·30 (0·18–0·55)	0·32 (0·16–0·60)	0·16 (0·08–0·32)
eGFR (mL/min per 1·73 m²)	98·2 (15·1)	104·5 (15·8)	78·2 (16·3)	93·1 (15·4)	87·9 (13·9)
Cystatin C (mg/L)	0·89 (0·13)	0·84 (0·13)	1·05 (0·19)	0·92 (0·16)	0·92 (0·14)
Total cholesterol (mg/dL)	186 (36)	199 (34)	198 (43)	200 (43)	199 (43)
LDL-cholesterol (mg/dL)	113 (32)	126 (32)	125 (38)	129 (36)	127 (36)
HDL-cholesterol (mg/dL)	57 (17)	51 (13)	43 (10)	45 (13)	50 (13)
Triglycerides (mg/dL)	87 (60–128)	148 (68–205)	160 (119–226)	139 (96–189)	120 (86–167)
FFA (µmol/L)	616 (252)	687 (279)	611 (203)	671 (230)	628 (241)
GADA >2 U/mL	247/247 (100%)	0/28 (0%)	0/121 (0%)	0/323 (0%)	0/386 (0%)
ICA >20 JDF	210/247 (85%)	3/28 (11%)	3/118 (3%)	13/312 (4%)	8/378 (2%)
IAA >0·4 U/mL	97/203 (48%)	3/17 (18%)	7/98 (7%)	18/250 (7%)	8/286 (3%)

Data are n (%), mean (SD), or median (IQR). SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes. HOMA-B=homoeostatic assessment model for β-cell function. HOMA-IR=homoeostatic assessment model for insulin resistance. hsCRP=high-sensitivity C-reactive protein. eGFR=estimated glomerular filtration rate. FFA=free fatty acids. GADA=glutamic acid decarboxylase autoantibodies. ICA=islet-cell autoantibodies. JDF=Juvenile Diabetes Foundation units. IAA=insulin autoantibodies.

Table 1: **Patients' characteristics at baseline, by cluster allocation**

cluster. Similar to patients with SIRD, individuals in the MOD cluster were characterised by obesity and substantial adipose tissue insulin resistance, but they had moderate whole-body insulin resistance. 386 (35%) of 1105 patients were assigned to the MARD cluster. Patients with MARD were generally older than those in other clusters and showed only minor metabolic abnormalities. By measuring islet-cell autoantibodies and insulin autoantibodies, 59 (7%) of 858 patients in clusters other than SAID were identified as having autoimmune diabetes at baseline.

Of 421 eligible patients, 54 (13%) dropped out during the period between baseline and the 5-year reassessment. 367 patients with baseline data were reassessed in our study after 5 years; baseline data for these individuals are shown in the appendix (p 3). Patients' characteristics at the 5-year reassessment are shown in table 2, stratified by cluster at baseline. Six (2%) of 279 patients in clusters other than SAID developed glutamic acid decarboxylase antibodies during this 5-year period.

Drugs taken by the study population at baseline and follow-up are shown in the appendix (p 2). After 5 years of disease progression, overall use of glucose-lowering drugs (p<0·0001), lipid-lowering drugs (p<0·0001), and blood pressure-lowering drugs (p<0·0001) increased in all clusters.

The metabolic characteristics of patients with newly diagnosed diabetes, and the changes in these variables over 5 years, are shown in figure 1; p values for all comparisons are shown in the appendix (pp 4–6). Fasting blood glucose was highest in patients assigned to the SIDD cluster at baseline compared with all other clusters (figure 1A). After 5 years of disease progression, patients in all clusters had achieved similar fasting blood glucose levels, with decreased levels in patients with SIDD and increased levels in all other clusters (figure 1A). At baseline, C-peptide secretory capacity during the intravenous glucose tolerance test was lowest in the SAID cluster (median 29 ng/dL [IQR 13–61]) and SIDD cluster (38 ng/dL [23–53]) compared with other clusters (SIRD 175 ng/dL [107–235], p<0·0001; MOD 104 ng/dL [71–163], p<0·0001; and MARD 94 [58–129], p<0·0001; figure 1B). These results were similar at 5-year follow-up, with patients in the SAID cluster showing lower total C-peptide secretion (median 5 ng/dL [IQR 0–11]) compared with those with SIRD (124 ng/dL [58–196], p<0·0001), MOD (71 ng/dL [38–117], p<0·0001), and MARD (94 ng/dL [58–129], p<0·0001), and similar values in the SIDD cluster (18 ng/dL [4–37], p=0·80), indicating the progressive reduction of β-cell reserve in these two clusters. After 5 years of disease progression, C-peptide secretion had declined significantly in patients with SAID and MARD (figure 1B). Whole-body insulin sensitivity was lowest in patients with SIRD at baseline (mean 4·3 mg/kg per min [SD 2·0]) compared with those

	SAID (n=88)	SIDD (n=10)	SIRD (n=35)	MOD (n=106)	MARD (n=128)
Female	36 (41%)	1 (10%)	8 (23%)	46 (43%)	31 (24%)
Male	52 (59%)	9 (90%)	27 (77%)	60 (57%)	97 (76%)
Age (years)	39·6 (31·3–54·6)	43·0 (34·4–50·4)	60·7 (56·6–69·8)	50·2 (43·3–56·7)	64·4 (57·6–69·5)
BMI (kg/m²)	27·1 (5·5)	27·4 (5·9)	35·0 (4·7)	34·7 (5·9)	28·3 (3·7)
Waist-to-hip ratio	0·88 (0·08)	0·93 (0·05)	1·03 (0·06)	0·96 (0·08)	0·96 (0·06)
HOMA-B	32·3 (22·4–62·6)	34·5 (19·8–49·3)	109·3 (77·3–143·0)	61·5 (42·0–93·8)	72·6 (50·0–94·4)
HOMA-IR	1·3 (0·8–2·0)	1·1 (0·8–1·5)	4·1 (3·4–5·8)	2·7 (2·1–3·7)	2·1 (1·7–2·8)
Fasting blood glucose (mg/dL)	156 (55)	140 (45)	149 (42)	169 (59)	140 (33)
HbA_{1c} (%)	7·1% (1·0)	7·3% (1·6)	6·7% (0·8)	7·3% (1·4)	6·7% (0·8)
HbA_{1c} (mmol/mol)	54 (11)	56 (17)	49 (9)	56 (16)	49 (9)
hsCRP (mg/dL)	0·12 (0·06–0·33)	0·18 (0·05–0·23)	0·31 (0·19–0·55)	0·27 (0·13–0·39)	0·15 (0·08–0·29)
eGFR (mL/min per 1·73 m²)	97·8 (16·2)	98·2 (8·1)	72·9 (17·3)	92·3 (16·2)	84·8 (14·3)
Cystatin C (mg/L)	0·90 (0·13)	0·91 (0·08)	1·15 (0·21)	0·96 (0·17)	0·96 (0·15)
Total cholesterol (mg/dL)	187 (39)	186 (34)	202 (49)	205 (39)	204 (44)
LDL-cholesterol (mg/dL)	114 (34)	121 (32)	126 (39)	131 (35)	131 (40)
HDL-cholesterol (mg/dL)	64 (20)	58 (12)	43 (11)	45 (14)	52 (16)
Triglycerides (mg/dL)	76 (61–116)	73 (51–95)	186 (132–298)	161 (102–258)	131 (93–194)
FFA (µmol/L)	644 (337)	611 (311)	619 (246)	688 (257)	613 (234)
GADA >2 U/mL	73/89 (82%)	2/10 (20%)	1/35 (3%)	1/106 (1%)	2/128 (2%)
ICA >20 JDF	68/87 (78%)	4/10 (40%)	2/34 (6%)	7/105 (7%)	4/123 (3%)
IAA >0·4 U/mL	69/89 (78%)	8/10 (80%)	0/35 (0%)	15/106 (14%)	6/128 (5%)

Data are n (%), mean (SD), or median (IQR). SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes. HOMA-B=homoeostatic assessment model for β-cell function. HOMA-IR=homoeostatic assessment model for insulin resistance. hsCRP=high-sensitivity C-reactive protein. eGFR=estimated glomerular filtration rate. FFA=free fatty acids. GADA=glutamic acid decarboxylase autoantibodies. ICA=islet-cell autoantibodies. JDF=Juvenile Diabetes Foundation units. IAA=insulin autoantibodies.

Table 2: **Patients' characteristics at 5-year follow-up, by cluster allocation**

with SAID (8·4 mg/kg per min [3·2], p<0·0001), MARD (7·5 mg/kg per min [2·5], p<0·0001), MOD (6·6 mg/kg per min [2·6], p=0·0011), and SIDD (5·5 mg/kg per min [2·4]; p=0·0035). Whole-body insulin sensitivity was similar to HOMA-IR estimates, even after adjustments for medication at baseline. At 5-year follow-up, insulin sensitivity was lowest in patients with SIRD (mean 3·9 mg/kg per min [SD 1·3]) and MOD (5·2 mg/kg per min [2·3]) compared with the other clusters, but insulin sensitivity also decreased significantly from baseline over the 5-year period in patients with SAID (8·9 mg/kg per min [2·9] *vs* 6·5 mg/kg per min [2·5], p=0·0071) and MARD (7·4 mg/kg per min [2·3] *vs* 6·2 mg/kg per min [2·2], p<0·0001) irrespective of statistical adjustments for baseline medication (figure 1C). Patients with MOD and SIRD had the highest levels of high-sensitivity C-reactive protein at both baseline and 5-year follow-up compared with individuals assigned to other clusters (figure 1D). Levels of high-sensitivity C-reactive protein decreased significantly over 5 years in patients with MOD (figure 1D). Serum triglycerides were lowest in patients assigned to the SAID cluster compared with those in other clusters (figure 1E). Amounts of serum triglycerides increased significantly in patients with MOD and MARD over 5 years (figure 1E). The fasting adipose tissue insulin resistance index was highest in patients with SIRD and MOD at baseline (respectively, median 15·6 [IQR 9·3–20·9] and 11·6 [7·4–17·9]) compared with those with MARD (6·0 [3·9–10·3], both p<0·0001) and SAID (6·0 [3·0–9·5], both p<0·0001), but not SIDD (9·4 [6·5–14·8]), p=0·096 and p=0·78, respectively). At 5-year follow-up, adipose tissue insulin resistance was similar across groups (median 17·3 [IQR 7·4–24·3] for SIRD, 11·7 [6·8–19·9] for MOD, 6·6 [4·0–11·1] for MARD, 4·6 [1·8–21·8] for SIDD, and 4·5 [2·4–20·4] for SAID; differences were only significant for SIRD *vs* MARD [p=0·0081] and SAID [p<0·0001]).

Markers of liver steatosis and fibrosis are shown in figure 2; p values for all comparisons are shown in the appendix (pp 7, 8). In patients with newly diagnosed diabetes, hepatocellular lipid content was highest at baseline in patients assigned to the SIRD cluster (median 19% [IQR 11–22]) compared with all other clusters (7% [2–15] for MOD, p=0·00052; 5% [2–11] for MARD, p<0·0001; 2% [0–13] for SIDD, p=0·0083; and 1% [0–3] for SAID, p<0·0001), even after adjustments for baseline medication. After 5 years, hepatocellular lipid content was comparable between patients with SIRD (median 22% [IQR 8–24]), MOD (12% [6–20]), and MARD (8% [1–14]), lower in SIDD (1% [0–2]), but remained lowest in patients with SAID (median 0% [IQR 0–2], p<0·0001 *vs* SIRD, p<0·0001 *vs* MOD, and p=0·028 *vs* MARD). Clustering showed a predictive value (AUC) of 0·61 (95% CI 0·56–0·66] for NAFLD, defined as hepatocellular lipid content greater

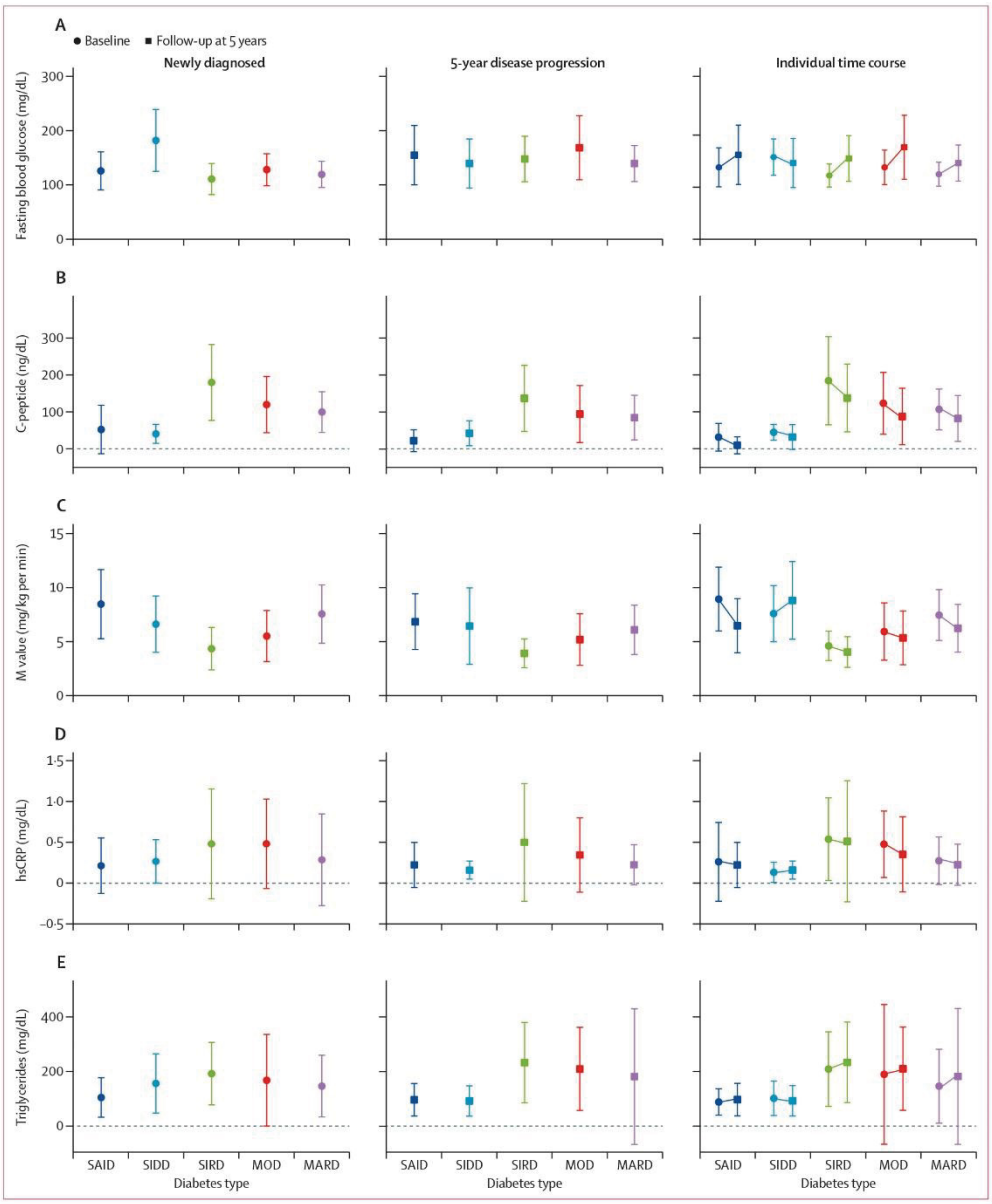


Figure 1: **Progression of metabolic variables over 5 years**
Metabolic characteristics of patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD were assessed at baseline and after 5 years of disease progression. Plots show fasting blood glucose (A), total C-peptide secretion during the intravenous glucose tolerance test (B), insulin sensitivity (C), hsCRP levels (D), and triglycerides (E). The individual time course of each of the parameters is shown for patients who had data available at both baseline (circles) and follow-up (squares). Data are mean; whiskers show SD. Data were ln-transformed when applicable. Exact p values for all comparisons are provided in the appendix (pp 4–6). SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes. hsCRP=high-sensitivity C-reactive protein.

www.thelancet.com/diabetes-endocrinology **Published online July 22, 2019 http://dx.doi.org/10.1016/S2213-8587(19)30187-1**

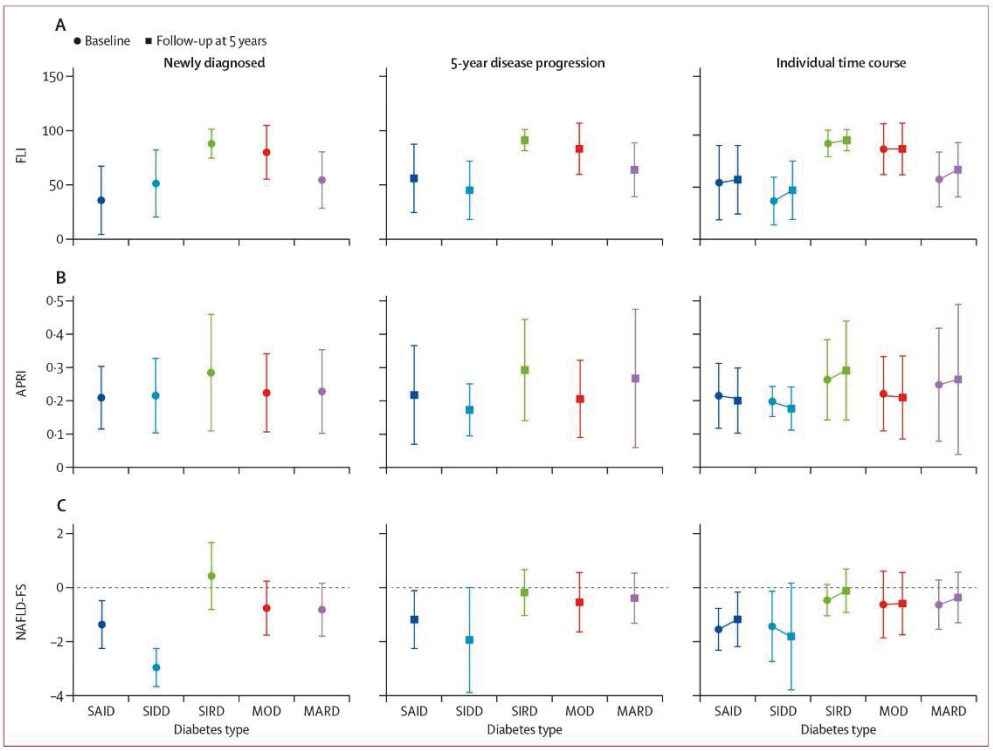


Figure 2: **Progression of liver steatosis and liver fibrosis over 5 years**
Liver testing was done at baseline and after 5 years of disease progression in patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD. Liver steatosis was assessed non-invasively using FLI (A), liver fibrosis was measured by calculating APRI (B), and NAFLD was assessed with the NAFLD-FS (C). The individual time course of each of the variables is shown for patients who had data available at both baseline (circles) and follow-up (squares). Data are mean; whiskers show SD.
Exact p values for all comparisons are provided in the appendix (pp 7, 8). SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes. FLI=fatty liver index. APRI=aspartate aminotransferase-to-platelet ratio index. NAFLD-FS=non-alcoholic fatty liver disease fibrosis score.

than 5%. The fatty liver index was highest at baseline in patients assigned to the SIRD cluster (mean 88 [SD 13]) compared with all other clusters (80 [25] for MOD, p=0·0004; 54 [27] for MARD, p<0·0001; 52 [31] for SIDD, p<0·0001; and 36 [31] for SAID, p<0·0001; figure 2A). At 5 years, patients assigned to the SIRD cluster (mean 91 [SD 10]) and MOD cluster (83 [24]) had the highest fatty liver index values compared with patients in the MARD cluster (64 [25], both p<0·0001), SIDD cluster (45 [27], p=0·0016 and p=0·017, respectively), and SAID cluster (54 [31], p<0·0001 and p=0·00084, respectively). Patients with MARD showed a significant increase over 5 years in the fatty liver index (p=0·00079; figure 2A). The fatty liver index value correlated positively with hepatocellular lipid content (r=0·69; p<0·0001). Patients with MARD showed a significant increase over 5 years in the aspartate aminotransferase-to-platelet ratio index (figure 2B), whereas the NAFLD fibrosis score increased significantly in patients with SAID, SIRD, and MARD (figure 2C). Both the aspartate aminotransferase-to-platelet ratio index (figure 2B) and NAFLD fibrosis score (figure 2C) showed highest estimates of hepatic fibrosis at baseline for patients assigned to SIRD cluster. At 5-year follow-up, the prevalence of hepatic fibrosis (NAFLD fibrosis score >0·6) was highest in patients with SIRD (n=7 [26%]) compared with those with SAID (n=5 [7%]; p=0·0011), MARD (n=12 [12%]; p=0·012), MOD (n=13 [15%]; p=0·050), and SIDD (n=0 [0%]; p value not available).

The prevalence of nephropathy and neuropathy is presented for each cluster in figure 3, at baseline and at 5-year follow-up; numbers of patients are shown in the appendix (pp 9, 10). Patients in the SIRD cluster had the lowest eGFR at baseline (mean 78·2 mL/min per 1·73 m² [SD 16·3]) compared with those with SAID (98·2 mL/min per 1·73 m² [15·1], p<0·0001), SIDD (104·5 mL/min per 1·73 m² [15·8], p<0·0001), MOD (93·1 mL/min per 1·73 m² [15·4], p<0·0001), and MARD (87·9 mL/min per 1·73 m² [13·9], p<0·0001; table 1). Results were similar at 5-year follow-up, with the lowest eGFR in the SIRD cluster (mean 72·9 mL/min

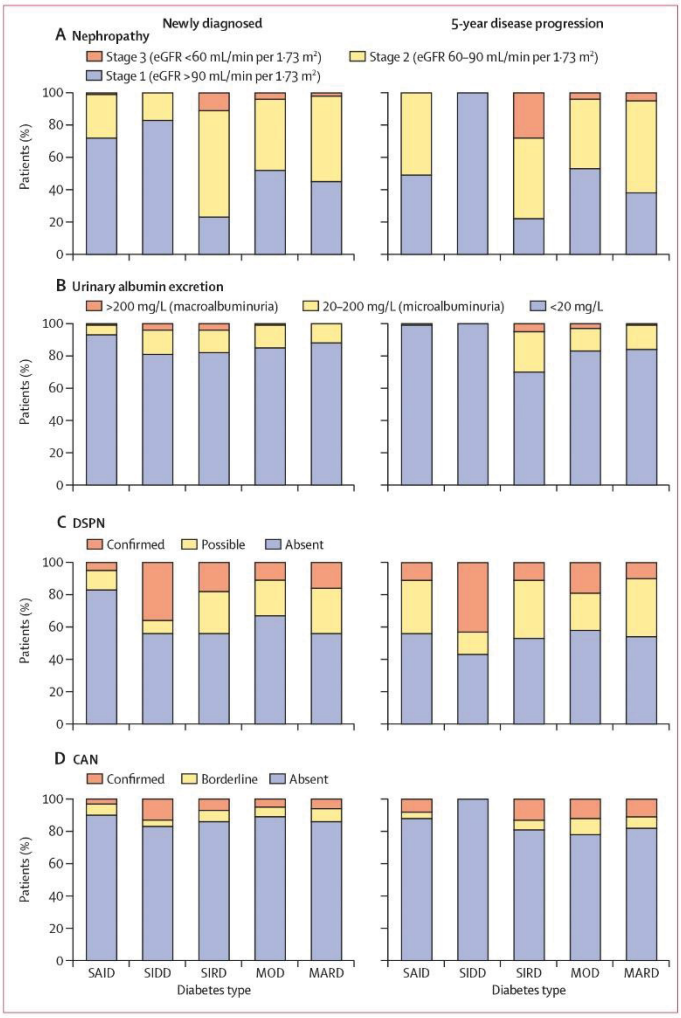


Figure 3: **Progression of diabetes-associated nephropathy and neuropathy over 5 years**
Diabetic nephropathy (A, B) was assessed non-invasively by eGFR at baseline and after 5 years of disease progression in patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD. DSPN (C) and CAN (D) were assessed in patients with newly diagnosed diabetes and after 5 years of disease progression, and these disorders were categorised according to international criteria.[21] Numbers of patients (%) are shown in the appendix (pp 9, 10). eGFR=estimated glomerular filtration rate. SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes. DSPN=diabetic sensorimotor polyneuropathy. CAN=cardiac autonomic neuropathy.

per 1·73 m² [SD 17·3]) compared with those with SAID (97·8 mL/min per 1·73 m² [16·2], p<0·0001), SIDD (98·2 mL/min per 1·73 m² [8·1], p<0·0001), MOD (92·3 mL/min per 1·73 m² [16·2], p<0·0001), and MARD (84·8 mL/min per 1·73 m² [14·3], p=0·0045; table 2). This finding is consistent with having the highest prevalence of stage 2 and stage 3 nephropathy at baseline in the SIRD cluster (n=86 [77%]) compared with SAID (n=64 [30%]; p<0·0001), MARD (n=183 [55%]; p=0·0010), SIDD (n=4 [17%]; p<0·0001) and MOD (n=125 [45%]; p<0·0001; figure 3A). Similar results were noted for urinary albumin excretion in the SIRD cluster at 5-year follow-up (figure 3B). The AUC for diabetic nephropathy was 0·64 (95% CI 0·61–0·67).

The prevalence of diabetic sensorimotor polyneuropathy is shown in figure 3C, and the prevalence of cardiac autonomic neuropathy is shown in figure 3D, both stratified for confirmed and borderline neuropathy. At baseline, patients with SIDD showed the highest prevalence of confirmed diabetic sensorimotor polyneuropathy and cardiac autonomic neuropathy. At baseline, confirmed diabetic sensorimotor polyneuropathy (figure 3C) was most prevalent in patients with SIDD (n=9 [36%]) compared with those with SAID (n=10 [5%]; p<0·0001), SIRD (n=10 [17%]; p<0·0001), MOD (n=26 [11%]; p<0·0001), and MARD (n=39 [15%]; p=0·00066). At 5-year follow-up, patients with SIDD also had the highest prevalence of confirmed diabetic sensorimotor polyneuropathy (n=3 [50%]) compared with those with SAID (n=2 [12%], p<0·0001), SIRD (n=3 [12%], p<0·0001), MOD (n=14 [17%], p<0·0001), and MARD (n=9 [9%], p<0·0001). Clustering had a predictive value of 63% for diabetic sensorimotor polyneuropathy (AUC 0·63, 95% CI 0·59–0·66). At baseline, borderline and confirmed cardiac autonomic neuropathy (figure 3D) were most prevalent in patients with SIDD (n=4 [18%]) compared with those with SAID (n=18 [10%], p=0·0045), MOD (n=26 [10%], p=0·014), and MARD (n=33 [13%], p=0·029), and prevalence was similar to SIRD (n=8 [15%]; p=0·11). After 5 years, the prevalence of confirmed cardiac autonomic neuropathy (figure 3D) was similar in patients with SAID (n=2 [8%]), SIRD (n=4 [13%]), MOD (n=9 [11%]), and MARD (n=10 [11%]), whereas no patients with SIDD had this disorder, most likely attributable to the low number of observations in patients with SIDD at the 5-year timepoint.

Because of the few overt cases of diabetic retinopathy in our study population, this disorder could not be comparatively assessed.

The pattern of cluster distribution at 5-year follow-up is shown in a Sankey diagram (figure 4). Overall, of all patients with available data at both baseline and follow-up, 85 (23%) switched cluster allocation at 5-year follow-up. Analysing specific metabolic variables showed that changes in glycaemia (fasting blood glucose and HbA_{1c}) and fasting serum triglycerides were associated with migration from the moderate clusters (ie, MOD and MARD) to SIDD. Furthermore, migration from MARD to SIDD was linked to increases in fatty liver index value.

Discussion

The findings of our study, which used comprehensive phenotyping, show four key points. First, patients who would usually be classified with type 2 diabetes present in clusters with different whole-body and adipose-tissue insulin resistance and with distinct patterns of

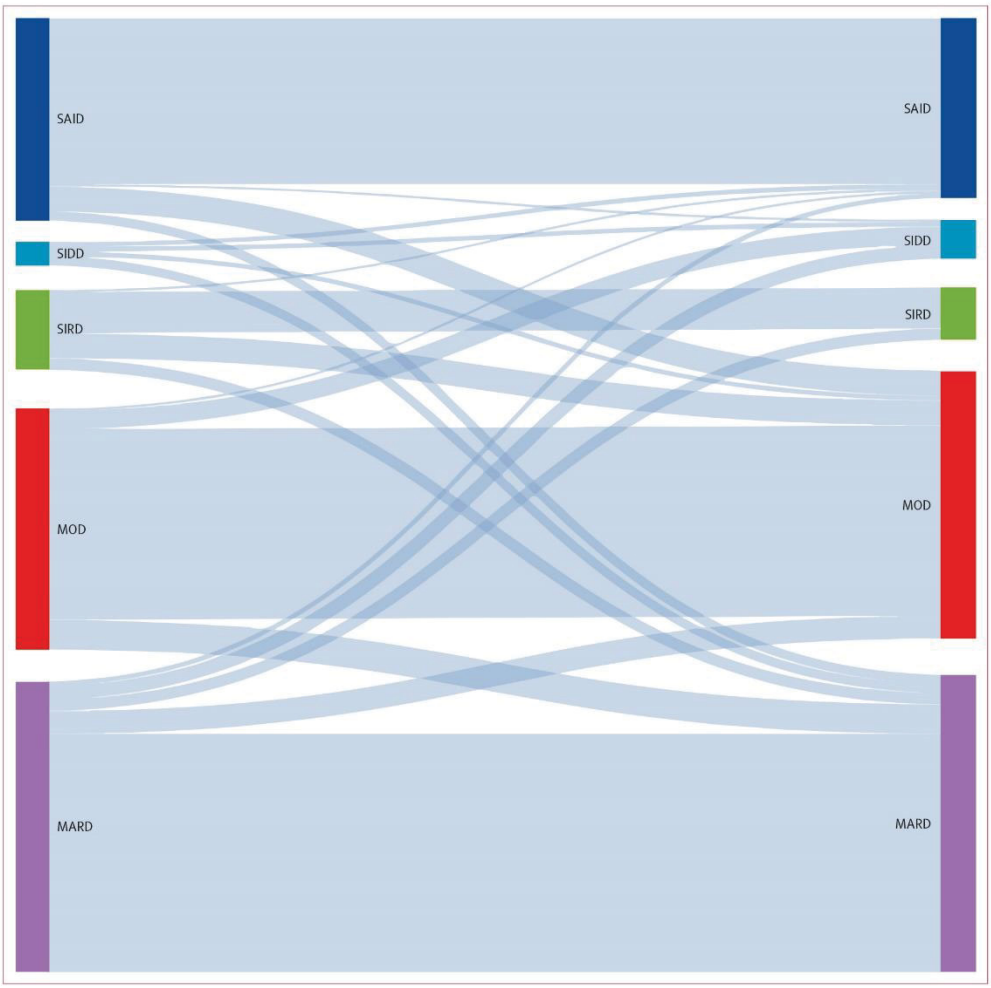


Figure 4: **Cluster redistribution at 5-year follow-up**
The Sankey diagram shows the redistribution and migration pattern of the study population from baseline to 5-year follow-up. Cluster reproducibility at follow-up (ie, the proportion of patients allocated to the same cluster at baseline and follow up) was 20% SIDD, 82% SAID, 51% SIRD, 79% MOD, and 82% MARD. SAID=severe autoimmune diabetes. SIDD=severe insulin-deficient diabetes. SIRD=severe insulin-resistant diabetes. MOD=moderate obesity-related diabetes. MARD=moderate age-related diabetes.

diabetes-related comorbidities early after diagnosis. Second, patients within these clusters show different progression of diabetes-related complications within 5 years after diagnosis. Third, cluster membership can change as the disease progresses. Finally, measurement of more than one islet-directed antibody can lead to more patients being classified with autoimmune diabetes.

A major novel finding of our study is that whole-body insulin resistance endorses the findings of the cluster algorithm reported in the Swedish study[1] and these clusters show differences in the prevalence of NAFLD. The SIRD cluster presented with striking whole-body insulin resistance as assessed by the hyperinsulinaemic-euglycaemic clamp test. Of note, the M value mainly reflects insulin-stimulated skeletal muscle glucose uptake,[25] whereas HOMA-IR (used for the cluster analysis) serves as an index of fasting hepatic insulin resistance.[3] These data also show that patients with SIRD are truly insulin-resistant and increased C-peptide and HOMA-IR are not merely a result of reduced C-peptide clearance in patients with impaired kidney function. Our findings further show that fasting adipose-tissue insulin sensitivity is also lowest in patients with SIRD, indicative of whole-body insulin resistance under both fasting and

insulin-stimulated conditions. Patients in the MOD cluster also showed severe adipose-tissue insulin resistance but only moderate whole-body (muscle) insulin resistance. This finding underlines the importance of adipose-tissue function for the development of whole-body insulin resistance and diabetes in obese people. Moreover, amounts of fasting serum triglycerides and—to some extent—high-sensitivity C-reactive protein were increased in patients with SIRD and MOD, highlighting increased lipid availability and low-grade inflammation as key drivers in the pathogenesis of these specific clusters. Indeed, there is growing evidence from preclinical studies that supports the idea of a primary role of the adipose tissue and lipotoxicity[26] in the development of insulin resistance. The higher insulin sensitivity of patients with SAID could also be attributable to reduction of lipid-induced insulin resistance accredited to the recorded favourable lipid profile,[27] which might in turn be linked to preserved insulin action and signalling.[28]

Cross-sectional analyses have shown that patients with type 2 diabetes frequently have increased hepatocellular lipid content,[26] which is also associated with increased insulin resistance. In turn, SIRD is associated with an increased prevalence of NAFLD and liver fibrosis, even after adjustments for baseline medication, reinforcing previous observations. Mechanistically, this finding could be attributable to impaired mitochondrial biogenesis linked to NAFLD progression.[29] Notably, although the recorded differences suggest early hepatic alterations, non-invasive scores such as the fatty liver index are only moderate estimates,[30] which are used in the absence of liver biopsy findings[8] and do not represent definitive diagnostic methods.

Insulin resistance has also been associated with impaired renal function,[31] and patients with SIRD show accelerated progression of diabetic kidney disease.[1] Our findings confirm this association, in that patients assigned to the SIRD cluster had decreased eGFR and increased cystatin-C levels, both at baseline and 5-year follow-up, despite good metabolic control, suggesting a superior role of insulin resistance compared with glycaemia for the onset and early progression of diabetic nephropathy.

Moreover, our findings indicate that insulin deficiency or hyperglycaemia are important triggers of diabetic neuropathy, with the highest prevalence recorded in patients with SIDD. Patients assigned to the SAID and SIDD clusters had the lowest β-cell reserves and were treated preferentially with insulin; yet, preserved glucose homoeostasis in patients with SIDD at 5-year follow-up did not restore neuronal signalling and nerve function. Thus, patients in the SIDD cluster would benefit from use of sensitive diagnostic methods for early detection and prediction of diabetic neuropathy and prevention of major clinical outcomes.[7] However, options for preventing diabetic neuropathy remain scarce and the predictive value and the possible efficacy of targeted treatment using the clustering approach will require future controlled intervention trials. In our study, the predictive value of diabetes-related complications deriving from cluster analysis showed only modest results. Of note, applying the clustering algorithm in patients with 5-year disease duration yielded a reproducibility of only 77%. This finding indicates that cluster membership is not an immutable feature, but could be affected by alterations in triglycerides, liver steatosis, and glucose homoeostasis and heterogeneous treatment over time.

In our study, the distribution of clusters differed slightly from that reported in the population-based Swedish cohort;[1] the prevalence of SAID was higher in our study, whereas the distribution of the other clusters largely followed the Swedish pattern. This higher prevalence results from active recruitment of patients with autoimmune diabetes. In the Swedish study per cluster definition,[1] patients with SAID were included based on positive glutamic acid decarboxylase antibodies alone. In our study, by using more than one islet-cell directed autoantibody, another 59 patients (7%) were identified with autoimmune diabetes. Of note, six (2%) patients with negative glutamic acid decarboxylase antibodies at baseline tested positive at 5-year follow-up. Taken together, these findings support the need for comprehensive islet-cell autoantibody screening in patients with newly diagnosed diabetes to ensure a targeted therapeutic approach and to avoid inadequate treatment of hyperglycaemia.[32]

Our study benefits from comprehensive neurofunctional testing and gold standard metabolic phenotyping of insulin sensitivity and steatosis. Conversely, the recruitment strategy of the German Diabetes Study cohort is not population-based with distinct inclusion and exclusion criteria, which probably affects the number of patients allocated to specific clusters. For example, the lower prevalence of SIDD could be linked to the exclusion criterion of HbA_{1c} less than 9%.[11] Moreover, few patients have been followed up because the German Diabetes Study is ongoing. Because the German Diabetes Study is not representative of the general population,[11] our results cannot be generalised to community-based practice. Furthermore, psychosocial variables—eg, personal motivation and the varied treatment among participants—could have affected cluster membership and its change over time. Despite drug withdrawal before the metabolic tests and suitable statistical adjustments, glucose-lowering medication with specific effects on insulin secretion or action, as well as lipid-lowering and antihypertensive medication indirectly modulating insulin sensitivity, might have affected phenotypic measurements.

In conclusion, our study shows that patients with newly diagnosed diabetes can be allocated to specific clusters that show distinct metabolic alterations and different risk patterns for development of diabetes-related comorbidities and complications. These results underline the need for comprehensive diabetes-related autoimmunity screening in all patients with diabetes. In particular, we identified the highest prevalence of NAFLD in patients with SIRD and

the highest prevalence of diabetic neuropathy in the SIDD cluster, and these findings advocate for targeted prevention and early treatment in these subgroups of patients.

Contributors

MR is the primary investigator of the German Diabetes Study and had the idea for this cluster analysis. OPZ wrote the first draft of the report. KS, OA, and OK did statistical analyses. AS, GJB, YK, SA, KB, DFM, VB, KM, J-HH, LG, EA, JSe, PN, SKo, SMS, MS, AFHP, SKa, ST, HUH, DZ, JSz, and MR researched data, contributed to the discussion, and reviewed and edited the report. All authors contributed to data acquisition, data analysis, data interpretation, revision of the report, and have read and approved the final report.

Declaration of interests

MR reports personal fees from Eli Lilly, Novo Nordisk, Poxel SA, Boehringer-Ingelheim Pharma, Terra Firma, Sanofi US, Servier Labatories, Prosciento, and Fishawack Group. AFHP reports personal fees from Boehringer-Ingelheim and Lilly. PN reports grants from the German Diabetes Center (funded by the German Federal Ministry of Education and Research); and grants from Novo Nordisk. SMS reports a grant from the German Center for Diabetes Research. DZ reports grants and personal fees from Mitsubishi Tanube and Wörwag; and personal fees from Pfizer, TrigoCare, Allergan, Berlin-Chemie, Astellas, Meda, Novartis, Novaremed, Takeda, AstraZeneca, and Impeto Medical. OPZ, KS, AS, GJB, YK, SA, KB, DFM, VB, KM, J-HH, OA, LG, EA, JSe, SKo, MS, Ska, ST, HUH, OK, and JSz declare no competing interests.

Data sharing

The datasets generated or analysed during the current study are not available publicly because they are subject to national data protection laws and restrictions imposed by the ethics committee to ensure privacy of study participants. However, they can be applied for through an individual project agreement with the principal investigator of the German Diabetes Study. The study protocol and the individual methods have been published in the cohort profile[11] and are unrestrictedly available.

Acknowledgments

We thank staff involved in the conduct of the German Diabetes Study for technical help and support. The German Diabetes Study was initiated and financed by the German Diabetes Center (which is funded by the German Federal Ministry of Health and the Ministry of Culture and Science of the state of North Rhine-Westphalia), the German Diabetes Association, the German Federal Ministry of Education and Research (to the German Center for Diabetes Research), Research Network SFB 1116 of the German Research Foundation, and the Schmutzler Stiftung.

References

1 Ahlqvist E, Storm P, Käräjämäki 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:** 361–69.
2 American Diabetes Association. 2: classification and diagnosis of diabetes—standards of medical care in diabetes, 2018. *Diabetes Care* 2018; **41** (suppl 1): S13–27.
3 Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; **27:** 1487–95.
4 Stidsen JV, Henriksen JE, Olsen MH, et al. Pathophysiology-based phenotyping in type 2 diabetes: a clinical classification tool. *Diabetes Metab Res Rev* 2018; **34:** e3005.
5 Prasad RB, Groop L. Precision medicine in type 2 diabetes. *J Intern Med* 2019; **285:** 40–48.
6 Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol* 2012; **11:** 521–34.
7 Bönhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging biomarkers, tools, and treatments for diabetic polyneuropathy. *Endocr Rev* 2019; **40:** 153–92.
8 European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64:** 1388–402.
9 Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017; **14:** 32–42.
10 Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology* 2017; **65:** 1557–65.
11 Szendroedi J, Saxena A, Weber KS, et al. Cohort profile: the German Diabetes Study (GDS). *Cardiovasc Diabetol* 2016; **15:** 59.
12 Zaharia OP, Bobrov P, Strassburger K, et al. Metabolic characteristics of recently diagnosed adult-onset autoimmune diabetes mellitus. *J Clin Endocrinol Metab* 2018; **103:** 429–37.
13 Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367:** 20–29.
14 Levey AS, Coresh J, Balk E, et al. National kidney foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139:** 137–47.
15 Gastaldelli A, Gaggini M, DeFronzo RA. Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the San Antonio Metabolism Study. *Diabetes* 2017; **66:** 815–22.
16 Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. *Hepatology* 2017; **66:** 1486–501.
17 Kahl S, Nowotny B, Piepel S, et al. Estimates of insulin sensitivity from the intravenous-glucose-modified-clamp test depend on suppression of lipolysis in type 2 diabetes: a randomised controlled trial. *Diabetologia* 2014; **57:** 2094–102.
18 Laufs A, Livingstone R, Nowotny B, et al. Quantitative liver 31P magnetic resonance spectroscopy at 3T on a clinical scanner. *Magn Reson Med* 2014; **71:** 1670–75.
19 Bonhof GJ, Strom A, Puttgen S, et al. Patterns of cutaneous nerve fibre loss and regeneration in type 2 diabetes with painful and painless polyneuropathy. *Diabetologia* 2017; **60:** 2495–503.
20 Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 1993; **36:** 150–54.
21 Dyck PJ, Albers JW, Andersen H, et al. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev* 2011; **27:** 620–28.
22 Ziegler D, Dannehl K, Muhlen H, Spuler M, Gries FA. Prevalence of cardiovascular autonomic dysfunction assessed by spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses at various stages of diabetic neuropathy. *Diabet Med* 1992; **9:** 806–14.
23 Ziegler D, Strom A, Bonhof G, et al. Differential associations of lower cardiac vagal tone with insulin resistance and insulin secretion in recently diagnosed type 1 and type 2 diabetes. *Metabolism* 2018; **79:** 1–9.
24 Solomon SD, Chew E, Duh EJ, et al. Diabetic retinopathy: a position statement by the American Diabetes Association. *Diabetes Care* 2017; **40:** 412.
25 Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; **148:** 852–71.
26 Gancheva S, Jelenik T, Alvarez-Hernandez E, Roden M. Interorgan metabolic crosstalk in human insulin resistance. *Physiol Rev* 2018; **98:** 1371–415.
27 American Diabetes Association. Dyslipidemia management in adults with diabetes. *Diabetes Care* 2004; **27** (suppl 1): s68–71.
28 Biddinger SB, Hernandez-Ono A, Rask-Madsen C, et al. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab* 2008; **7:** 125–34.
29 Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab* 2015; **21:** 739–46.
30 Kahl S, Strassburger K, Nowotny B, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. *PLoS One* 2014; **9:** e94059.
31 Spoto B, Pisano A, Zoccali C. Insulin resistance in chronic kidney disease: a systematic review. *Am J Physiol Renal Physiol* 2016; **311:** F1087–108.
32 Pieralice S, Pozzilli P. Latent autoimmune diabetes in adults: a review on clinical implications and management. *Diabetes Metab J* 2018; **42:** 451–64.

5. Discussion

In summary, the first study shows that adult patients with recently diagnosed autoimmune diabetes have normal insulin sensitivity, but specifically a subgroup of patients who do not require insulin treatment despite presenting diabetes-associated autoantibodies, present with higher beta-cell function at onset of diabetes. The distinct metabolic features of these patients warrant appropriate therapeutic strategies aiming at preserving of beta-cell function and sustaining insulin sensitivity.

The second study supports the evidence for an updated categorization of patients with diabetes using an established clustering approach. Our results suggest this approach is successful at identifying patients at increased risk of diabetic complications and associated diseases. We show that patients of the SIRD cluster present with significant insulin resistance which associates an increased prevalence of NAFLD and liver fibrosis. Patients in the SIDD cluster showed increased prevalence of DSPN and CAN, making this subgroup a target for diabetic neuropathy prevention and therapy strategies.

Collectively, the studies indicate that improved phenotyping would help for a more precise identification of groups with different pathology and distinct risk of diabetes progression.

5.1. New avenues for diabetes classification

It has been noted that incidence rates of both type 1 diabetes and type 2 diabetes have been increasing (92; 93), which can be attributed to increased life expectancy but also to altered lifestyle. Patients with diabetes generally present with a high heterogeneity of metabolic features and with large variation in the relative contributions of insulin resistance and beta-cell dysfunction between subgroups and individuals. Furthermore, alterations in glucose regulation exist among individuals even in those with comparable diagnostic glucose levels (94). These features represent primary factors in the diagnostic and therapeutic decision and differences are reflected in the way patients are diagnosed and in the choice of medication. Furthermore, advances have been made in identifying subgroups of patients with specific susceptibility to diabetes complications which should pave the road for precise, individualized prevention and therapy.

5.2. Novel subgroups of patients with type 1 diabetes

Type 1 diabetes is a disease characterized by destruction of pancreatic beta-cells, which leads to absolute deficiency of insulin secretion. The clinical onset and progression, can be fulminant with acute-onset or slowly progressive. The primordial trigger of the autoimmune reaction in type 1 diabetes remains unclear and so does the reason for variation in the severity of the response.

Moreover, it has become apparent, that beta-cell destruction is not the same in all type 1 diabetes patients, as C-peptide is measurable in some individuals throughout the disease progression. This questions whether the decreased beta-cell function in type 1 diabetes is a result of loss of beta-cell mass or a functional defect reflected by loss of beta-cell secretory response to glucose or other stimuli (95). Numerous features of diabetes are constant, including the requirement for exogenous insulin administration, and the ongoing autoimmunity directed against the islets (96). Yet it should be emphasized that the rate of diabetes development is heterogeneous, with some individuals developing diabetes soon after the appearance of two antibodies and others having the disease evolve more slowly over time.

In our study we identified 42 patients with diabetes that did not require insulin treatment for at least 6 months after diabetes diagnosis. Despite their positivity for diabetes-related autoantibodies (GADA and ICA), these patients had still preserved C-peptide secretion capacity. This patient contingent represented 8.3% of the GDS population and the prevalence was along the lines previously described in the literature

As the presence of beta-cell–directed autoantibodies is the hallmark of type 1 diabetes, studies have tried to stratify patients by the rate of immune response of number of positive diabetes-related autoantibodies (97).

The patients reflecting so-called LADA in our cohort had higher insulin sensitivity than matched patients with type 2 diabetes and presented with better beta-cell function parameters than patients with type 1 diabetes, independent of BMI. As the term ‘LADA’ is not used in current guidelines, we point to the characteristics of the population and refer the so-called LADA patients in our cohort as antibody positive diabetes patients without insulin treatment (AB+/INS-). In AB+/INS- patients GAD antibody titers were found to inversely correlate with both C-peptide release after glucagon stimulation and BMI. Of note, the high prevalence of diabetes-related complications is noteworthy,

despite the lower prevalence of components of the metabolic syndrome in these newly diagnosed AB+/INS- patients (98).

While AB+/INS- patients have a lower body weight and younger age at onset of diabetes than patients with type 2 diabetes (99), previous studies have attributed differences in metabolic parameters to BMI (100). Also, similar to the study of Hawa et al. (99), we found an inverse association between BMI and the GAD autoantibody titer, indicating that AB+/INS- patients with lower beta-cell directed immunoreactivity are closer to the typical phenotype of type 2 diabetes.

Previous studies implied that the onset of disease in patients with adult-onset autoimmune diabetes can be insidious, similar as observed in patients with type 2 diabetes, which promotes a postponed medical attendance and possibly a delayed initiation of adequate treatment (40). Nevertheless, we showed that latency of diagnosis following the first appearance of diabetes-related symptoms was not different between diabetes patients. The assessment tool regarding diabetes-related symptoms in the GDS uses a questionnaire regarding polydipsia, polyuria, nicturia, weight loss, visual impairment, and fatigue. There was no difference between groups related to either symptom severity, symptom duration or frequency. Furthermore, family history of diabetes was not different between groups nor were HbA1c levels. Therefore, the clinical appearance does not show clear criteria to distinguish latent autoimmune diabetes from type 2 diabetes.

The methods of achieving glycemic control have been viewed with controversy in patients with the diabetes form formerly termed LADA, since there is no consensus on therapeutic strategies (101). Previous studies have reported insufficient glycemic control in these patients endorsing immediate insulin treatment (46). In contrast, in our cohort, HbA1c levels in AB+/INS- patients were comparable to those with type 2 diabetes and well within the guideline-imposed limits for glycemic control (14). While guidelines (14) advocate for distinguishing patients presenting with diabetes-associated antibodies from type 2 diabetes, in our cohort, the majority of AB+/INS- patients were initially diagnosed as type 2 diabetes. Even without insulin treatment 6 months from disease onset 73% of AB+/INS- patients achieve excellent glycemic control being treated with biguanides (Metformin), sulphonylureas and/or dipeptidylpeptidase-4 inhibitor (42; 102).

Previous studies on patients with so-called LADA have described pronounced dyslipidemia (103) which is regarded as an important atherogenic risk factor. This,

coupled with reduced insulin sensitivity and hypertension, can contribute to an increased cardiovascular risk (104), increasing the hazard for vascular events. Our findings are in line with a Spanish study, which showed only satisfactory lipid control (low-density lipoprotein (LDL) levels <100 mg/dl) in patients with diabetes even after several years of disease duration (105). Similarly, AB+/INS- patients of our study (106) had a heterogeneous lipid profile, with lower LDL levels than type 1 diabetes patients independent of age, BMI and waist-to-hip ratio, and presumably benefit from the lipid-lowering therapy, but at the same time have lower HDL-levels than patients with type 2 diabetes. The prevalence of diabetes-related complications and cardiovascular risk factors is noteworthy, afflicting as much as 12% of the recently diagnosed AB+/INS- patients. As cardiovascular disease is the number one cause of morbidity or mortality in diabetes (107), risk stratification and subsequent targeted management of cardiovascular complications is highly recommended in patients with autoimmune diabetes (108; 109).

Up to date, patients with autoimmune diabetes have been scarcely investigated with regard to insulin sensitivity. There is compelling evidence that insulin resistance can be present in both type 2 diabetes and type 1 diabetes (25), although to a lesser degree in the latter. Our results suggest that a higher BMI and insufficient glycemic control are not the main contenders in determining insulin resistance in adult-onset autoimmune diabetes. Insulin resistance in different target organs such as skeletal muscle, liver and adipose tissue is one of the most common metabolic disorders (52). It is associated with ectopic fat storage and inflammatory events.

Even though AB+/INS- patients had higher whole-body insulin sensitivity than their type 2 diabetes counterparts of similar age, they also have a lower insulin sensitivity than age- and BMI-matched controls. There is contradicting data on describing so-called LADA as a distinct disease entity with unique metabolism, as it encompasses features that are present in both type 1 and type 2 diabetes. Interestingly, our AB+/INS- patients showed both better insulin sensitivity and preserved beta-cell function, distinct from patients with type 2 diabetes, independent of age, sex or BMI. The gold standard method for detecting insulin sensitivity is the hyperinsulinemic-euglycemic clamp test (110). The test determines the whole-body glucose uptake at rest under a constant insulin infusion, from which the M value can be calculated as a measure of insulin sensitivity. However, the clamp test is a complex examination, which is difficult to implement in clinical practice. Consequently, there are several indices, such as HOMA-

B, HOMA-IR, OGIS (oral glucose insulin sensitivity) or PREDIM (predicted M), which can be used to determine insulin sensitivity and beta cell function in good approximation (111; 112).

The preserved residual capacity of C-peptide stimulation in AB+/INS- patients reflects a decelerated loss of beta-cell functionality when compared to type 1 diabetes. Nonetheless, AB+/INS- patients who have a reduced C-peptide secretion will be prone to a shorter interval of insulin-free therapy than patients with type 2 diabetes (113). Despite the presence of beta-cell directed autoimmunity, confirmed by the presence of diabetes-associated autoantibodies, there seems to be only a mild autoimmune reactivity.

It is important to identify this risk group, which may develop early insulin resistance as a result of obesity. In this context, screening strategies could be useful to better identify at-risk patients and to introduce adequate prevention and therapy (114).

5.3. Diabetes clusters in the population of the German Diabetes Study

Participants of the GDS (78) undergo extensive metabolic and clinical examinations allowing for an ample evaluation of therapeutic management and of the occurrence of (pre)clinical diabetes-related complications. The established clustering algorithm (64) was applied on eligible volunteers of the GDS recruited from all participating German centers (Düsseldorf, Tübingen, Heidelberg, Dresden, Berlin, Leipzig, Lübeck). The distribution of patients is shown in Fig. 2 in comparison to the traditional classification of type 1 and type 2 diabetes.

Figure 2. Comparison between the traditional taxonomy and the new cluster classification in the German Diabetes Study

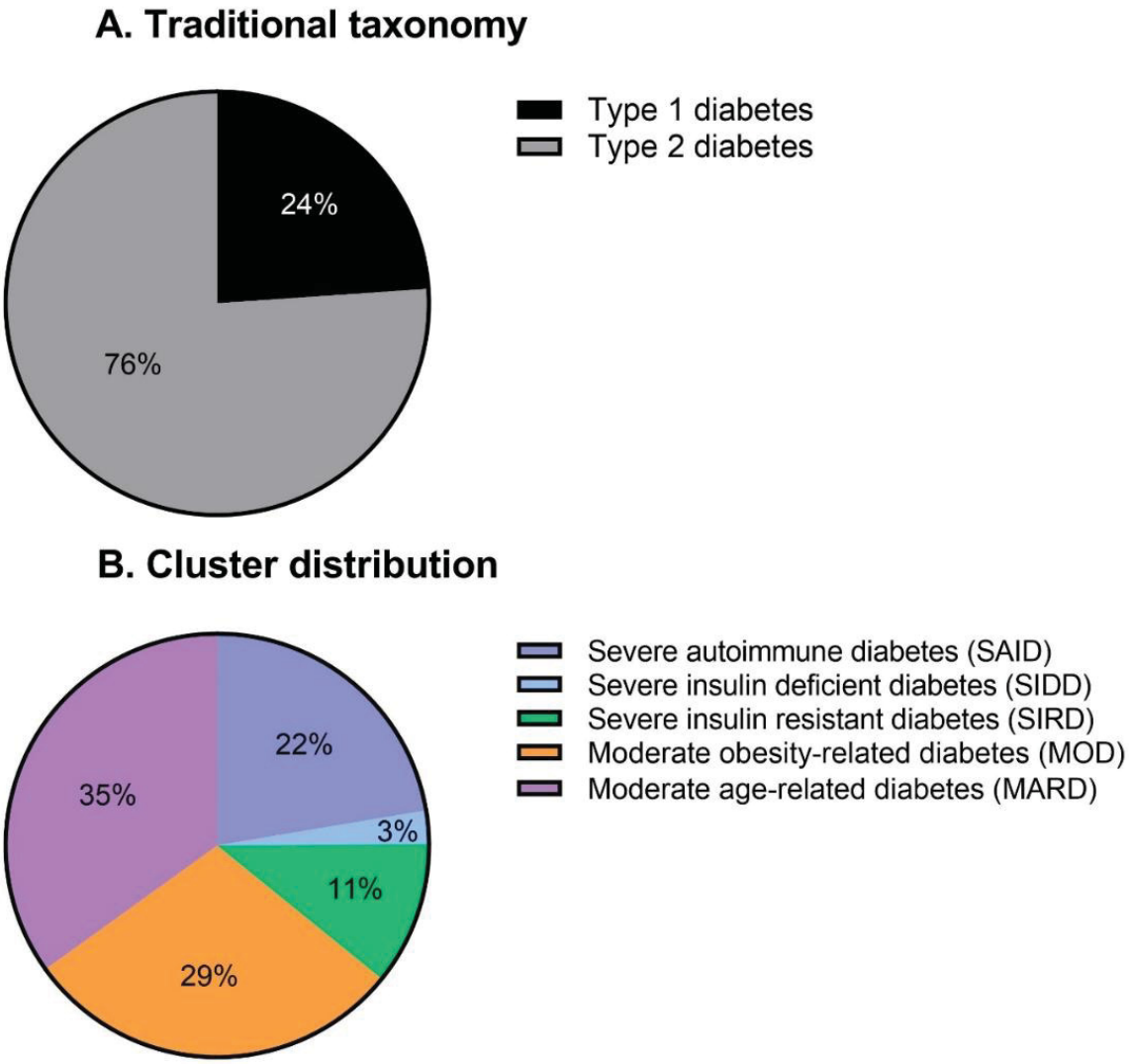


Figure Legends. Pie charts of the different distribution of diabetes types following the tradition type 1 and type 2 diabetes taxonomy in comparison with the new proposed cluster distribution. Percentages refer to the frequency of the diagnosis in patients of the German Diabetes Study. Adapted from Zaharia et al. (116).

We assessed the anthropometric and clinical features of the patients stratified by cluster and observed differences in key features, reinforcing previous observations. Distribution of the clustering variables is presented in Fig. 3.

Figure 3. Distribution of cluster variables in clusters of patients with diabetes from the German Diabetes Study

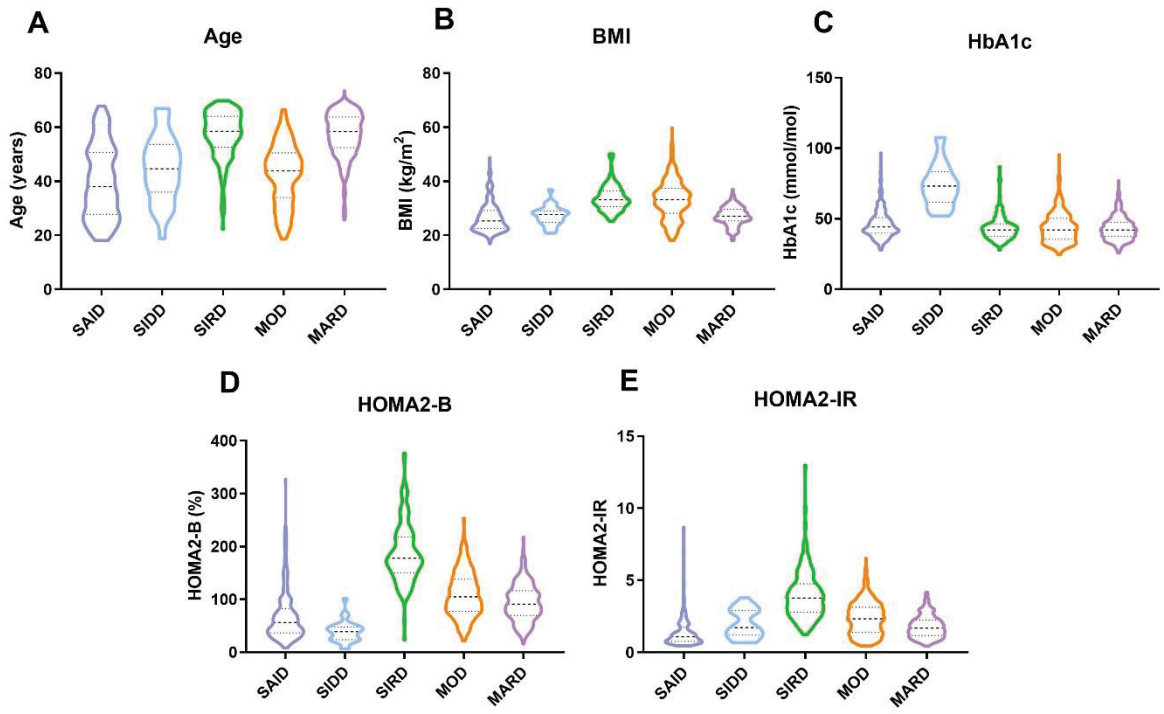


Figure Legends. Violin plots of the distribution of the different variables included in the clustering algorithm (age at diagnosis (A); BMI (B); glycemic control (C); beta-cell function (D) and insulin sensitivity (E) in patients of the German Diabetes Study. SAID, severe autoimmune diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes; MOD, mild obesity-related diabetes; MARD, mild age-related diabetes; BMI, body mass index; HOMA-IR/-B, homeostatic model assessment of insulin resistance and beta-cell function. Adapted from Zaharia et al. (116).

5.3.1. Severe autoimmune diabetes cluster

SAID, as identified by the presence of GADA, tended to occur at younger age, associated with relatively low BMI, poor glycemic control and overt insulin deficiency (Fig. 4). Patients with SAID correspond to patients with type 1 diabetes. Along these lines, we observed that patients with SAID had higher insulin sensitivity and lower liver fat content. The higher insulin sensitivity of patients with SAID compared to other clusters could also be due to the observed favorable lipid profile with high LDL levels and low HDL and triglyceride levels (115) and the consequent reduction of lipid-induced insulin resistance. Of SAID patients, 67% received insulin upon diagnosis. Importantly, our study (116) shows that measurement of more than one islet-directed antibody

increases the percentage of patients usually determined as having type 1 diabetes (SAID).

5.3.2. Cluster-derived subphenotypes of patients with type 2 diabetes

The clustering algorithm has been validated in different cohorts and populations of different ethnicities. Figure 4 shows the distribution of the clusters in the Swedish (64), Chinese and American population (117), and the distribution in the population of the German Diabetes Study (116). The original algorithm was replicated in two cross-sectional population-based datasets. 2316 participants newly diagnosed with diabetes from the 2007–08 China National Diabetes and Metabolic Disorders Study (CNDMDS) and 685 participants newly diagnosed with diabetes from the 1988–94 National Health and Nutrition Examination Survey (NHANES III) were analyzed (117). These studies, as well as the All New Diabetics In Scania (ANDIS) cohort in which the algorithm was initialized, are based on data from registries of patients with newly-diagnosed diabetes, representative of the diseased population. In contrast, the GDS enforces strict inclusion and exclusion criteria which can moderately preselect the study population. This is why we observed a reduction in the prevalence of SIDD in the GDS population. Furthermore, in contrast to the GDS, the data derived from CNDMDS and NHANES III cohorts only allowed cross-sectional analyses which do not reflect the impact of clusters on the development of possible diabetes-related metabolic alteration in the course of the disease.

Figure 4. Comparison between the cluster distribution of patients with non-autoimmune diabetes in the Swedish, Chinese and American population as well as in the population of the German Diabetes Study

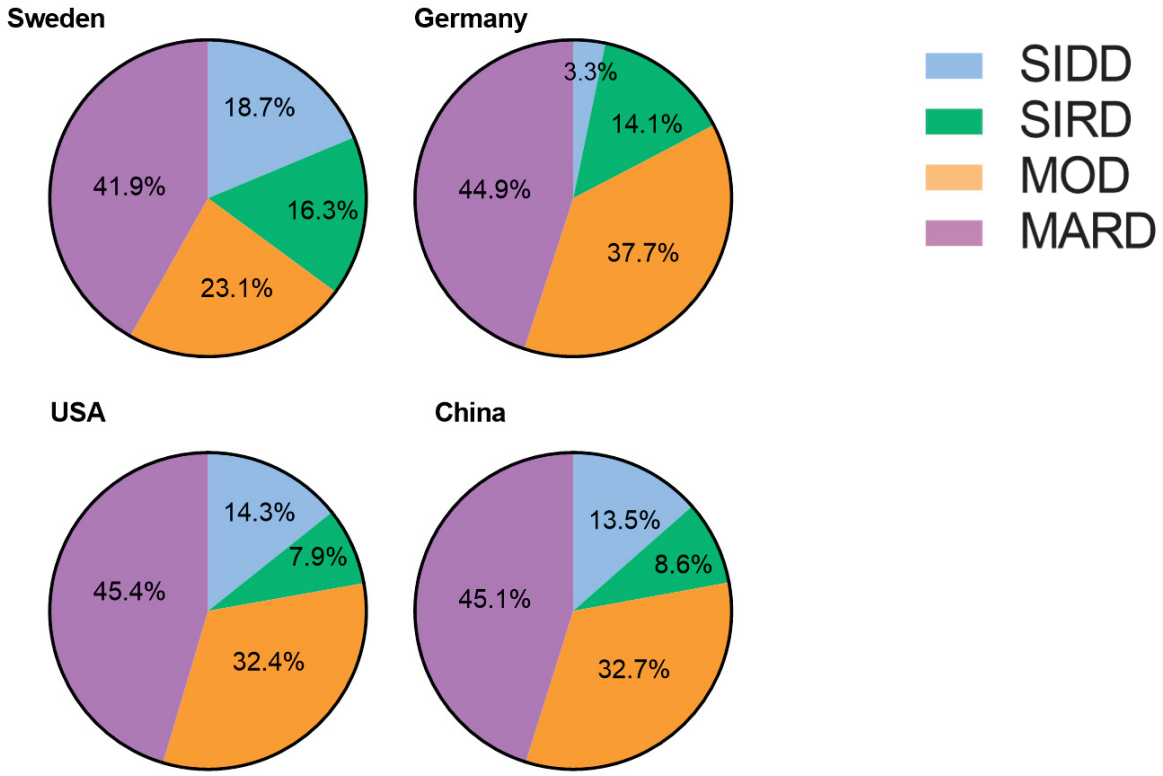


Figure Legends. Pie charts of the different distribution of diabetes types following the new proposed cluster distribution in the Swedish, German, Chinese and American population. Percentages refer to the frequency of the respective diagnosis in patients with non-autoimmune diabetes. SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes; MOD, mild obesity-related diabetes; MARD, mild age-related diabetes. Adapted from Ahlqvist et al. (64), Zaharia et al. (116) and Zou et al. (117).

In our study, SIDD showed clinical similarities to SAID but encompassed GADA-negative patients. Due to the overt insulin deficiency, 67% of these patients had insulin therapy. This cluster represented 3% of the individuals in the study population. Of SIDD patients 44% were treated with insulin at diabetes diagnosis, straying away from current guidelines which recommend metformin as first-line therapy. SIRD was characterized by insulin resistance and high BMI and was present in 11% of the patients. Patients with SIRD had lowest eGFR compared to other clusters. MOD was characterized by obesity but not insulin resistance and represented 29% of the cohort population. MOD and SIRD patients both had increased levels of high sensitivity C-

reactive protein (hsCRP), indicating subclinical inflammation, compared to the other groups. MARD encompassed patients that were generally older than those in other clusters and had only mild problems with glucose control, similar to MOD. This cluster comprised 35% of the patients.

Using comprehensive phenotyping, our study further shows that whole-body, but also adipose-tissue insulin resistance identifies clusters of patients in a cohort usually termed type 2 diabetes early after diagnosis. A major novel finding of this study (116) is that the SIRD cluster indeed presented with marked whole-body insulin resistance as assessed from the hyperinsulinemic-euglycemic clamp. Of note, the M-value mainly reflects insulin-stimulated skeletal muscle glucose uptake (118), while the HOMA-IR, used for the cluster analysis, serves as index of fasting hepatic insulin resistance (65). Our study (119) further shows that fasting adipose-tissue insulin sensitivity (79) is also lowest in SIRD indicating that this cluster indeed exhibits general or whole-body insulin resistance both under fasting and insulin-stimulated conditions. Interestingly, the MOD cluster also showed severe adipose-tissue insulin resistance, but only moderate whole-body (muscle) insulin resistance. This underlines the importance of adipose-tissue function for the development of whole-body insulin resistance and diabetes in obese persons. Moreover, fasting serum triglycerides and to a certain extent hsCRP were increased in SIRD and MOD, highlighting increased lipid availability and low-grade inflammation as key drivers in the pathogenesis of these specific clusters. Indeed, there is growing evidence from preclinical studies that supports the concept of a primary role of the adipose-tissue and lipotoxicity (52) in the development of insulin resistance.

5.4. Cluster-specific susceptibility to diabetes-related complications and comorbidities

The clustering algorithm based on simple clinical criteria allows to allocate patients with type 2 diabetes into clusters with different prevalence of diabetes comorbidities and has previously shown differences for diabetic nephropathy and cardiovascular disease (64). We expanded those observations by tackling other so far unexplored comorbidities such as diabetic neuropathy and non-alcoholic fatty liver disease.

Figure 5. Prevalence of diabetes-related complications and comorbidities in clusters of patients with diabetes from the German Diabetes Study

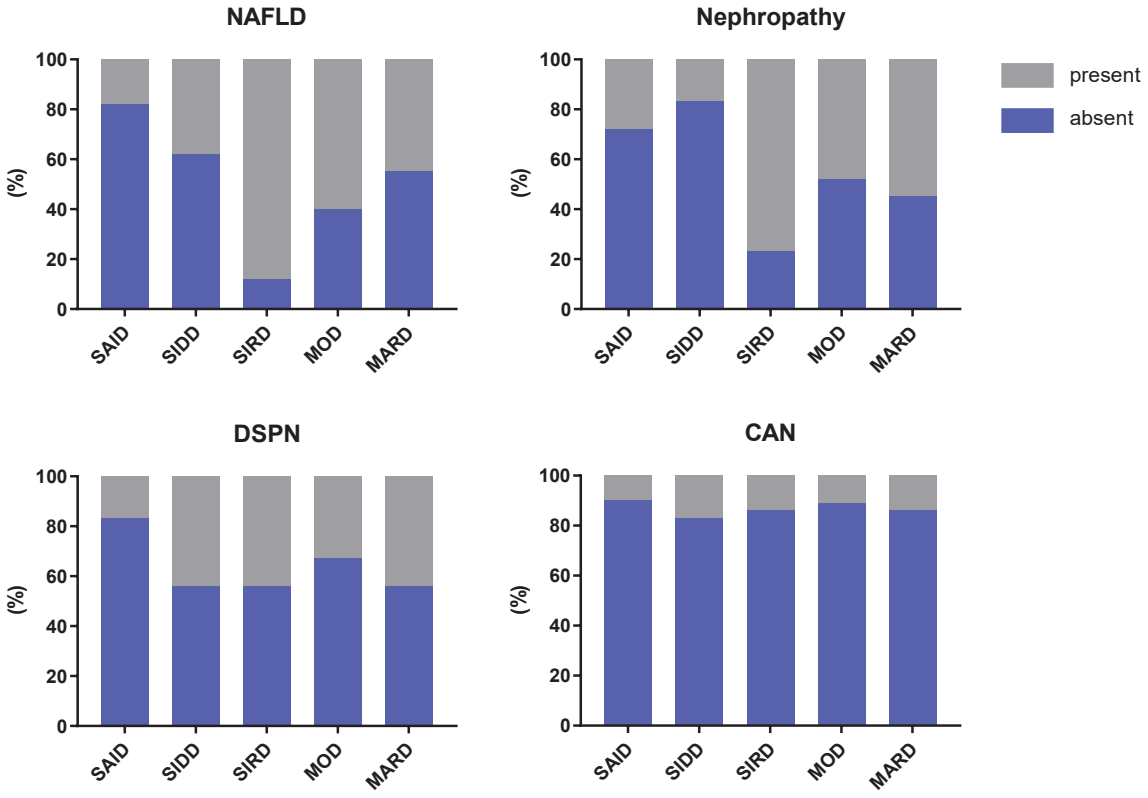


Figure Legends. Bar graphs of the prevalence of diabetes-related complications in patients with newly diagnosed diabetes of the German Diabetes Study. SAID, severe autoimmune diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes; MOD, mild obesity-related diabetes; MARD, mild age-related diabetes; DSPN, distal-symmetric polyneuropathy*; CAN,* cardiovascular autonomous neuropathy*; NAFLD, non-alcoholic fatty liver disease. Adapted from Zaharia et al. (116).*

The prevalence of diabetes-related complications and comorbidities in the study population is presented in Fig. 5 stratified by cluster. Liver steatosis was assessed noninvasively in patients with newly diagnosed patients with diabetes using MRS. The cut-off for NAFLD was set at 5.5% liver fat content (120; 121). Diabetic nephropathy was assessed noninvasively using the estimated glomerular filtration rate (eGFR) at baseline and after 5 years disease progression. Nephropathy was categorized as stage 1 (normal, eGFR > 90, blue), or impaired (stage 2, or eGFR 60-90 or stage 3 (eGFR < 60). Columns show percentages of the diagnosed patients of the total number of examined patients. Distal-symmetric polyneuropathy (DSPN) and cardiovascular

autonomous neuropathy (CAN) were assessed by clinical and functional tests and classified according to current international guidelines (122).

5.4.1. Non-alcoholic fatty liver disease

Excessive accumulation of hepatic triglycerides >5.5% defines non-alcoholic fatty liver disease (NAFLD) (120; 121), which is considered as the liver manifestation of the metabolic syndrome and associates tightly with obesity and type 2 diabetes (67). The prevalence of NAFLD in type 2 diabetes is estimated to be between 28 and 55% (123). NAFLD encompasses a spectrum of liver diseases ranging from simple steatosis in the absence of inflammation to steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC). The causal relationship between hyperlipidemia, ectopic lipid storage in the liver and insulin resistance has been studied intensively, but remains not completely understood. NAFLD has been suggested to ensue in the setting of or secondary to prevailing insulin resistance (124), but increased lipid availability achieved by lipid infusion also induces hepatic lipid accumulation and insulin resistance (125).

The development of NAFLD is significantly determined by disturbances in the energy metabolism. Adequate mitochondrial function contributes to the regulation of insulin sensitivity and secretion. Processes impairing mitochondrial function lead to disturbed energy homeostasis with insulin resistance and deficiency and disrupt the metabolic function of the liver (126). The metabolic function of the liver is modulated by insulin and other metabolic factors. In the fasted state, the liver releases glucose through both glycogenolysis and gluconeogenesis. During pronged fasting, hepatic gluconeogenesis is the primary source for endogenous glucose production. Fasting also promotes lipolysis in adipose tissue, resulting in release of FFA. Increased fatty acid oxidation in cases of lipid overload can lead to reduced glucose transport and higher intracellular glucose (127). With regard to energy metabolism, previous studies show that in spite of similar mitochondrial content, obese humans with or without NAFLD had higher mitochondrial respiration rates than lean persons. NASH patients featured higher mitochondrial mass, but lower mitochondrial respiration, which associated with greater hepatic insulin resistance, mitochondrial uncoupling, and leaking activity. Furthermore, increased hepatic oxidative stress and oxidative DNA damage were accompanied by reduced anti-oxidant defense capacity and increased inflammation. These data suggest that at early stages of obesity-related insulin

resistance there is preserved hepatic mitochondrial plasticity, which is subsequently lost in NASH. Mitochondrial plasticity refers to the ability of the mitochondria to respond to altered metabolic conditions by modulating their activity, number or oxidative phosphorylation capacity (128).

Cross-sectional analyses showed that patients with type 2 diabetes frequently have increased HCL content (52), which associated also with increased insulin resistance. The association also showed a possible genetic link, where a TM6SF2 gene polymorphism (129), usually related to NAFLD, was associated with SIRD (64). TM6SF2-related NAFLD (130; 131) has not been shown to be associated with insulin resistance. Recent reviews on the topic did not identify any studies, showing that gene variants influenced the future risk of type 2 diabetes (132). These variants do predict NASH, cirrhosis and HCC worldwide, but have not turned out to be significant predictors of type 2 diabetes in the multiple genome-wide association studies searching for genetic risk markers for type 2 diabetes (132). Consequently, it is not surprising that the SIRD cluster associates with an increased prevalence of NAFLD and liver fibrosis, reinforcing previous observations. Mechanistically this could be due to impaired mitochondrial biogenesis which was linked to NAFLD progression (133). NAFLD was associated with type 2 diabetes also in previous studies. The Verona Diabetes Study, a prospective population-based observational study on type 2 diabetes patients, has reported an increased risk of death from gastrointestinal diseases, particularly from chronic liver cirrhosis and a higher risk of mortality from HCC (123). This risk increased significantly in obese patients. However, this study did not exclude other pathophysiological mechanisms, unrelated to metabolic changes due to diabetes, i.e. alcohol consumption, hepatitis infections or others.

Although not measured in all participants, the MRS data confirmed the results of the fatty liver index (FLI), suggesting an acceptable performance of this non-invasive index as reported for nondiabetic humans (134). In our cohort (119) HCL correlated positively with FLI ($r=0.69$, $p<0.001$). As to fibrosis scores, challenges still remain in using predictive scores in patients at low risk of NASH/fibrosis. Nevertheless, patients with diabetes are already at higher risk of progressive NAFLD or may even present with NASH. The observed differences would point to such early hepatic alterations, but cannot be proven without the current gold standard, liver biopsy. FLI is one of the best-validated steatosis scores for the general population and obese persons and recommended by international guidelines for NAFLD screening (66; 135). Thus, the

FLI is of clinical and practical relevance. Nevertheless, we are aware that the FLI offers modest efficacy to detect steatosis and cannot substitute the gold-standard quantification methods. Our results were in line with previous observations where AROC of FLI was 0.70 vs. MRS (134); 0.80 vs. ultrasound (136) and 0.83 vs. hepatic biopsy (135). Thus, FLI can serve as surrogate parameter for liver fat content and consequently as a modest clinical estimate of abnormal liver fat content, in the absence of routine MRS or liver biopsy.

Ultrasonography-diagnosed NAFLD predicted type 2 diabetes in a majority of studies (132), even after adjustment for potential confounders. However, ultrasonography is unreliable and difficult to use in obese subjects, it is possible that it is more sensitive to detecting type 2 diabetes risk in lean subjects (137). Ultrasonography is also inaccurate at quantifying liver fat percentages below 20-30% which may influence estimation of disease risk. On the other hand, ultrasonography is widely available and can detect focal lesions in addition to providing a semi-quantitative estimate of steatosis, which makes it easier to use in clinical practice.

5.4.2. Nephropathy

Diabetic nephropathy refers to the decline of kidney function seen in patients with diabetes. The progression of the disease is known to occur in a series of stages and is linked to glycemic and blood pressure control. However, despite aggressive glycemic control the prevalence of chronic kidney disease in diabetic patients has not witnessed any decrease over time, which warrants the search of additional associated factors in its progression (138) and a superior screening for patients at risk.

Development of albuminuria or renal impairment was independently associated with increased baseline systolic blood pressure, urinary albumin, plasma creatinine, and Indian-Asian ethnicity. In a prospective cohort of British patients with type 2 diabetes, nearly 40% developed albuminuria and nearly 30% developed renal impairment (139). The study revealed distinct sets of risk factors are associated with the development of albuminuria and renal impairment, consistent with the concept that they are not linked unalterably in type 2 diabetes (139). Previous studies identified male sex, increased waist circumference, plasma triglycerides, LDL cholesterol, HbA1c, increased leucocyte count, smoker status, and previous retinopathy as independent risk factors for albuminuria. Additional independent risk factors for renal impairment were female

sex, decreased waist circumference, age, increased insulin sensitivity, and previous sensory neuropathy.

Insulin resistance has also been associated with impaired renal function (140) and SIRD patients exhibit an accelerated progression of diabetic kidney disease (64). The present study confirms this association in that the SIRD cluster had decreased eGFR and increased cystatin-C levels, both at baseline and follow-up, despite good metabolic control, suggesting a superior role of insulin resistance compared to glycemia for the onset and early progression of diabetic nephropathy. Arguably, the Cockroft-Gault formula is not ideal for overweight and obese patients, possibly overestimating true glomerular filtration rates (141). Yet, the Cockroft-Gault formula is recommended by current guidelines for diagnosing nephropathy (142) especially for patients with higher glomerular filtration rate, as is the case for the patients of the German Diabetes Study.

5.4.3. Neuropathy

Diabetic neuropathy is a prevalent, disabling disorder with a wide pattern of symptoms, a still controversially discussed cause and a broad spectrum of risk factors (143; 144). Although subclinical abnormalities, some related to oxidative stress, can be already present at the onset of diabetes (144), little is known about the clinical and metabolic features predicting the development or progression of diabetic neuropathy. In our study we assessed DSPN and CAN.

Experimental studies suggest a multifactorial pathogenesis of DSPN driven by hyperglycemia, reduced insulin signaling, dyslipidemia and insulin resistance which can trigger DNA damage, endoplasmic reticulum stress, mitochondrial dysfunction, cellular injury, and irreversible damage. The relative importance of the pathways in this network will vary with cell type, disease profile, and time (122). Considering this, a multifactorial approach targeting glycemia, dyslipidemia and insulin resistance to prevent cardiovascular autonomic neuropathy, especially in people with type 2 diabetes, is currently recommended by guidelines (122).

Congruently, the present findings implicate that insulin deficiency or hyperglycemia are important triggers of diabetic neuropathy, both DSPN and CAN, with the highest prevalence observed in SIDD. It was speculated that impaired insulin signaling promotes cellular injury by decreasing gene expression of essential proteins, blocking protein synthesis, and promoting apoptosis (145). Patients of the SAID and SIDD cohorts had lowest beta-cell reserve and were treated preferentially with insulin.

Nevertheless, supplementation with insulin and/or glucose lowering medication which restored glucose homeostasis in SIDD patients at follow-up failed to restore neuronal signaling and nerve function in these patients. Thus, SIDD patients would benefit from the use of sensitive diagnostic tools for an early detection and prediction of diabetic neuropathy and prevention of major clinical sequels such as pain, foot ulcers, and autonomic dysfunction, which are associated with increased morbidity and mortality (144). Overall prevention of diabetic neuropathies focuses on glucose control and lifestyle modifications. Available evidence pertaining to DSPN and CAN, is scarce. Current guidelines primarily rely on optimizations of glucose control as early as possible to prevent or delay the development of distal symmetric polyneuropathy and cardiovascular autonomic neuropathy in people with type 1 and type 2 diabetes (122).

5.5. Reproducibility of the clustering algorithm

The innovative use of cluster assignment may pave the way for a novel diabetes classification. According to the current recommendations, classification of a patient is permanent. Thus, the question is, whether this would also hold true also after longer disease duration for a future new classification according to the clusters. The study design fortunately allows for clustering both at baseline and follow up and we could therefore show the differences in cluster allocation and migration pattern. In the GDS, patients are followed over the course of several years, with regular clinical assessments every 5 years (78). Of note, applying the clustering algorithm in patients with 5 years of disease duration, yielded a reproducibility of only 77%. This was in part related to metabolic parameters independent of the cluster algorithm. We identified that changes in triglycerides, liver steatosis and glucose homeostasis were associated with migration from the moderate to severe diabetes clusters. This finding indicates that cluster membership is not an immutable feature, but may be affected by additional metabolic alterations and treatment over time. However, when performed in newly-diagnosed patients it serves as an early indication of disease progression and development of complications. The lowest reproducibility was in the SIDD cluster, most likely due to efficient glucose-lowering treatment which decreased the severity of hyperglycemia and the associated risks.

However, it appears that the original primary cluster membership defines the progression of diabetes-related complications.

Several patients changed their titers of diabetes-related autoantibodies and tested positive/negative at follow up compared to baseline. Following studies will require longitudinal follow-up of the high-risk group to establish whether the autoimmune phenotypes are stable over time and indicate a fixed pathway of disease progression, which would be important as stratification tools in prevention studies.

5.6. Clinical implications of genetic phenotyping in diabetes

Studies have been focused to find a more accurate way of assessing which ranges of factors are most likely to determine the development of type 2 diabetes. The treatment options for diabetes have different action mechanisms and represent a challenge for the physician in order to prescribe the right therapeutic approach to a specific individual. Therefore, previous studies recommend a more granular approach that addresses the many different molecular processes leading to hyperglycemia, in order to link these to the proper treatment. While guidelines are continuously improving based on newest evidence, further studies are needed in distinguishing groups of patients that will respond to specific treatments (13).

In contrast to previous cluster analyses based on clinical parameters, which can change throughout a person's lifetime and as the condition progresses (116), this study suggests a more reliable way of identifying which relevant factors play a more important role in disease progression by considering genetic factors. Recent studies led to the identification of five clusters of genetic variants that may influence distinct subtypes of type 2 diabetes, grouped based on different diabetes-related mechanisms (77). Of the five genetic clusters that the team identified, two are linked beta-cell dysfunction, though each of them impacts proinsulin — the precursor of insulin — to a different degree. The other clusters are all linked to insulin resistance. However, one is obesity-mediated, another is mediated by lipodystrophy, and the third is mediated by the altered hepatic lipid metabolism. Almost a third of all the participants scored highly for just one cluster, which is suggestive of a single mechanism responsible for facilitating type 2 diabetes. The study claims to provide the most detailed overview of the genetic factors that underlie the development of type 2 diabetes in different individuals (77). The findings were verified by analyzing relevant population-based registries. It remains to be determined whether these clusters translate to differences in disease progression, complications, and response to treatment.

Further studies show a genetic link to response to therapeutic strategies, not limited to pharmacological treatment but also in response to lifestyle interventions, such as dietary changes or physical activity.

Physical inactivity, however, is associated with increased mortality rates and elevated risk for various diseases such as diabetes, obesity and cardiovascular diseases (146; 147). Consequently, international guidelines uniformly recommend regular physical activity as part of lifestyle modification in the prevention and management of type 2 diabetes (148). Previous studies showed a considerable response variability, with up to 7–63% non-responders following exercise training interventions for several endpoints, including glycemic control and insulin sensitivity in individuals with metabolic diseases (149). An impaired response to exercise training may not only result from acquired, but also from inherited factors (150; 151). Currently, polymorphisms of only few genes, i.e. peroxisome proliferator-activated receptor (PPAR)-γ (152), β3-adrenergic receptor (β3AR) (153) as well as PPARδ and PPARγ coactivator-1α (PGC1α) (154), have been associated with variable responsiveness to exercising. Studies showed that a single nucleotide polymorphism (SNP) in the NADH dehydrogenase (ubiquinone)-1ß subcomplex subunit 6 (*NDUFB6*) of the mitochondrial complex I relates to impaired muscle mitochondrial plasticity after exercise interventions (150; 155). Presence of the G/G allele (rs540467) correlated with exercise-mediated increases in muscle ATP synthase flux (150), in line with previous observations showing that mitochondrial function can predict insulin resistance (156) and the response to exercising (157).

Similarly, the response of serum lipids to diet shows large inter-individual variation, which might be caused by genes whose products affect lipoprotein metabolism such as apolipoprotein ApoE and ApoA5 (158). Carriers of genetic variants of ApoE is associated with elevated LDL-cholesterol levels and cardiovascular risk (159). Increased cardiovascular risk due to increased serum triglyceride concentrations is also present in distinct genetic variants of ApoA5 (158). Studies show that associations of dietary patterns with triglycerides and LDL-cholesterol differ by ApoE and ApoA5 haplotype in patients with recently diagnosed type 2 diabetes (160).

Taken together, these results suggest that subdividing patients based on genetic traits might further improve precision treatment targets and help identify responders and non-responders not only with regard to pharmacological treatment, but also regarding

lifestyle interventions. It remains to be investigated if cluster analyses may bring additional value to targeted lifestyle interventions, improving responsiveness.

6. Strengths and limitations of the novel classification tools

The strength of the study design (106) relies on the well-characterized metabolic phenotyping and the implementation of gold-standard tools to assess insulin sensitivity, beta cell function and steatosis.

In the first study, the patients defined as AB+/INS- are phenotyped in comparison with matched type 1 and type 2 diabetes patients and healthy controls. Patients included in the study had similar disease duration and have undergone gold-standard methods following standard operating procedures to evaluate beta-cell function and insulin sensitivity. While the 8.3% prevalence of so-called LADA in the type 2 diabetes patients of the GDS cohort, lies in the range of previously described studies, the relatively small number of subjects is a limitation of the study.

In the second study, the distribution of the clusters slightly differs from that of the population-based Swedish cohort by a higher prevalence of the severe autoimmune diabetes, while the distribution of the other clusters largely followed the previously reported pattern (64). This higher prevalence results from the active recruitment of patients with autoimmune diabetes and the intensified diagnosis of autoimmune diabetes by employing more than one islet-cell directed autoantibody. By this approach, another 59 patients (7%) were identified as suffering from autoimmune diabetes by measuring islet-cell antibodies and insulin autoantibodies in addition to GAD autoantibodies. Of note, 6 patients (2%) with negative GAD antibodies at baseline tested positive for GAD autoantibodies after 5 years of follow-up. The aim of this study was to employ the identical cluster definition as used by Ahlqvist et al. (64), by which SAID was categorized by positive GAD antibodies alone. We therefore used the other islet antibodies not for cluster definition, but only exploratory. Of note, employing more than one islet-cell directed autoantibody, another 59 patients (7%) were identified as suffering from autoimmune diabetes by measuring islet-cell antibodies and insulin autoantibodies in addition to GAD autoantibodies. Thus, these findings represent another - to our opinion - clinically relevant result of the study supporting the need for comprehensive islet-cell autoantibody screening in patients with newly diagnosed diabetes to avoid inadequate treatment of hyperglycemia. Taken together, these findings support the need for comprehensive islet-cell autoantibody screening in

patients with newly diagnosed diabetes to ensure a targeted therapeutic approach and to avoid inadequate treatment of hyperglycemia (161). Further limitation consists in the uniform presentation of patients with autoimmune diabetes. As previous studies have shown a high heterogeneity in metabolic features within patients with autoimmune diabetes, the clustering algorithm lacks the ability to differentiate between these patients. It appears paradoxical that autoimmune diabetes does not merit the same clinical-based cluster differentiation as type 2 diabetes. However, it should be noted that the etiology of type 1 diabetes is still largely considered mono-causal due to autoimmune destruction of the beta-cells, regardless of the high heterogeneity of clinical presentation. Nevertheless, it seems probable that future studies for the prevention of beta-cell destruction are likely to benefit from a greater emphasis on stratification of patients with autoimmune diabetes and prospective follow up.

As the GDS is an ongoing observational study, there were more data available of patients at baseline than at follow up. We have performed additional analyses revealing that from the population eligible for a follow up assessment after 5 years, 367 indeed participated yielding a drop-out rate of 13%, which is rather low compared to similar cohort studies (162). All other patients had a disease duration of still <5 years at the time of this analysis or did not meet all criteria for clustering. Therefore, the limited number of follow-up data does not reflect any selection bias.

Cluster-analyses are generally created for large populations, and should be applied to population-based patient groups. This way it can be ensured that patients represent the targeted population and that cluster allocation is not artificially randomized. In the context of the appropriate sample size for cluster analyses, we would like to refer to a previous paper (163) reporting that under all simulated circumstances a sample size of 70-times the number of variables proved adequate for cluster analyses. Therefore, considering that the current analyses used 5 variables (age, BMI, HbA1c, HOMA-B, HOMA-IR), we achieved the required sample size at both baseline and follow up (n=1105 and n=367, respectively). While using cutting-edge techniques to assess the metabolic subphenotypes makes this research particularly valuable and reliable, this approach limits the number of patients in selected clusters, e. g. the lower prevalence of SIDD could be linked to the exclusion criteria for glycemic control (HbA1c < 9%) (78). Thus, the present results cannot necessarily be generalized to the general population and community-based practice.

7. The future of precision medicine in the field of diabetes

With regard to precision medicine in diabetes (72) it is generally accepted that this approach will improve prevention and treatment of common, multifactorial diseases, such as type 2 diabetes, but there are few examples to date. The emerging novel risk variables shown to be associated with type 2 diabetes have modest effect sizes, and limited predictive value, particularly when compared with the performance of classic risk factors such as age, BMI, and blood glucose (164). Recently, there has been a resurgence of interest in the translational potential offered by genetic risk scores (165). This raises the prospect that the rollout of medical genotyping and sequencing will provide clinically actionable information on diabetes risk, particularly if genetic information is combined with other relevant clinical or exposure data. Great advances have been made in the exploration of the genetic architecture of diabetes and its complications, which have provided the foundations for the development of genomic medicine in diabetes (58). Genetic analysis can help discriminate subgroups of patients with specific molecular defects who otherwise are classified under the broad umbrella of type 1 diabetes or type 2 diabetes, and provide the basis for optimal preventive or therapeutic interventions (73). Genetic testing is considered to be especially effective in increasing the precision of diagnosis and treatment selection in individuals with known monogenic diabetes including MODY. It further serves as a promising framework for precision medicine in diabetes.

Adding on to the advances in genotyping, together with increasing sample sizes, new technologies, such as pharmacogenomics, will likely lead to the discovery of new risk variants for diabetes and its complications, as well as drug responses.

Questions remain regarding the additional information required to define the clinical utility of these data, and how best to incorporate them into routine diabetes care. Future areas of research include defining the functional impact of these identified variants on gene regulation or expression, further exploration of the extent to which genetic factors contribute to the observed phenotype, gene–environment interactions and gene–treatment responses. In addition to advances in genomics, there is also a need to integrate other types of “omics” (proteomics, metabolomics, lipidomics, transcriptomics or epigenetic changes) to provide a full landscape of the correlations between disease pathways, phenotypes and treatment response. Developments in these fields have the potential to predict individual response to preventive or therapeutic tactics. To this end, it is required to provide an optimized framework incorporating such –omics,

biomarkers, and genomic data into clinic records for evaluation of the validity, efficacy and cost-effectiveness of clinical testing. Most importantly, these initiatives must be accompanied by ongoing training and education of clinicians on how to use these data appropriately (166).

Additional research efforts are required to build the clinical evidence and roadmap for achieving consensus and developing guidelines (167) in the pursuit of precision medicine for making a precise diagnosis and giving the correct drug for the optimal outcome. Along these lines, Fitipaldi et al. recently proposed a comprehensive model for the development of precision medicine in diabetes. This model considers understanding disease physiopathology and natural disease progression at the basis of the development of precision medicine in diabetes. Following this, research should aim at identifying diagnostic and prognostic disease-specific biomarkers and integrate them in reliable algorithms. These diagnostic and prognostic algorithms should further be tested and validated in controlled clinical trials. Should the methods prove their validity, the next step is obtaining regulatory approval. Succeeding this, the approved algorithms should be implemented in primary and secondary healthcare practice after rigorous clinical training of the medical personnel.

Precision medicine for type 2 diabetes could require even more accurate profiling of individuals belonging to a given subphenotype, by integrating genetic and -omics data, digital or sensor-based behavioral and clinical monitoring, and pharmacodynamics. Most relevant to current translational objectives in diabetes are the potential implications of new studies for therapeutic strategies. The described heterogeneity, or the existence of subphenotypes, defined by distinct pathophysiological mechanisms with the potential to explain distinct clinical features and response to treatment, could open up the prospect of personalized medicine, which is an emerging aspiration for the complex management of diabetes.

8. Conclusion

In conclusion, adult patients with recently diagnosed autoimmune diabetes have higher insulin sensitivity than patients with type 2 diabetes but specifically a subgroup of patients with autoimmune diabetes have uncharacteristically higher beta-cell function at disease onset. The distinct metabolic features of this subphenotype warrant appropriate therapeutic strategies aiming at sustaining the remaining beta-cell function, preserving insulin sensitivity and preventing diabetes-related comorbidities.
Furthermore, our studies show that patients with diabetes can be allocated to specific clusters, which exhibit distinct metabolic alterations and different risk patterns at the time of diabetes diagnosis and for the development of diabetes-related comorbidities and complications. The results also underline the need of comprehensive diabetes-related autoimmunity screening in all diabetes patients. In particular, we identified the highest risk of NAFLD progression in patients with severe insulin resistant diabetes and of diabetic neuropathy in patients with severe insulin deficient diabetes. Our research could thereby pave the road for precision medicine in diabetes leading to targeted prevention and early treatment in subgroups of diabetes patients.

References

1. Krall LP, Levine R, Barnett D: The history of diabetes. Joslin's Diabetes Mellitus 13th ed Kahn CR, Weir GC, Eds Philadelphia, Pa, Lea and Febiger 1994:2
2. Lancereaux E: Le diabete maigre, ses symptomes, son evolution, son pronostic et son traitement. Union Medicale 1880;29:161
3. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E: Milestones in the history of diabetes mellitus: The main contributors. World journal of diabetes 2016;7:1-7
4. Naunyn B: *Der diabetes melitus*. Hölder, 1906
5. Himsworth H: The mechanism of diabetes mellitus. The Lancet 1939;234:171-176
6. Falta W: *Die Zuckerkrankheit*. Urban & Schwarzenberg, 1936
7. Joslin EP: *The treatment of diabetes mellitus: with observations upon the disease based upon thirteen hundred cases*. Lea & Febiger, 1917
8. White P: Classification of obstetric diabetes. American Journal of Obstetrics & Gynecology 1978;130:228-230
9. Lawrence RD: Lipodystrophy and hepatomegaly with diabetes, lidaemia, and other metabolic disturbances: a case throwing new light on the action of insulin. The Lancet 1946;247:724-731
10. World Health Organization WHO: Classification of diabetes mellitus. 2019;
11. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183
12. American Diabetes Association: 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care 2018;41:S13-s27
13. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC, Group ESD: 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD). European heart journal 2019;
14. Ryden L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, Marre M, Marx N, Mellbin L, Ostergren J, Patrono C, Seferovic P, Uva MS, Taskinen MR, Tendera M, Tuomilehto J, Valensi P, Zamorano JL, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, De Backer G, Sirnes PA, Ezquerra EA, Avogaro A, Badimon L, Baranova E, Baumgartner H, Betteridge J, Ceriello A, Fagard R, Funck-Brentano C, Gulba DC, Hasdai D, Hoes AW, Kjekshus JK, Knuuti J, Kolh P, Lev E, Mueller C, Neyses L, Nilsson PM, Perk J, Ponikowski P, Reiner Z, Sattar N, Schachinger V, Scheen A, Schirmer H, Stromberg A, Sudzhaeva S, Tamargo JL, Viigimaa M, Vlachopoulos C, Xuereb RG: ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). European heart journal 2013;34:3035-3087

15. Van Belle TL, Coppieters KT, Von Herrath MG: Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. Physiological reviews 2011;91:79-118
16. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, Lavant E, Louey A, Moonsamy P: HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes 2008;57:1084-1092
17. Tosur M, Geyer SM, Rodriguez H, Libman I, Baidal DA, Redondo MJ: Ethnic differences in progression of islet autoimmunity and type 1 diabetes in relatives at risk. Diabetologia 2018;61:2043-2053
18. Aviles-Santa L, Maclaren N, Raskin P: The relationship between immune-mediated Type 1 diabetes mellitus and ethnicity. Journal of diabetes and its complications 2004;18:1-9
19. Noble JA, Valdes AM: Genetics of the HLA region in the prediction of type 1 diabetes. Current diabetes reports 2011;11:533-542
20. Irvine WJ: Classification of idiopathic diabetes. The Lancet 1977;309:638-642
21. Kawasaki E, Maruyama T, Imagawa A, Awata T, Ikegami H, Uchigata Y, Osawa H, Kawabata Y, Kobayashi T, Shimada A, Shimizu I, Takahashi K, Nagata M, Makino H, Hanafusa T: Diagnostic criteria for acute-onset type 1 diabetes mellitus (2012): Report of the Committee of Japan Diabetes Society on the Research of Fulminant and Acute-onset Type 1 Diabetes Mellitus. J Diabetes Investig 2014;5:115-118
22. Eisenbarth GS: Type I Diabetes Mellitus. New England Journal of Medicine 1986;314:1360-1368
23. Katsarou A, Gudbjornsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark A: Type 1 diabetes mellitus. Nat Rev Dis Primers 2017;3:17016
24. Rawshani A, Sattar N, Franzen S, Rawshani A, Hattersley AT, Svensson AM, Eliasson B, Gudbjornsdottir S: Excess mortality and cardiovascular disease in young adults with type 1 diabetes in relation to age at onset: a nationwide, register-based cohort study. Lancet 2018;392:477-486
25. Kaul K, Apostolopoulou M, Roden M: Insulin resistance in type 1 diabetes mellitus. Metabolism: clinical and experimental 2015;64:1629-1639
26. Bergman BC, Howard D, Schauer IE, Maahs DM, Snell-Bergeon JK, Eckel RH, Perreault L, Rewers M: Features of hepatic and skeletal muscle insulin resistance unique to type 1 diabetes. The Journal of clinical endocrinology and metabolism 2012;97:1663-1672
27. Kacerovsky M, Jones J, Schmid AI, Barosa C, Lettner A, Kacerovsky-Bielesz G, Szendroedi J, Chmelik M, Nowotny P, Chandramouli V, Wolzt M, Roden M: Postprandial and fasting hepatic glucose fluxes in long-standing type 1 diabetes. Diabetes 2011;60:1752-1758
28. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: A common feature of Type 2 (non-insulin-dependent) and Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1982;23:313-319
29. Yki-Jarvinen H: Glucose toxicity. Endocr Rev 1992;13:415-431
30. Cleland SJ, Fisher BM, Colhoun HM, Sattar N, Petrie JR: Insulin resistance in type 1 diabetes: what is 'double diabetes' and what are the risks? Diabetologia 2013;56:1462-1470
31. Giuffrida FM, Bulcao C, Cobas RA, Negrato CA, Gomes MB, Dib SA: Double-diabetes in a real-world sample of 2711 individuals: associated with insulin treatment or part of the heterogeneity of type 1 diabetes? Diabetology & metabolic syndrome 2016;8:28

32. Raj SM, Howson JM, Walker NM, Cooper JD, Smyth DJ, Field SF, Stevens HE, Todd JA: No association of multiple type 2 diabetes loci with type 1 diabetes. Diabetologia 2009;52:2109-2116
33. Zimmet PZ: The pathogenesis and prevention of diabetes in adults: genes, autoimmunity, and demography. Diabetes care 1995;18:1050-1064
34. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM: Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? Diabetes 2005;54 Suppl 2:S62-67
35. Palmer JP, Hirsch IB: What's in a Name. Diabetes Care 2003;26:536
36. Stenstrom G, Gottsater A, Bakhtadze E, Berger B, Sundkvist G: Latent autoimmune diabetes in adults: definition, prevalence, beta-cell function, and treatment. Diabetes 2005;54 Suppl 2:S68-72
37. Laugesen E, Ostergaard JA, Leslie RD, Danish Diabetes Academy W, Workshop S: Latent autoimmune diabetes of the adult: current knowledge and uncertainty. Diabet Med 2015;32:843-852
38. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC: Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005;48:2206-2212
39. Atkinson MA, Eisenbarth GS, Michels AW: Type 1 diabetes. Lancet 2014;383:69-82
40. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L: The many faces of diabetes: a disease with increasing heterogeneity. Lancet 2014;383:1084-1094
41. Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C, Capizzi M, Arpi ML, Bazzigaluppi E, Dotta F, Bosi E: High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 2007;30:932-938
42. Leslie RD, Palmer J, Schloot NC, Lernmark A: Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. Diabetologia 2016;59:13-20
43. Blaslov K, Bulum T, Knezevic-Cuca J, Duvnjak L: Relationship between autoantibodies combination, metabolic syndrome components and diabetic complications in autoimmune diabetes in adults. Endocrine 2015;48:551-556
44. Mollo A, Hernandez M, Marsal JR, Esquerda A, Rius F, Blanco-Vaca F, Verdaguer J, Pozzilli P, de Leiva A, Mauricio D, Action L: Latent autoimmune diabetes in adults is perched between type 1 and type 2: evidence from adults in one region of Spain. Diabetes Metab Res Rev 2013;29:446-451
45. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR: Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993;42:359-362
46. Kumar A, de Leiva A: Latent autoimmune diabetes in adults (LADA) in Asian and European populations. Diabetes Metab Res Rev 2017;
47. Machann J, Thamer C, Stefan N, Schwenzer NF, Kantartzis K, Haring HU, Claussen CD, Fritsche A, Schick F: Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. Radiology 2010;257:353-363
48. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, Meigs JB, Sutherland P, D'Agostino RB, Sr., O'Donnell CJ, Fox CS: Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. Obesity (Silver Spring, Md) 2010;18:2191-2198

49. Tonjes A, Fasshauer M, Kratzsch J, Stumvoll M, Bluher M: Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. PLoS One 2010;5:e13911
50. Bloomgarden ZT: Type 2 Diabetes in the Young. Diabetes Care 2004;27:998
51. Riddy DM, Delerive P, Summers RJ, Sexton PM, Langmead CJ: G Protein–Coupled Receptors Targeting Insulin Resistance, Obesity, and Type 2 Diabetes Mellitus. Pharmacological Reviews 2018;70:39
52. Gancheva S, Jelenik T, Alvarez-Hernandez E, Roden M: Interorgan Metabolic Crosstalk in Human Insulin Resistance. Physiological reviews 2018;98:1371-1415
53. Lean ME, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, Peters C, Zhyzhneuskaya S, Al-Mrabeh A, Hollingsworth KG, Rodrigues AM, Rehackova L, Adamson AJ, Sniehotta FF, Mathers JC, Ross HM, McIlvenna Y, Stefanetti R, Trenell M, Welsh P, Kean S, Ford I, McConnachie A, Sattar N, Taylor R: Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet 2018;391:541-551
54. American Diabetes Association: 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes—2019. Diabetes Care 2019;42:S90
55. Jiang G, Luk AO, Yang X, Wang Y, Tam CHT, Lau SH, Ozaki R, Kong APS, Tong PC, Chow CC, Chan JCN, So WY, Ma RCW: Progression to treatment failure among Chinese patients with type 2 diabetes initiated on metformin versus sulphonylurea monotherapy--The Hong Kong Diabetes Registry. Diabetes Res Clin Pract 2016;112:57-64
56. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G: Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. The New England journal of medicine 2006;355:2427-2443
57. Weng J, Li Y, Xu W, Shi L, Zhang Q, Zhu D, Hu Y, Zhou Z, Yan X, Tian H, Ran X, Luo Z, Xian J, Yan L, Li F, Zeng L, Chen Y, Yang L, Yan S, Liu J, Li M, Fu Z, Cheng H: Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet 2008;371:1753-1760
58. Xie F, Chan JC, Ma RC: Precision medicine in diabetes prevention, classification and management. J Diabetes Investig 2018;9:998-1015
59. Rathmann W, Strassburger K, Bongaerts B, Bobrov P, Kuss O, Mussig K, Markgraf DF, Szendroedi J, Herder C, Roden M, Group GDS: Impact of insulin sensitivity, beta-cell function and glycaemic control on initiation of second-line glucose-lowering treatment in newly diagnosed type 2 diabetes. Diabetes Obes Metab 2017;
60. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC, Testa MA, Weiss R: Type 2 diabetes mellitus. Nature Reviews Disease Primers 2015;1:15019
61. Bailey CJ, Aschner P, Del Prato S, LaSalle J, Ji L, Matthaei S: Individualized glycaemic targets and pharmacotherapy in type 2 diabetes. Diabetes & vascular disease research 2013;10:397-409
62. Cersosimo E, Johnson EL, Chovanes C, Skolnik N: Initiating therapy in patients newly diagnosed with type 2 diabetes: Combination therapy vs a stepwise approach. Diabetes Obes Metab 2018;20:497-507
63. Stidsen JV, Henriksen JE, Olsen MH, Thomsen RW, Nielsen JS, Rungby J, Ulrichsen SP, Berencsi K, Kahlert JA, Friborg SG, Brandslund I, Nielsen AA, Christiansen JS, Sorensen HT, Olesen TB, Beck-Nielsen H: Pathophysiology-based

phenotyping in type 2 diabetes: A clinical classification tool. Diabetes Metab Res Rev 2018;34:e3005
64. Ahlqvist E, Storm P, Karajamaki A, Martinell M, Dorkhan M, Carlsson A, Vikman P, Prasad RB, Aly DM, Almgren P, Wessman Y, Shaat N, Spegel P, Mulder H, Lindholm E, Melander O, Hansson O, Malmqvist U, Lernmark A, Lahti K, Forsen T, Tuomi T, Rosengren AH, Groop L: Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol 2018;6:361-369
65. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-1495
66. European Association for the Study of the Liver (EASL): EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of hepatology 2016;64:1388-1402
67. Tilg H, Moschen AR, Roden M: NAFLD and diabetes mellitus. Nature reviews Gastroenterology & hepatology 2017;14:32-42
68. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R: Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology 2017;65:1557-1565
69. Prasad RB, Groop L: Precision medicine in type 2 diabetes. J Intern Med 2019;285:40-48
70. Faerch K, Johansen NB, Witte DR, Lauritzen T, Jorgensen ME, Vistisen D: Relationship between insulin resistance and beta-cell dysfunction in subphenotypes of prediabetes and type 2 diabetes. J Clin Endocrinol Metab 2015;100:707-716
71. Faerch K, Witte DR, Tabak AG, Perreault L, Herder C, Brunner EJ, Kivimaki M, Vistisen D: Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: a post-hoc analysis of the longitudinal Whitehall II cohort study. Lancet Diabetes Endocrinol 2013;1:43-51
72. Fitipaldi H, McCarthy MI, Florez JC, Franks PW: A Global Overview of Precision Medicine in Type 2 Diabetes. Diabetes 2018;67:1911-1922
73. Florez JC: Precision Medicine in Diabetes: Is It Time? Diabetes Care 2016;39:1085-1088
74. Bowman P, Flanagan SE, Hattersley AT: Future Roadmaps for Precision Medicine Applied to Diabetes: Rising to the Challenge of Heterogeneity. Journal of Diabetes Research 2018;2018:12
75. Hattersley AT, Patel KA: Precision diabetes: learning from monogenic diabetes. Diabetologia 2017;60:769-777
76. McCarthy MI: Painting a new picture of personalised medicine for diabetes. Diabetologia 2017;60:793-799
77. Udler MS, Kim J, von Grotthuss M, Bonàs-Guarch S, Cole JB, Chiou J, Christopher DAoboM, the I, Boehnke M, Laakso M, Atzmon G, Glaser B, Mercader JM, Gaulton K, Flannick J, Getz G, Florez JC: Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: A soft clustering analysis. PLOS Medicine 2018;15:e1002654
78. Szendroedi J, Saxena A, Weber KS, Strassburger K, Herder C, Burkart V, Nowotny B, Icks A, Kuss O, Ziegler D, Al-Hasani H, Mussig K, Roden M, Group GDS: Cohort profile: the German Diabetes Study (GDS). Cardiovasc Diabetol 2016;15:59
79. Gastaldelli A, Gaggini M, DeFronzo RA: Role of Adipose Tissue Insulin Resistance in the Natural History of Type 2 Diabetes: Results From the San Antonio Metabolism Study. Diabetes 2017;66:815-822

80. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G: Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. Hepatology 2017;66:1486-1501
81. Seissler J, de Sonnaville JJ, Morgenthaler NG, Steinbrenner H, Glawe D, Khoo-Morgenthaler UY, Lan MS, Notkins AL, Heine RJ, Scherbaum WA: Immunological heterogeneity in type I diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. Diabetologia 1998;41:891-897
82. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS: Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 1998;47:1857-1866
83. Weber KS, Nowotny B, Strassburger K, Pacini G, Mussig K, Szendroedi J, Herder C, Roden M, Group GDS: The Role of Markers of Low-Grade Inflammation for the Early Time Course of Glycemic Control, Glucose Disappearance Rate, and beta-Cell Function in Recently Diagnosed Type 1 and Type 2 Diabetes. Diabetes Care 2015;38:1758-1767
84. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H, Type 1 Diabetes Trial Net Research G, European CPTSG: Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. Diabetes Care 2008;31:1966-1971
85. Menart-Houtermans B, Rutter R, Nowotny B, Rosenbauer J, Koliaki C, Kahl S, Simon MC, Szendroedi J, Schloot NC, Roden M: Leukocyte profiles differ between type 1 and type 2 diabetes and are associated with metabolic phenotypes: results from the German Diabetes Study (GDS). Diabetes Care 2014;37:2326-2333
86. Laufs A, Livingstone R, Nowotny B, Nowotny P, Wickrath F, Giani G, Bunke J, Roden M, Hwang JH: Quantitative liver 31P magnetic resonance spectroscopy at 3T on a clinical scanner. Magn Reson Med 2014;71:1670-1675
87. Bonhof GJ, Strom A, Puttgen S, Ringel B, Bruggemann J, Bodis K, Mussig K, Szendroedi J, Roden M, Ziegler D: Patterns of cutaneous nerve fibre loss and regeneration in type 2 diabetes with painful and painless polyneuropathy. Diabetologia 2017;60:2495-2503
88. Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Bruggemann J, Strom A, Peschel S, Kohler B, Stachs O, Guthoff RF, Roden M: Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 2014;63:2454-2463
89. Dyck PJ, Albers JW, Andersen H, Arezzo JC, Biessels GJ, Bril V, Feldman EL, Litchy WJ, O'Brien PC, Russell JW: Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev 2011;27:620-628
90. Herder C, Schamarek I, Nowotny B, Carstensen-Kirberg M, Strassburger K, Nowotny P, Kannenberg JM, Strom A, Puttgen S, Mussig K, Szendroedi J, Roden M, Ziegler D, German Diabetes Study G: Inflammatory markers are associated with cardiac autonomic dysfunction in recent-onset type 2 diabetes. Heart 2017;103:63-70
91. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S, Low P, Valensi P: Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes/Metabolism Research and Reviews 2011;27:639-653
92. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, Imperatore G, Linder B, Marcovina S, Pettitt DJ, Pihoker C, Saydah S, Wagenknecht L:

Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. The New England journal of medicine 2017;376:1419-1429
93. Chatterjee S, Khunti K, Davies MJ: Type 2 diabetes. The Lancet 2017;389:2239-2251
94. Faerch K, Hulman A, Solomon TP: Heterogeneity of Pre-diabetes and Type 2 Diabetes: Implications for Prediction, Prevention and Treatment Responsiveness. Curr Diabetes Rev 2016;12:30-41
95. Skyler JS: Characterizing Subgroups of Type 1 Diabetes. Diabetes 2014;63:3578-3580
96. Liu E, Eisenbarth GS: Type 1A diabetes mellitus-associated autoimmunity. Endocrinol Metab Clin North Am 2002;31:391-410, vii-viii
97. Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estorninho M, Kronenberg-Versteeg D, Bingley PJ, Todd JA, Guy C, Dunger DB, Powrie J, Willcox A, Foulis AK, Richardson SJ, de Rinaldis E, Morgan NG, Lorenc A, Peakman M: Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes 2014;63:3835-3845
98. Lee SH, Kwon HS, Yoo SJ, Ahn YB, Yoon KH, Cha BY, Lee KW, Son HY: Identifying latent autoimmune diabetes in adults in Korea: the role of C-peptide and metabolic syndrome. Diabetes Res Clin Pract 2009;83:e62-65
99. Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R, Mauricio D, De Leiva A, Yderstraede K, Beck-Neilsen H, Tuomilehto J, Sarti C, Thivolet C, Hadden D, Hunter S, Schernthaner G, Scherbaum WA, Williams R, Brophy S, Pozzilli P, Leslie RD: Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. Diabetes Care 2013;36:908-913
100. Juhl CB, Bradley U, Holst JJ, Leslie RD, Yderstraede KB, Hunter S, Action LC: Similar weight-adjusted insulin secretion and insulin sensitivity in short-duration late autoimmune diabetes of adulthood (LADA) and type 2 diabetes: Action LADA 9 [corrected]. Diabet Med 2014;31:941-945
101. Pipi E, Marketou M, Tsirogianni A: Distinct clinical and laboratory characteristics of latent autoimmune diabetes in adults in relation to type 1 and type 2 diabetes mellitus. World J Diabetes 2014;5:505-510
102. Brophy S, Brunt H, Davies H, Mannan S, Williams R: Interventions for latent autoimmune diabetes (LADA) in adults. Cochrane Database Syst Rev 2007:CD006165
103. Li X, Zhou Z, Huang G, Su H, Yan X, Yang L: Metabolic syndrome in adult-onset latent autoimmune diabetes. Metab Syndr Relat Disord 2005;3:174-180
104. Marini MA, Fiorentino TV, Succurro E, Pedace E, Andreozzi F, Sciacqua A, Perticone F, Sesti G: Association between hemoglobin glycation index with insulin resistance and carotid atherosclerosis in non-diabetic individuals. PLoS One 2017;12:e0175547
105. Arranz Martin A, Lecumberri Pascual E, Brito Sanfiel MA, Andia Melero V, Nattero Chavez L, Sanchez Lopez I, Canovas Molina G, Arrieta Blanco F, Gonzalez Perez Del Villar N: Clinical and metabolic profile of patients with latent autoimmune diabetes in adults in specialized care in Madrid. Endocrinologia, diabetes y nutricion 2017;64:34-39
106. Zaharia OP, Bobrov P, Strassburger K, Bodis K, Karusheva Y, Scholz M, Markgraf DF, Burkart V, Schloot NC, Mussig K, Szendroedi J, Roden M: Metabolic Characteristics of Recently Diagnosed Adult-Onset Autoimmune Diabetes Mellitus. J Clin Endocrinol Metab 2018;103:429-437
107. Bertoluci MC, Rocha VZ: Cardiovascular risk assessment in patients with diabetes. Diabetology & metabolic syndrome 2017;9:25

108. Roh MO, Jung CH, Kim BY, Mok JO, Kim CH: The prevalence and characteristics of latent autoimmune diabetes in adults (LADA) and its relation with chronic complications in a clinical department of a university hospital in Korea. Acta Diabetol 2013;50:129-134
109. Martinell M, Dorkhan M, Stalhammar J, Storm P, Groop L, Gustavsson C: Prevalence and risk factors for diabetic retinopathy at diagnosis (DRAD) in patients recently diagnosed with type 2 diabetes (T2D) or latent autoimmune diabetes in the adult (LADA). Journal of diabetes and its complications 2016;30:1456-1461
110. Kahl S, Nowotny B, Piepel S, Nowotny PJ, Strassburger K, Herder C, Pacini G, Roden M: Estimates of insulin sensitivity from the intravenous-glucose-modified-clamp test depend on suppression of lipolysis in type 2 diabetes: a randomised controlled trial. Diabetologia 2014;57:2094-2102
111. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ: A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539-548
112. Tura A, Chemello G, Szendroedi J, Gobl C, Faerch K, Vrbikova J, Pacini G, Ferrannini E, Roden M: Prediction of clamp-derived insulin sensitivity from the oral glucose insulin sensitivity index. Diabetologia 2018;61:1135-1141
113. Nambam B, Aggarwal S, Jain A: Latent autoimmune diabetes in adults: A distinct but heterogeneous clinical entity. World J Diabetes 2010;1:111-115
114. Zaharia OP, Szendrödi J: Subphänotypen des Diabetes. Der Diabetologe 2019;15:288-295
115. American Diabetes A: Dyslipidemia Management in Adults With Diabetes. Diabetes Care 2004;27:s68-s71
116. Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, Bodis K, Markgraf DF, Burkart V, Mussig K, Hwang JH, Asplund O, Groop L, Ahlqvist E, Seissler J, Nawroth P, Kopf S, Schmid SM, Stumvoll M, Pfeiffer AFH, Kabisch S, Tselmin S, Haring HU, Ziegler D, Kuss O, Szendroedi J, Roden M: Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol 2019;7:684-694
117. Zou X, Zhou X, Zhu Z, Ji L: Novel subgroups of patients with adult-onset diabetes in Chinese and US populations. The Lancet Diabetes & Endocrinology 2019;7:9-11
118. Samuel VT, Shulman GI: Mechanisms for insulin resistance: common threads and missing links. Cell 2012;148:852-871
119. Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, Bodis K, Markgraf DF, Burkart V, Mussig K, Hwang JH, Asplund O, Groop L, Ahlqvist E, Seissler J, Nawroth P, Kopf S, Schmid SM, Stumvoll M, Pfeiffer AFH, Kabisch S, Tselmin S, Haring HU, Ziegler D, Kuss O, Szendroedi J, Roden M: Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol 2019;
120. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT: Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. The American journal of physiology 1999;276:E977-989
121. De Lucia Rolfe E, Brage S, Sleigh A, Finucane F, Griffin SJ, Wareham NJ, Ong KK, Forouhi NG: Validity of ultrasonography to assess hepatic steatosis compared to magnetic resonance spectroscopy as a criterion method in older adults. PloS one 2018;13:e0207923-e0207923

122. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, Sosenko JM, Ziegler D: Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care 2017;40:136-154
123. Trombetta M, Spiazzi G, Zoppini G, Muggeo M: Review article: type 2 diabetes and chronic liver disease in the Verona diabetes study. Alimentary pharmacology & therapeutics 2005;22 Suppl 2:24-27
124. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844-1850
125. Roden M: Mechanisms of Disease: hepatic steatosis in type 2 diabetes--pathogenesis and clinical relevance. Nature clinical practice Endocrinology & metabolism 2006;2:335-348
126. Jelenik T, Roden M: Mitochondrial plasticity in obesity and diabetes mellitus. Antioxidants & redox signaling 2013;19:258-268
127. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet (London, England) 1963;1:785-789
128. Szendroedi J, Phielix E, Roden M: The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol 2012;8:92-103
129. Liu Y-L, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JBS, Allison MED, Alexander GJ, Piguet A-C, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour J-F, Day CP, Daly AK, Anstee QM: TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nature Communications 2014;5:4309
130. Sookoian S, Castaño GO, Scian R, Mallardi P, Fernández Gianotti T, Burgueño AL, San Martino J, Pirola CJ: Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515-525
131. Zhou Y, Llauradó G, Orešič M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H: Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. Journal of hepatology 2015;62:657-663
132. Lallukka S, Yki-Jarvinen H: Non-alcoholic fatty liver disease and risk of type 2 diabetes. Best practice & research Clinical endocrinology & metabolism 2016;30:385-395
133. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, Herder C, Carstensen M, Krausch M, Knoefel WT, Schlensak M, Roden M: Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab 2015;21:739-746
134. Kahl S, Strassburger K, Nowotny B, Livingstone R, Kluppelholz B, Kessel K, Hwang JH, Giani G, Hoffmann B, Pacini G, Gastaldelli A, Roden M: Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One 2014;9:e94059
135. Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V: Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Alimentary pharmacology & therapeutics 2014;40:1209-1222
136. Koehler EM, Schouten JN, Hansen BE, Hofman A, Stricker BH, Janssen HL: External validation of the fatty liver index for identifying nonalcoholic fatty liver disease in a population-based study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2013;11:1201-1204

137. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM: Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: A meta-analysis. Hepatology 2011;54:1082-1090
138. Sulaiman MK: Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. Diabetology & metabolic syndrome 2019;11:7-7
139. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR: Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. Diabetes 2006;55:1832-1839
140. Spoto B, Pisano A, Zoccali C: Insulin resistance in chronic kidney disease: a systematic review. American Journal of Physiology-Renal Physiology 2016;311:F1087-F1108
141. Pai MP: Estimating the glomerular filtration rate in obese adult patients for drug dosing. Advances in chronic kidney disease 2010;17:e53-62
142. Levey AS, Coresh J, Balk E, et al.: National kidney foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Annals of Internal Medicine 2003;139:137-147
143. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL: Diabetic neuropathy: clinical manifestations and current treatments. The Lancet Neurology 2012;11:521-534
144. Bonhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D: Emerging biomarkers, tools, and treatments for diabetic polyneuropathy. Endocrine reviews 2018;
145. Feldman EL, Nave KA, Jensen TS, Bennett DLH: New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. Neuron 2017;93:1296-1313
146. Paffenbarger RS, Jr., Hyde RT, Wing AL, Hsieh CC: Physical activity, all-cause mortality, and longevity of college alumni. The New England journal of medicine 1986;314:605-613
147. Qin L, Knol MJ, Corpeleijn E, Stolk RP: Does physical activity modify the risk of obesity for type 2 diabetes: a review of epidemiological data. European journal of epidemiology 2010;25:5-12
148. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, Castorino K, Tate DF: Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. Diabetes care 2016;39:2065-2079
149. Böhm A, Weigert C, Staiger H, Häring HU: Exercise and diabetes: relevance and causes for response variability. Endocrine 2016;51:390-401
150. Kacerovsky-Bielesz G, Kacerovsky M, Chmelik M, Farukuoye M, Ling C, Pokan R, Tschan H, Szendroedi J, Schmid AI, Gruber S, Herder C, Wolzt M, Moser E, Pacini G, Smekal G, Groop L, Roden M: A single nucleotide polymorphism associates with the response of muscle ATP synthesis to long-term exercise training in relatives of type 2 diabetic humans. Diabetes Care 2012;35:350-357
151. Herrmann SD, Willis EA, Honas JJ, Lee J, Washburn RA, Donnelly JE: Energy intake, nonexercise physical activity, and weight loss in responders and nonresponders: The Midwest Exercise Trial 2. Obesity 2015;23:1539-1549
152. Kahara T, Takamura T, Hayakawa T, Nagai Y, Yamaguchi H, Katsuki T, Katsuki K, Katsuki M, Kobayashi K: PPARgamma gene polymorphism is associated with exercise-mediated changes of insulin resistance in healthy men. Metabolism 2003;52:209-212
153. Kahara T, Takamura T, Hayakawa T, Nagai Y, Yamaguchi H, Katsuki T, Katsuki K, Katsuki M, Kobayashi K: Prediction of exercise-mediated changes in metabolic markers by gene polymorphism. Diabetes research and clinical practice 2002;57:105-110

154. Stefan N, Thamer C, Staiger H, Machicao F, Machann J, Schick F, Venter C, Niess A, Laakso M, Fritsche A, Haring HU: Genetic variations in PPARD and PPARGC1A determine mitochondrial function and change in aerobic physical fitness and insulin sensitivity during lifestyle intervention. The Journal of clinical endocrinology and metabolism 2007;92:1827-1833
155. Kacerovsky-Bielesz G, Chmelik M, Ling C, Pokan R, Szendroedi J, Farukuoye M, Kacerovsky M, Schmid AI, Gruber S, Wolzt M, Moser E, Pacini G, Smekal G, Groop L, Roden M: Short-term exercise training does not stimulate skeletal muscle ATP synthesis in relatives of humans with type 2 diabetes. Diabetes 2009;58:1333-1341
156. Apostolopoulou M, Strassburger K, Herder C, Knebel B, Kotzka J, Szendroedi J, Roden M, group GDS: Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. Diabetologia 2016;59:2203-2207
157. Stephens NA, Xie H, Johannsen NM, Church TS, Smith SR, Sparks LM: A transcriptional signature of "exercise resistance" in skeletal muscle of individuals with type 2 diabetes mellitus. Metabolism 2015;64:999-1004
158. Tamasawa N, Murakami H, Yamato K, Matsui J, Tanabe J, Suda T: Influence of apolipoprotein E genotype on the response to caloric restriction in type 2 diabetic patients with hyperlipidaemia. Diabetes, Obesity and Metabolism 2003;5:345-348
159. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J: Association of apolipoprotein E genotypes with lipid levels and coronary risk. Jama 2007;298:1300-1311
160. Weber KS, Knebel B, Strassburger K, Kotzka J, Stehle P, Szendroedi J, Mussig K, Buyken AE, Roden M, Group GDS: Associations between explorative dietary patterns and serum lipid levels and their interactions with ApoA5 and ApoE haplotype in patients with recently diagnosed type 2 diabetes. Cardiovasc Diabetol 2016;15:138
161. Pieralice S, Pozzilli P: Latent Autoimmune Diabetes in Adults: A Review on Clinical Implications and Management. Diabetes & metabolism journal 2018;42:451-464
162. Lawton J, Fox A, Fox C, Kinmonth AL: Participating in the United Kingdom Prospective Diabetes Study (UKPDS): a qualitative study of patients' experiences. The British journal of general practice : the journal of the Royal College of General Practitioners 2003;53:394-398
163. Dolnicar S, Grün B, Leisch F, Schmidt K: Required Sample Sizes for Data-Driven Market Segmentation Analyses in Tourism. Journal of Travel Research 2014;53:296-306
164. Merino J, Udler MS, Leong A, Meigs JB: A Decade of Genetic and Metabolomic Contributions to Type 2 Diabetes Risk Prediction. Curr Diab Rep 2017;17:135
165. Knowles JW, Ashley EA: Cardiovascular disease: The rise of the genetic risk score. PLOS Medicine 2018;15:e1002546
166. Floyd JS, Psaty BM: The Application of Genomics in Diabetes: Barriers to Discovery and Implementation. Diabetes Care 2016;39:1858-1869
167. Schully SD, Lam TK, Dotson WD, Chang CQ, Aronson N, Birkeland ML, Brewster SJ, Boccia S, Buchanan AH, Calonge N, Calzone K, Djulbegovic B, Goddard KA, Klein RD, Klein TE, Lau J, Long R, Lyman GH, Morgan RL, Palmer CG, Relling MV, Rubinstein WS, Swen JJ, Terry SF, Williams MS, Khoury MJ: Evidence synthesis and guideline development in genomic medicine: current status and future prospects. Genetics in medicine : official journal of the American College of Medical Genetics 2015;17:63-67

Acknowledgements

First and foremost, I would like to thank my advisors, Prof. Dr. Michael Roden and PD Dr. Julia Szendrödi, for trusting me with scientific projects, giving me the opportunity to implement my ideas and for sharing their knowledge. You have been amazing mentors advising me, guiding me and inspiring me through all these years. This work would not have been possible without your continuous support, supervision and encouragement.

My gratitude goes also to the deputy heads of the Clinical Research Center, PD. Dr. Volker Burkart and Prof. Dr. Karsten Müssig and all group members and staff – thank you for your help, team work, professionalism and for creating an excellent atmosphere for doing research. I would also like to acknowledge Dr. Strassburger for his help with statistical analyses, Dr. Jelenik for his valuable insights, and Dr. Pesta for the enthusiastic constructive discussions. Because of your continuous support and your kind encouragements I was able to stay focused and motivated. Special thanks to my colleagues, Theresa Kössler, Yanislava Karusheva and Kálmán Bódis, for supporting me in every way. I will forever cherish the beautiful memories we made during our time working together.

To all volunteers, who took part in these studies – thank you for participating in the German Diabetes Study and for contributing to the progress in diabetes research. As the German Diabetes Study is a nation-wide mission, I would like to extend my gratitude to the centers across Germany involved in the conduct of this study and to the German Center for Diabetes Research (DZD) for their efforts.

At last, I want to thank my wonderful parents, Rodica Milena and Răzvan Zaharia, and my partner, Jason McCaffery, who have been supporting me throughout writing this thesis and my life in general. Thank you for your unconditional support, your love and the continuously motivating words.