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

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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.

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,

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

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See **Online** for appendix

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.

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 with SAID

	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**

(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 than 5%. The fatty liver index was highest at baseline in

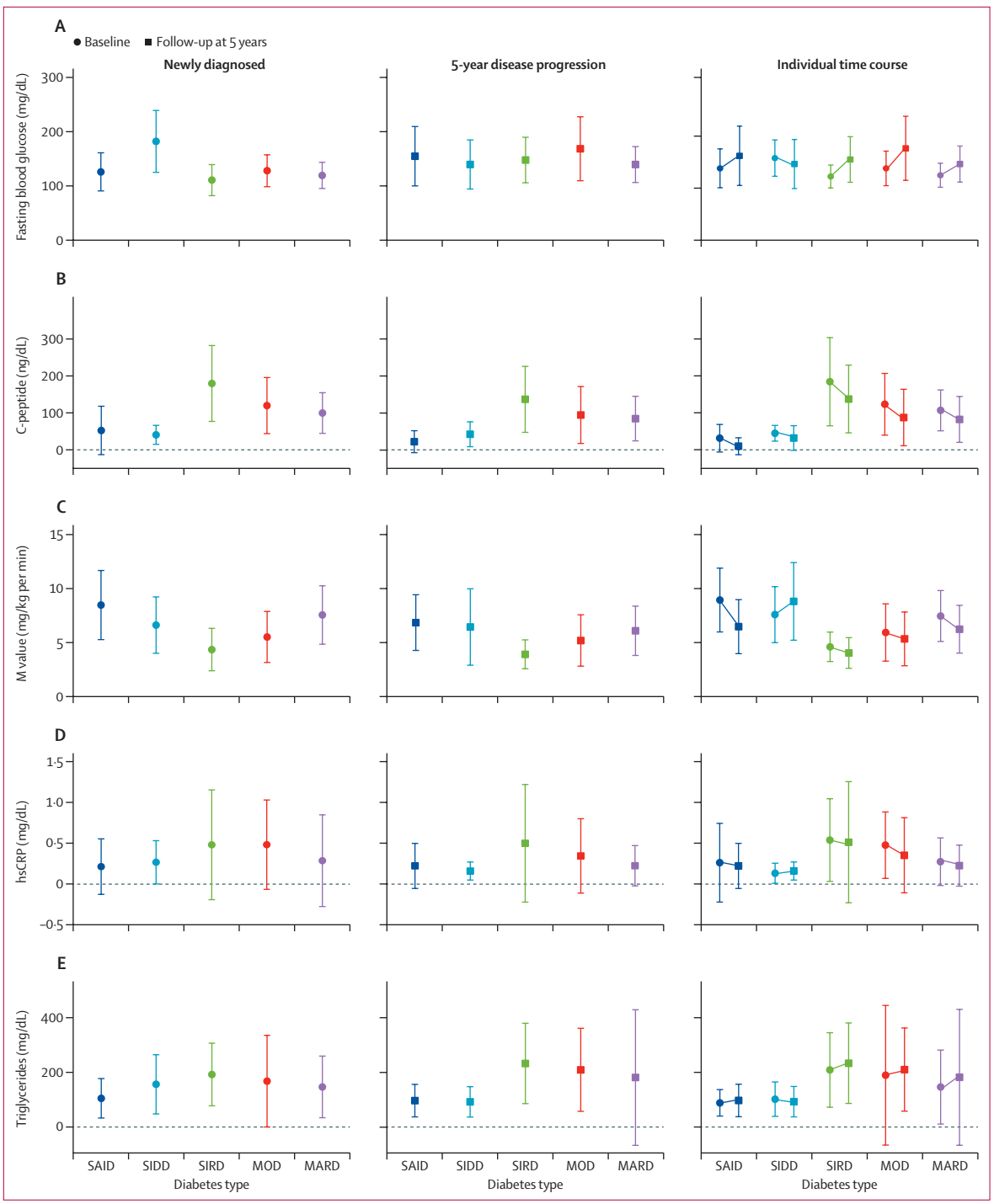

Figure 1: **Progression of metabolic variables over 5 years**
Metabolic characteristics of patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD were assessed at baseline and after 5 years of disease progression. Plots show fasting blood glucose (A), total C-peptide secretion during the intravenous 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.

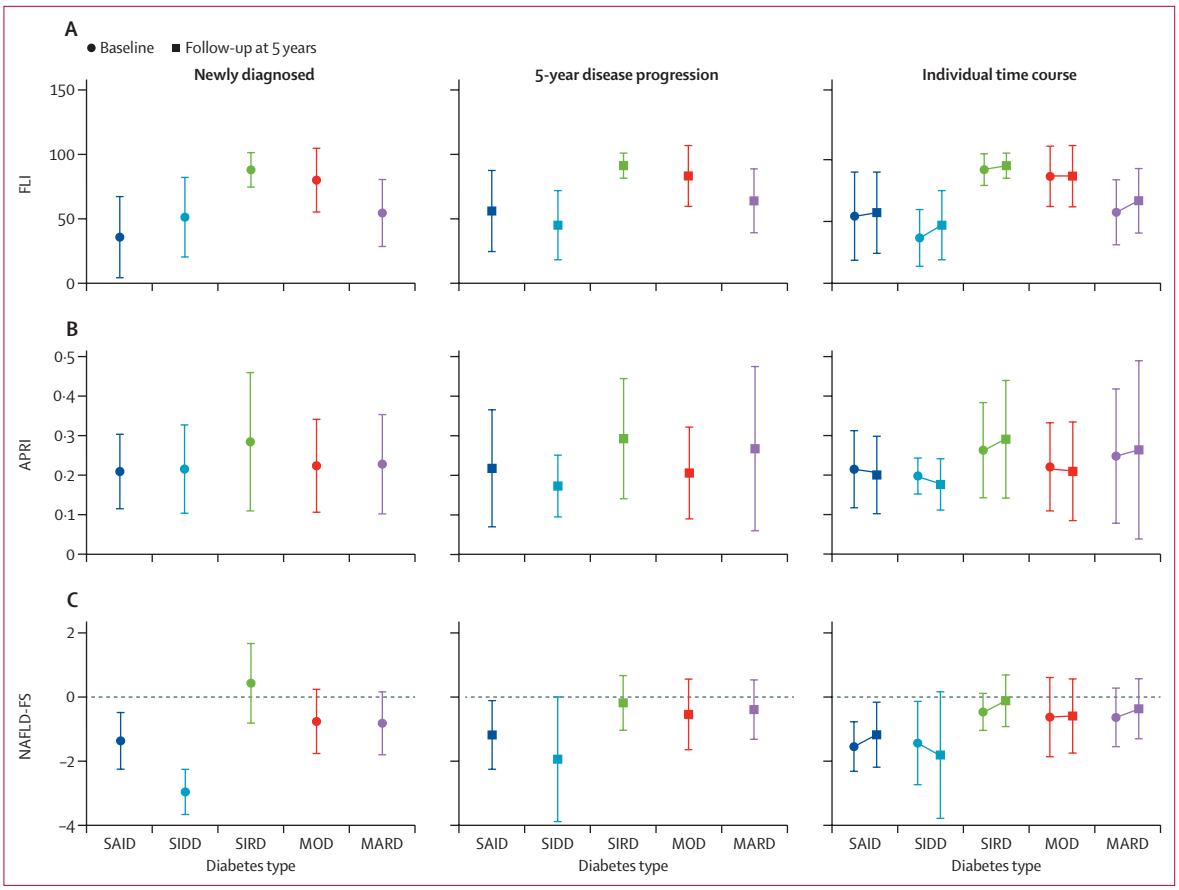

Figure 2: **Progression of liver steatosis and liver fibrosis over 5 years**
Liver testing was done at baseline and after 5 years of disease progression in patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD. Liver steatosis was assessed non-invasively using FLI (A), liver fibrosis was measured 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.

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 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.

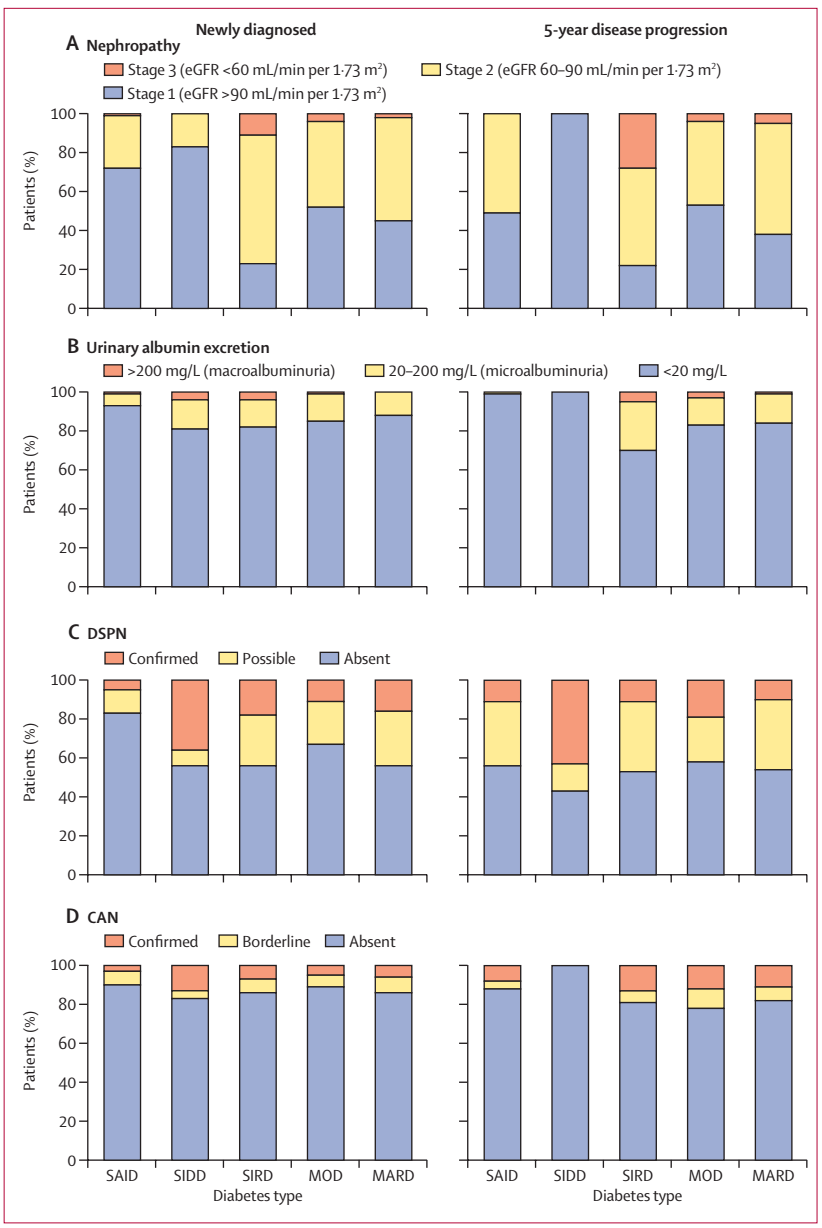

Figure 3: **Progression of diabetes-associated nephropathy and neuropathy over 5 years**
Diabetic nephropathy (A, B) was assessed non-invasively by eGFR at baseline and after 5 years of disease progression in patients with newly diagnosed SAID, SIDD, SIRD, MOD, and MARD. DSPN (C) and CAN (D) were assessed 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.

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 diabetes-related comorbidities early after diagnosis.

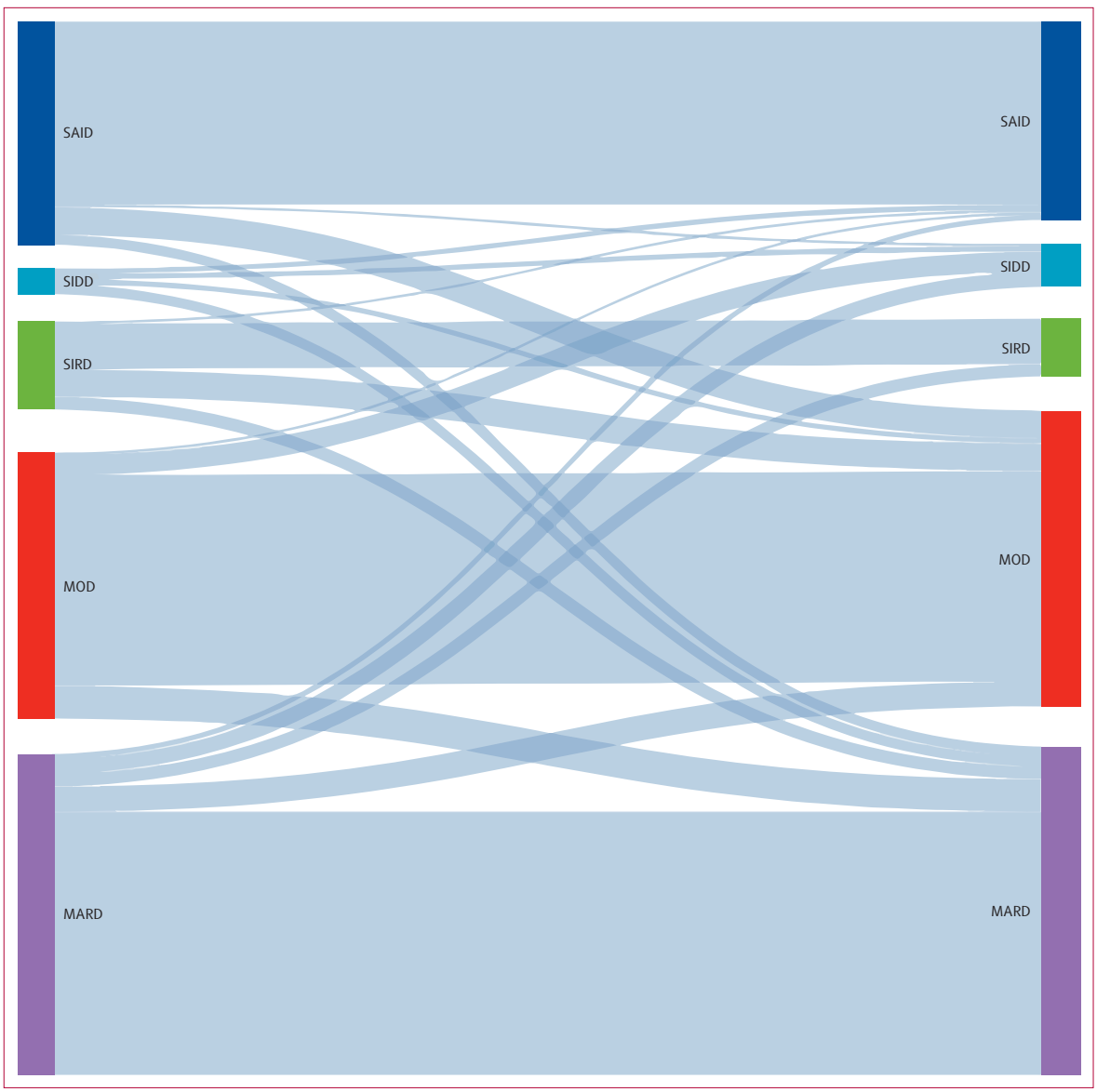

Figure 4: **Cluster redistribution at 5-year follow-up**
The Sankey diagram shows the redistribution and migration pattern of the study population from baseline to 5-year follow-up. Cluster reproducibility at follow-up (ie, the proportion of patients allocated to the same cluster at baseline and follow 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.

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.

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