



# Pathophysiology-based subphenotyping of individuals at elevated risk for type 2 diabetes

Robert Wagner<sup>1,2,3</sup>✉, Martin Heni<sup>1,2,3,4</sup>, Adam G. Tabák<sup>5,6,7</sup>, Jürgen Machann<sup>1,2,8</sup>, Fritz Schick<sup>2,8</sup>, Elko Randrianarisoa<sup>1,2</sup>, Martin Hrabě de Angelis<sup>1,2,9,10</sup>, Andreas L. Birkenfeld<sup>1,2,3</sup>, Norbert Stefan<sup>1,2,3,11</sup>, Andreas Peter<sup>1,2,4</sup>, Hans-Ulrich Häring<sup>1,2</sup> and Andreas Fritzsche<sup>1,2,3</sup>

The state of intermediate hyperglycemia is indicative of elevated risk of developing type 2 diabetes<sup>1</sup>. However, the current definition of prediabetes neither reflects subphenotypes of pathophysiology of type 2 diabetes nor is predictive of future metabolic trajectories. We used partitioning on variables derived from oral glucose tolerance tests, MRI-measured body fat distribution, liver fat content and genetic risk in a cohort of extensively phenotyped individuals who are at increased risk for type 2 diabetes<sup>2,3</sup> to identify six distinct clusters of subphenotypes. Three of the identified subphenotypes have increased glycemia (clusters 3, 5 and 6), but only individuals in clusters 5 and 3 have imminent diabetes risks. By contrast, those in cluster 6 have moderate risk of type 2 diabetes, but an increased risk of kidney disease and all-cause mortality. Findings were replicated in an independent cohort using simple anthropomorphic and glycemic constructs<sup>4</sup>. This proof-of-concept study demonstrates that pathophysiological heterogeneity exists before diagnosis of type 2 diabetes and highlights a group of individuals who have an increased risk of complications without rapid progression to overt type 2 diabetes.

Type 2 diabetes occurs when insulin secretion from pancreatic beta cells cannot sufficiently be increased to compensate for insulin resistance. Causes of beta-cell dysfunction and insulin resistance are heterogeneous, as are individual trajectories of hyperglycemia and subsequent manifestation of diabetes complications<sup>5</sup>. The currently used binary definition of type 2 diabetes is based solely on blood glucose and cannot differentiate between patients with mild or more-aggressive disease, the latter being prone to early development of complications. In addition to blood glucose, new proposed diabetes classifications<sup>6,7</sup> introduced additional variables, such as insulin secretion and insulin sensitivity, to subclassify the type 2 diabetes spectrum with the primary aim of a better prediction of metabolic dysfunction and complications.

The development of type 2 diabetes is a slow process, and its manifestation is preceded by a phase of prediabetes that often remains undiagnosed. Some diabetes complications, such as the unexpectedly frequent early diabetic kidney disease in the newly identified severe insulin-resistant diabetes cluster<sup>6</sup>, might require preventive

actions prior to the clinical manifestation of type 2 diabetes. The assessment of insulin secretion and insulin sensitivity could be hindered by secondary gluco-lipotoxicity, once diabetes has developed and glucose levels are continuously elevated<sup>8</sup>.

Determination of prediabetes subphenotypes prior to the manifestation of diabetes could improve detection of individuals at risk for diabetes and complications.

Using accurate measurements of insulin sensitivity and insulin secretion based on oral glucose tolerance test (OGTT)-derived variables, as well as variables linked to diabetes pathogenesis, we describe a new subphenotyping approach of metabolic risk before diabetes manifestation. Variables include high-density lipoprotein (HDL) cholesterol, which has been causally linked to type 2 diabetes<sup>9</sup>, magnetic resonance (MR)-imaging-derived measures of metabolically unfavorable and favorable fat compartments<sup>10</sup> and liver fat content measured with <sup>1</sup>H-MR spectroscopy. To assess genetic liability, we also incorporated a polygenic risk score for type 2 diabetes<sup>11</sup> as a partitioning variable. The clusters identified by the sophisticated phenotypes in the Tübingen Family study and Tübingen Lifestyle Program (TUEF/TULIP) cohort were replicated using simpler markers of similar anthropometric and glycemic constructs in a large prospective occupational cohort (the Whitehall II study)<sup>4</sup>. Our results suggest that stratification of populations at increased risk for type 2 diabetes using simple clinical features could allow for precise and efficient prevention strategies for individuals at increased risk of developing type 2 diabetes.

Initial clustering and identification of the subphenotypes was done using data from a subset of participants ( $n=899$ ) from the TUEF/TULIP study. Analysis was performed on data for participants who had no missing values for the preselected phenotyping variables: glucose challenge; insulin sensitivity; insulin secretion; HDL cholesterol; liver fat content; subcutaneous-fat volume; visceral fat volume; and a polygenic risk score for type 2 diabetes risk. The clustering was replicated in the Whitehall II cohort ( $n=6,810$ ) using conceptually similar variables: glycemia during glucose challenge, insulin sensitivity, insulin secretion, fasting insulin, fasting triglycerides, waist circumference, hip circumference, body-mass index (BMI) and HDL cholesterol (Extended Data Fig. 1 and Methods).

<sup>1</sup>Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany. <sup>2</sup>German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany. <sup>3</sup>Department of Internal Medicine, Division of Diabetology, Endocrinology and Nephrology, Eberhard-Karls University Tübingen, Tübingen, Germany. <sup>4</sup>Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital of Tübingen, Tübingen, Germany. <sup>5</sup>Department of Epidemiology and Public Health, University College London, London, UK. <sup>6</sup>Department of Internal Medicine and Oncology, Semmelweis University Faculty of Medicine, Budapest, Hungary. <sup>7</sup>Department of Public Health, Semmelweis University Faculty of Medicine, Budapest, Hungary. <sup>8</sup>University Department of Radiology, Section on Experimental Radiology, Eberhard-Karls University Tübingen, Tübingen, Germany. <sup>9</sup>Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Zentrum München, Neuherberg, Germany. <sup>10</sup>Chair of Experimental Genetics, TUM School of Life Sciences (SoLS), Technische Universität München, Freising, Germany. <sup>11</sup>Department of Pediatrics, Harvard Medical School, Boston, MA, USA. ✉e-mail: [robert.wagner@uni-tuebingen.de](mailto:robert.wagner@uni-tuebingen.de)

We identified six clusters with distinctive patterns of the variables in the TUEF/TULIP study (Fig. 1a,b), which were replicated in the Whitehall II cohort (Fig. 1c,d). Cluster characteristics and comparisons are shown in Table 1 and Supplementary Tables 1–3, and key features of the clusters are reported in Extended Data Fig. 2.

There was a cluster-specific enrichment of the diabetes-related genetic variant rs10830963 in melatonin receptor 1B (*MTNR1B*) (analysis of variance (ANOVA)  $P=0.02$  after Benjamini–Hochberg correction for multiple testing; Supplementary Table 4). Participants in cluster 3 had higher frequency of the diabetes-associated G allele than did those in cluster 1 (uncorrected  $P=0.00036$  for cluster 3 relative to cluster 1). Using the pathophysiological classification of diabetes-related genetic variants proposed by Udler et al.<sup>12</sup>, we found differences within the beta-cell group (uncorrected  $P=0.001$ ,  $P=0.007$  after Benjamini–Hochberg correction; Fig. 2a). Pairwise comparisons showed significant differences between cluster 6 and each of clusters 1, 2 and 3 (ANOVA with Tukey's post-hoc test  $P<0.05$ ), suggesting a lower abundance of beta-cell function related risk alleles in cluster 6.

In the longitudinal analysis, all participants with available data were followed for the development of diabetes, nephropathy, cardiovascular endpoints and all-cause mortality (Fig. 3). The proportional-hazards assessment in Whitehall II is shown in Supplementary Table 5. Diabetes incidence was the highest in cluster 5, followed by cluster 3 in both the TUEF/TULIP and Whitehall II cohorts. Mean follow-up was 4.1 and 16.3 yr, respectively. In TUEF/TULIP, participants in cluster 6 did not demonstrate an increased risk for diabetes (Fig. 3a). The diabetes risk in cluster 6 was only moderately elevated compared with that in cluster 1 in Whitehall II (hazard ratio (HR) 2.22, confidence interval (CI) 1.7–2.89). Clusters 3 and 5 also had elevated diabetes risk compared with that in cluster 1, with HRs of 3.45 (CI 2.76–4.31) and 6.62 (CI 5.06–8.67), respectively (Fig. 3c and Supplementary Table 5). By contrast, cluster 2 had a significantly lower risk of developing diabetes than did cluster 1 (HR 0.4, CI 0.33–0.47) in the Whitehall II cohort. Current smoking was a risk factor for diabetes in Whitehall II, but did not affect the risk of diabetes for participants in clusters 3, 5 and 6 (Supplementary Table 6).

In Whitehall II, there were 201 participants with incident diabetes and a defined Ahlqvist diabetes classification<sup>6</sup>. Relatively few participants developed diabetes in the metabolically healthy clusters (cluster 1, 48 of 817 (5.9%); cluster 2, 62 of 2,552 (2.4%); cluster 4, 14 of 314 (4.5%), out of those eligible for computation of the Ahlqvist classes). Of these participants, most (34 of 48 (70.8%), 59 of 62 (95.2%) and 12 of 14 (85.7%, respectively) transitioned into mild diabetes classes according to the Ahlqvist classification (mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD)). Thirteen of 23 participants (57%) in cluster 6 (13 of 23 (57%)) developed severe insulin-resistant diabetes (SIRD, Supplementary Table 7 and Extended Data Fig. 3).

We used two approaches to compare our multivariable clustering with glucose-based stratification alone. We first tested cumulative diabetes risk for the Hulman classes<sup>13</sup> that are computed from the glucose course during an OGTT (Extended Data Fig. 4). Next, we stratified the baseline area under the curve (AUC) glucose of

Whitehall II into 5 quintiles, (Extended Data Fig. 5). In head-to-head comparisons, the cumulative diabetes risk of the high-risk clusters 3 and 5 together was higher than that of Hulman classes 3 and 4 together ( $P=0.04$ , TUEF/TULIP) and also higher than that of the top 2 AUC glucose quintiles ( $P<0.0001$ , Whitehall II, both log-rank tests). Thus, our cluster-based approach was superior to both of these approaches in delineating groups with high cumulative risk for development of diabetes.

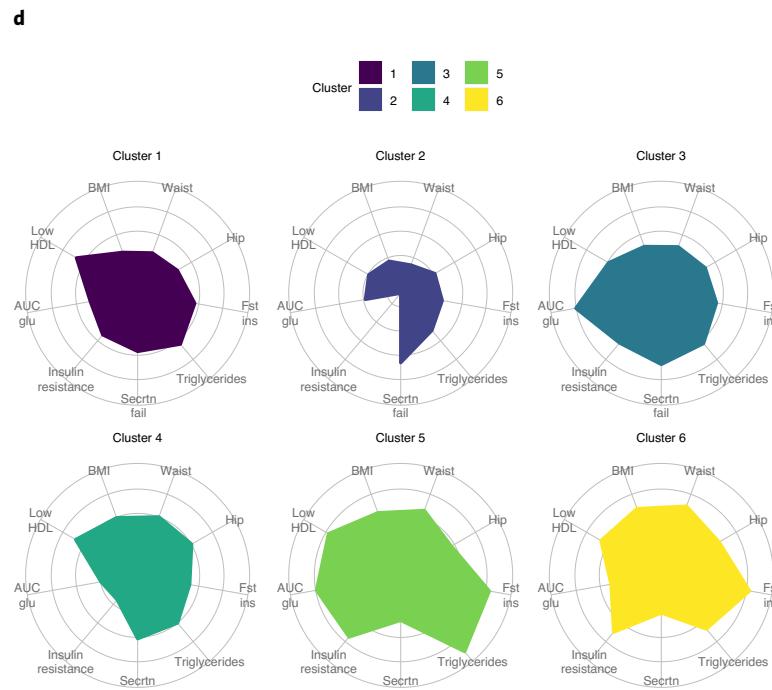
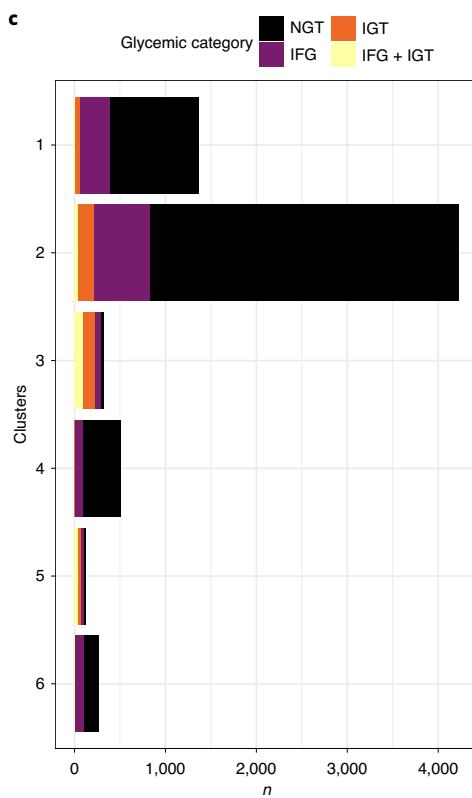
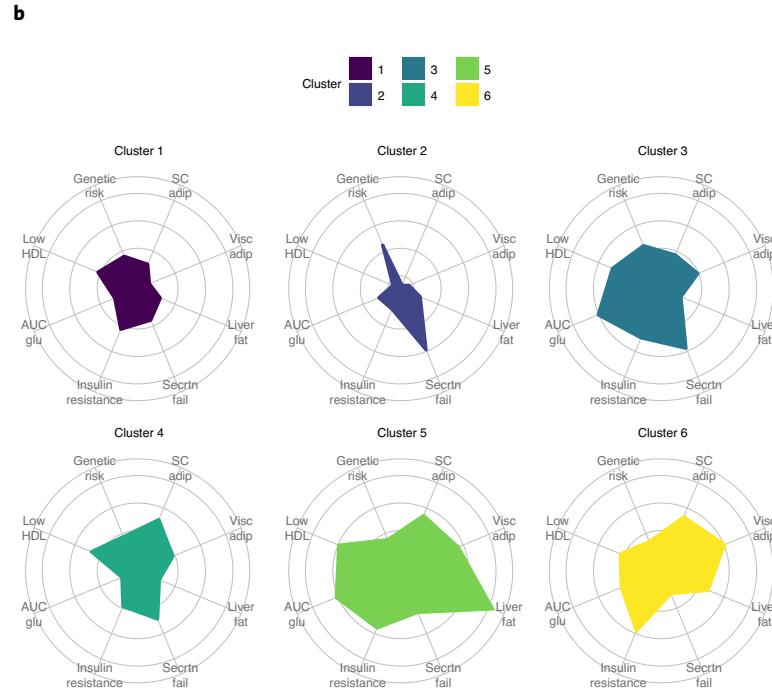
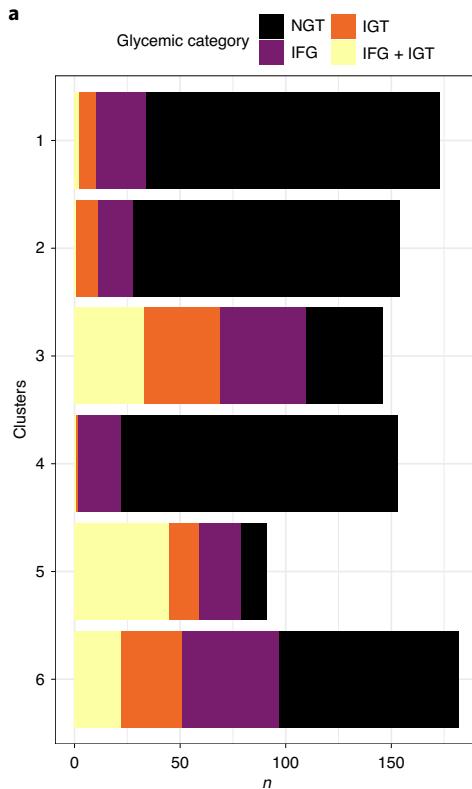
The overall difference in the Kaplan–Meier curves for microalbuminuria did not reach statistical significance in TUEF/TULIP (mean follow-up 4.3 yr, number of events = 71,  $P_{\text{log-rank, uncorrected}}=0.061$ ; Fig. 3b). In the proportional-hazard assessment, cluster 6 showed a significantly higher risk for microalbuminuria than did cluster 1 ( $P=0.01$ ). Results were similar, but not significant, for the Whitehall II participants with available baseline urine measurements ( $n=316$ , number of events = 58, uncorrected  $P=0.058$ ) when adjusting for baseline urinary albumin-to-creatinine ratio. In Whitehall II, participants in cluster 6 had a significantly higher risk for stage 3 chronic kidney disease or worse than did those in cluster 1 (uncorrected  $P=0.0003$ , mean follow-up 18.2 yr, number of events = 1,387; Fig. 3d and Extended Data Fig. 6). Individuals in the diabetes-susceptible clusters 3 and 5 also demonstrated higher risks for chronic kidney disease relative to cluster 1 in Whitehall II (uncorrected  $P=0.004$  and  $P=0.02$ , respectively; Supplementary Table 5). The fully adjusted model also controlled for smoking, cholesterol and triglycerides is shown in Supplementary Table 8. Given that participants in cluster 6 had elevated visceral fat, we hypothesized that this could be associated with fat in the renal sinuses, which is a risk factor for exercise-induced microalbuminuria<sup>14</sup>. TUEF/TULIP participants in cluster 6 had the most renal sinus fat compared with other clusters ( $P<0.05$  for all pairwise comparisons, Tukey's post-hoc test, Fig. 2b,  $n=199$ ). It was higher than in cluster 5 after adjusting for potential confounders (Supplementary Table 9).

In the TUEF/TULIP cohort, we used carotid intima-media thickness (IMT) as a proxy for cardiovascular endpoints due to a lack of a register-based assessment of clinical events. IMT was associated with cluster membership ( $F=14.55$ , degrees of freedom = 5,  $P<0.001$ ). Each of clusters 3, 5 and 6 had higher IMT values than each of clusters 1, 2 or 4 (Extended Data Fig. 7 and Supplementary Table 1,  $P<0.002$ ). After adjustment for sex, age, age<sup>2</sup> and BMI, clusters 3 and 5 had higher IMT than did cluster 1 ( $P<0.03$ ). In the Whitehall II cohort, we evaluated the incidence of coronary heart disease (CHD, mean follow-up 17.2 yr, 800 events; Fig. 2e). We also investigated, as a combined vascular endpoint, the incidence of CHD and stroke (mean follow-up 22.9 yr, 1,040 events, Supplementary Table 5). In the proportional-hazard assessment, the elevated cardiovascular risk in cluster 5 was not independent from sex, age and BMI, but was consistently lower in cluster 2 than in cluster 1, and this was also the case after adjustments (Supplementary Table 5). Compared with cluster 1 in Whitehall II, all-cause mortality was by about 40% higher for cluster 6 (Fig. 3f), while cluster 2 had a lower mortality rate, even after adjustments for covariates (Supplementary Table 5). The elevated mortality risk in cluster 6 (relative to cluster 1) was not affected by adjustment for smoking and lipids (full model in Supplementary Table 10).

**Fig. 1 | Distribution of the cluster feature variables.** **a–d**, Partitioning of participants into 6 clusters along 8 variables in the TUEF/TULIP ( $n=899$ ) (a,b) and 9 variables in the Whitehall II cohort ( $n=6,810$ ) (c,d). **a** and **c** show the number of participants in each cluster, with colors indicating glycemic categories (NGT, normal glucose tolerance; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; IFG + IGT, concomitant impaired fasting glycemia and impaired glucose tolerance). **b** and **d** show the medoids (the representative subject, TUEF/TULIP) or the medians (Whitehall II) of each cluster with the corresponding standardized level (z scores) of the feature variables (SC adip, subcutaneous adipose tissue; visc adip, visceral adipose tissue; secrtn fail, insulin secretion failure; AUC glu, area under the glucose curve during OGTT; waist, waist circumference; hip, hip circumference; fst ins, fasting insulin). Clusters in the Whitehall II cohort were identified using Euclidean distances from the median values of the proxy variables in TUEF/TULIP that have also been assessed in Whitehall II. For the radar charts (b,d), the z scores of insulin sensitivity, insulin secretion and HDL were directionally flipped ( $-1 \times z$  score) to yield polygon areas related to adverse variable effects.

The applied variable-based partitioning of individuals without type 2 diabetes yielded groups differing in risk for type 2 diabetes and its complications. We validated these findings using simple measures of the same pathophysiological constructs in a large occupational cohort.

Cluster 5 was identified as the subpopulation with the highest risk of type 2 diabetes, renal and vascular disease and all-cause mortality. Individuals in this cluster had obesity, insulin resistance, high levels of fatty liver and low insulin secretion. Cluster 6 represented an insulin-resistant phenotype, in which participants had high



**Table 1 | Cluster characteristics of the TUEF/TULIP cohort after stratification for the six clusters**

	1	2	3	4	5	6	P value
	Low risk	Very low risk	Beta-cell failure	Low risk obese	High risk insulin-resistant fatty liver	High risk visceral fat nephropathy	
n	173	154	146	153	91	182	
Sex = male (%)	64 (37.0)	59 (38.3)	65 (44.5)	56 (36.6)	35 (38.5)	67 (36.8)	0.72
Age (mean (s.d.))	39.05 (12.55)	41.75 (13.29)	52.26 (12.11)	40.14 (11.85)	49.74 (11.81)	47.38 (12.64)	8.7 × 10 <sup>-27</sup>
BMI (kg per m <sup>2</sup> ) (mean (s.d.))	26.82 (3.16)	23.45 (3.32)	29.15 (4.01)	31.54 (3.67)	34.45 (5.11)	34.94 (4.90)	1.6 × 10 <sup>-135</sup>
Waist circumference (cm) (mean (s.d.))	88.44 (9.63)	80.58 (9.80)	97.11 (11.21)	99.14 (10.59)	108.17 (12.88)	107.86 (12.34)	1 × 10 <sup>-111</sup>
Hip circumference (cm) (mean (s.d.))	101.62 (7.71)	95.66 (8.01)	105.80 (13.25)	112.61 (9.01)	115.17 (11.02)	117.06 (10.58)	3.1 × 10 <sup>-89</sup>
Total adipose tissue MRI (liter) (mean (s.d.))	27.71 (6.42)	20.75 (7.63)	33.27 (9.98)	42.34 (9.31)	46.20 (12.07)	48.28 (12.00)	4.4 × 10 <sup>-142</sup>
Subcutaneous adipose tissue MRI (liter) (mean (s.d.))	8.78 (3.13)	5.96 (3.50)	10.95 (4.33)	15.02 (4.79)	16.72 (5.75)	18.13 (6.19)	1.6 × 10 <sup>-111</sup>
Visceral adipose tissue MRI (liter) (mean (s.d.))	2.40 (1.48)	1.77 (1.21)	4.16 (1.92)	3.75 (1.97)	5.73 (2.34)	5.64 (2.44)	1.9 × 10 <sup>-87</sup>
Subcutaneous to visceral adipose ratio (mean (s.d.))	5.16 (3.13)	4.63 (2.84)	3.38 (2.18)	5.38 (3.18)	3.33 (1.56)	3.78 (1.95)	5 × 10 <sup>-15</sup>
Visceral adipose % of total (mean (s.d.))	0.09 (0.06)	0.09 (0.06)	0.13 (0.06)	0.09 (0.06)	0.13 (0.05)	0.12 (0.06)	5.9 × 10 <sup>-17</sup>
Liver fat content (mean (s.d.))	3.34 (3.25)	2.16 (2.90)	5.10 (3.72)	3.61 (3.51)	20.79 (5.73)	9.88 (5.49)	5.2 × 10 <sup>-193</sup>
Fatty liver disease = yes (%)	28 (16.2)	8 (5.2)	51 (34.9)	26 (17.0)	91 (100.0)	137 (75.3)	3.1 × 10 <sup>-82</sup>
Renal sinus fat (mean of r&l, %) (mean (s.d.))	5.20 (3.80)	5.77 (4.23)	9.42 (4.75)	7.15 (4.52)	10.02 (4.87)	12.07 (6.08)	1.3 × 10 <sup>-26</sup>
Systolic blood pressure (mmHg) (mean (s.d.))	126.26 (14.15)	123.36 (15.81)	135.73 (18.59)	126.32 (15.43)	143.66 (19.34)	137.86 (17.06)	6.3 × 10 <sup>-29</sup>
Diastolic blood pressure (mmHg) (mean (s.d.))	80.25 (10.64)	78.52 (11.09)	84.93 (12.07)	81.16 (10.14)	92.57 (13.62)	87.47 (12.07)	2.5 × 10 <sup>-24</sup>
Heart rate (mean (s.d.))	69.35 (10.00)	67.47 (10.85)	69.29 (9.91)	68.13 (10.76)	75.39 (12.26)	72.26 (9.64)	1.9 × 10 <sup>-8</sup>
Fasting glucose (mmol l <sup>-1</sup> ) (mean (s.d.))	5.12 (0.44)	5.04 (0.50)	5.64 (0.55)	5.14 (0.41)	5.93 (0.58)	5.48 (0.50)	1.6 × 10 <sup>-56</sup>
Postchallenge glucose (mmol/l) (mean (s.d.))	6.12 (1.09)	5.99 (1.26)	7.87 (1.38)	5.72 (0.86)	8.31 (1.54)	7.10 (1.38)	1.2 × 10 <sup>-80</sup>
Glycemic category (%)							2.3 × 10 <sup>-68</sup>
NGT	139 (80.3)	126 (81.8)	36 (24.7)	131 (85.6)	12 (13.2)	85 (46.7)	
IFG	24 (13.9)	17 (11.0)	41 (28.1)	20 (13.1)	20 (22.0)	46 (25.3)	
IGT	8 (4.6)	10 (6.5)	36 (24.7)	1 (0.7)	14 (15.4)	29 (15.9)	
IFG + IGT	2 (1.2)	1 (0.6)	33 (22.6)	1 (0.7)	45 (49.5)	22 (12.1)	
GAD antibody-positive participants	5 (3.2)	4 (2.8)	3 (2.5)	5 (3.7)	2 (2.6)	9 (5.7)	0.7
Glycated hemoglobin (mmol mol <sup>-1</sup> ) (mean (s.d.))	35.67 (4.47)	36.77 (4.03)	38.95 (6.37)	35.80 (3.95)	40.06 (3.60)	38.23 (3.86)	3.1 × 10 <sup>-19</sup>
Triglycerides (mmol l <sup>-1</sup> ) (mean (s.d.))	1.26 (0.57)	0.87 (0.35)	1.59 (1.17)	1.16 (0.63)	2.04 (1.13)	1.57 (0.79)	2.3 × 10 <sup>-30</sup>
Insulin sensitivity (Matsuda) (mean (s.d.))	14.54 (6.07)	24.33 (9.08)	11.52 (5.39)	17.63 (7.16)	5.99 (3.01)	7.46 (3.78)	5.3 × 10 <sup>-128</sup>
Fasting insulin (pmol l <sup>-1</sup> ) (mean (s.d.))	51.97 (22.02)	32.34 (14.35)	54.89 (25.73)	48.55 (22.18)	113.98 (64.09)	99.81 (48.55)	5.5 × 10 <sup>-93</sup>

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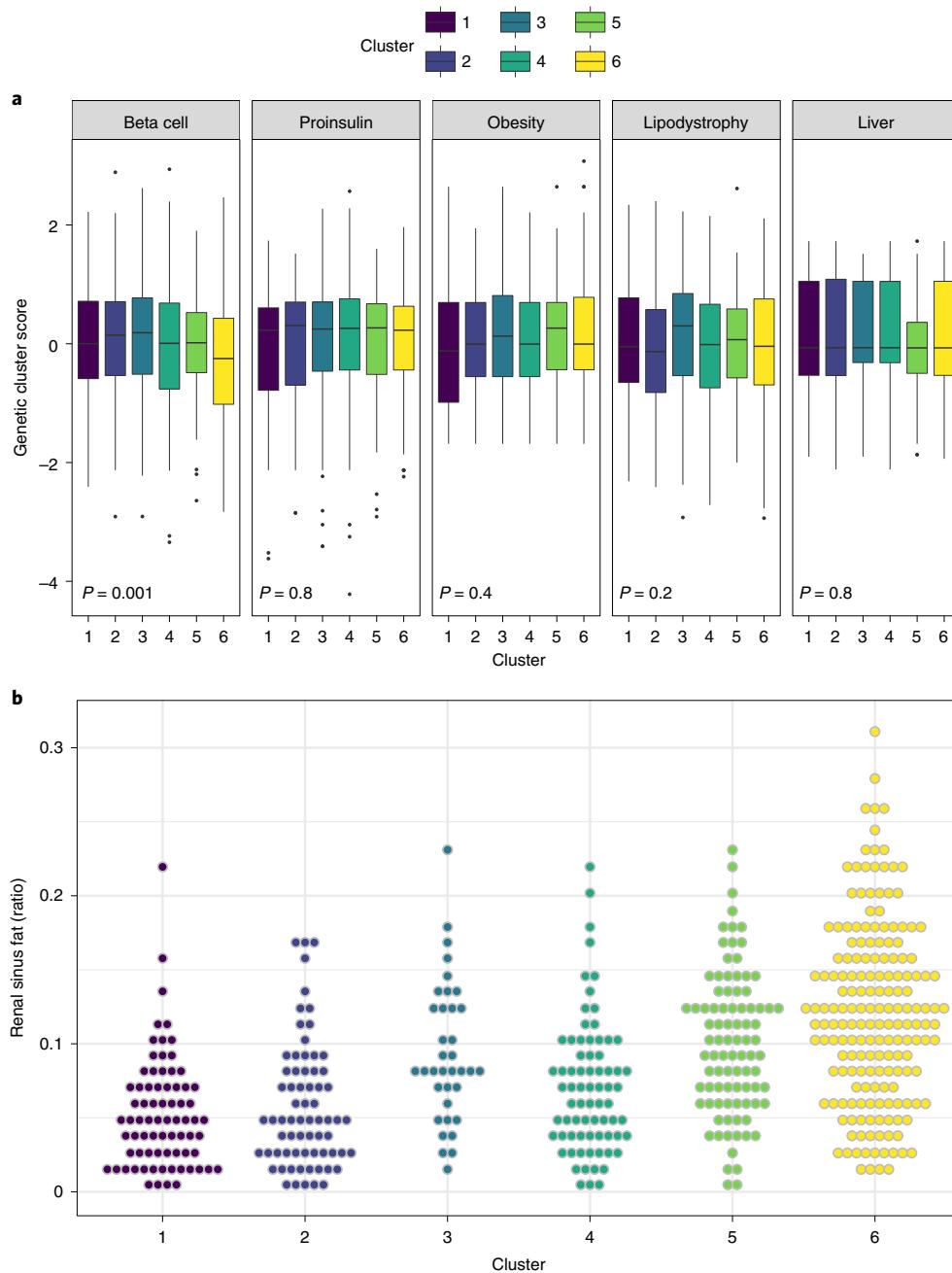
**Table 1 | Cluster characteristics of the TUEF/TULIP cohort after stratification for the six clusters (continued)**

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>P value</b>
	Low risk	Very low risk	Beta-cell failure	Low risk obese	High risk insulin-resistant fatty liver	High risk visceral fat nephropathy	
Insulinogenic index (mean (s.d.))	184.24 (274.80)	100.61 (139.56)	69.84 (37.14)	153.66 (136.01)	125.06 (69.77)	191.29 (136.68)	$1.9 \times 10^{-13}$
Disposition index (mean (s.d.))	2804.28 (6133.07)	2485.30 (5193.75)	701.59 (293.53)	2475.65 (2227.36)	653.97 (357.25)	1270.95 (979.49)	$1.5 \times 10^{-9}$
C-reactive protein (mg dl <sup>-1</sup> ) (mean (s.d.))	0.20 (0.34)	0.12 (0.25)	0.21 (0.34)	0.29 (0.32)	0.49 (0.47)	0.39 (0.42)	$2 \times 10^{-18}$
Cholesterol (mmol l <sup>-1</sup> ) (mean (s.d.))	4.91 (0.95)	4.88 (0.87)	5.27 (1.02)	4.82 (0.98)	5.34 (0.93)	5.14 (0.93)	$2.2 \times 10^{-6}$
LDL (mmol l <sup>-1</sup> ) (mean (s.d.))	3.04 (0.89)	2.73 (0.78)	3.22 (0.85)	2.97 (0.82)	3.41 (0.84)	3.15 (0.80)	$2.1 \times 10^{-9}$
HDL (mmol l <sup>-1</sup> ) (mean (s.d.))	1.34 (0.28)	1.69 (0.36)	1.32 (0.29)	1.27 (0.29)	1.18 (0.27)	1.28 (0.30)	$2.8 \times 10^{-45}$
Aspartate aminotransferase (U l <sup>-1</sup> ) (mean (s.d.))	22.38 (6.98)	22.79 (7.91)	22.52 (7.03)	22.14 (6.97)	32.73 (14.50)	25.18 (9.94)	$3.8 \times 10^{-21}$
Alanine aminotransferase (U l <sup>-1</sup> ) (mean (s.d.))	24.95 (13.39)	22.41 (10.12)	25.42 (10.48)	26.34 (15.06)	48.47 (34.70)	34.24 (18.43)	$3 \times 10^{-2}$
Gamma-glutamyl transferase (U l <sup>-1</sup> ) (mean (s.d.))	22.82 (19.52)	18.24 (15.11)	28.49 (26.08)	21.48 (14.03)	39.82 (34.49)	33.90 (26.46)	$8 \times 10^{-16}$
Serum creatinine (mg dl <sup>-1</sup> ) (mean (s.d.))	0.83 (0.18)	0.81 (0.17)	0.82 (0.18)	0.82 (0.15)	0.78 (0.15)	0.79 (0.17)	0.18
Urinary albumin/creatinine ratio (mean (s.d.))	17.31 (35.16)	18.46 (28.62)	16.05 (14.97)	17.58 (30.75)	24.11 (45.77)	16.51 (16.75)	0.53
Carotid intima-media thickness (mm) (mean (s.d.))	0.52 (0.12)	0.53 (0.10)	0.63 (0.13)	0.54 (0.12)	0.64 (0.12)	0.60 (0.12)	$2.8 \times 10^{-13}$
Polygenic risk score (mean (s.d.))	-0.09 (0.97)	0.15 (0.91)	0.24 (0.92)	-0.17 (0.91)	0.11 (0.81)	-0.07 (1.01)	0.00057
Family history of diabetes (%)							0.0084
No family history	64 (38.1)	58 (39.5)	42 (29.6)	64 (42.7)	27 (31.0)	72 (41.1)	
Second-degree relative	37 (22.0)	35 (23.8)	22 (15.5)	38 (25.3)	15 (17.2)	29 (16.6)	
First-degree relative	67 (39.9)	54 (36.7)	78 (54.9)	48 (32.0)	45 (51.7)	74 (42.3)	
Ever smoked = yes (%)	86 (49.7)	65 (42.2)	82 (56.2)	81 (52.9)	45 (49.5)	113 (62.1)	0.011
Current smoker = yes (%)	16 (9.9)	8 (5.7)	15 (11.0)	12 (8.5)	2 (2.4)	18 (10.7)	0.16
Cholesterol-lowering medication = yes (%)	6 (3.5)	0 (0.0)	8 (5.5)	2 (1.3)	1 (1.1)	6 (3.3)	0.038
Antihypertensive medication = yes (%)	10 (5.8)	3 (1.9)	21 (14.4)	2 (1.3)	25 (27.5)	44 (24.2)	$3.6 \times 10^{-17}$

P values were computed with one-way ANOVA for continuous variables and two-sided chi-squared tests for categorical variables. GAD, glutamic acid decarboxylase; LDL, low-density lipoprotein, HDL, high-density lipoprotein.

amounts of visceral fat, but less liver fat and higher insulin secretion compared with cluster 5. About half of the participants in cluster 6 had prediabetes on enrollment in the TUEF/TULIP study. However, mean glycemia (AUC glucose) was lower than in cluster 5, and the risk of type 2 diabetes was considered to be moderate. Nonetheless, participants in cluster 6 had high risk for microalbuminuria and chronic kidney disease. Cardiovascular risk was not elevated in this cluster; however, overall mortality was about 40% higher than in

the reference cluster 1, even after adjustment for confounders. Thus, clusters 5 and 6 both constitute obese, high-risk subpopulations with different glycemic, renal, cardiovascular and all-cause mortality risk profiles. Glucose does not seem to be the major driver of clinical events in cluster 6. Previous observations of an association of insulin resistance with diabetic nephropathy<sup>15–17</sup> highlight insulin resistance as a probable underlying factor. The discrepancy between moderate type 2 diabetes and high nephropathy risk for cluster 6 is



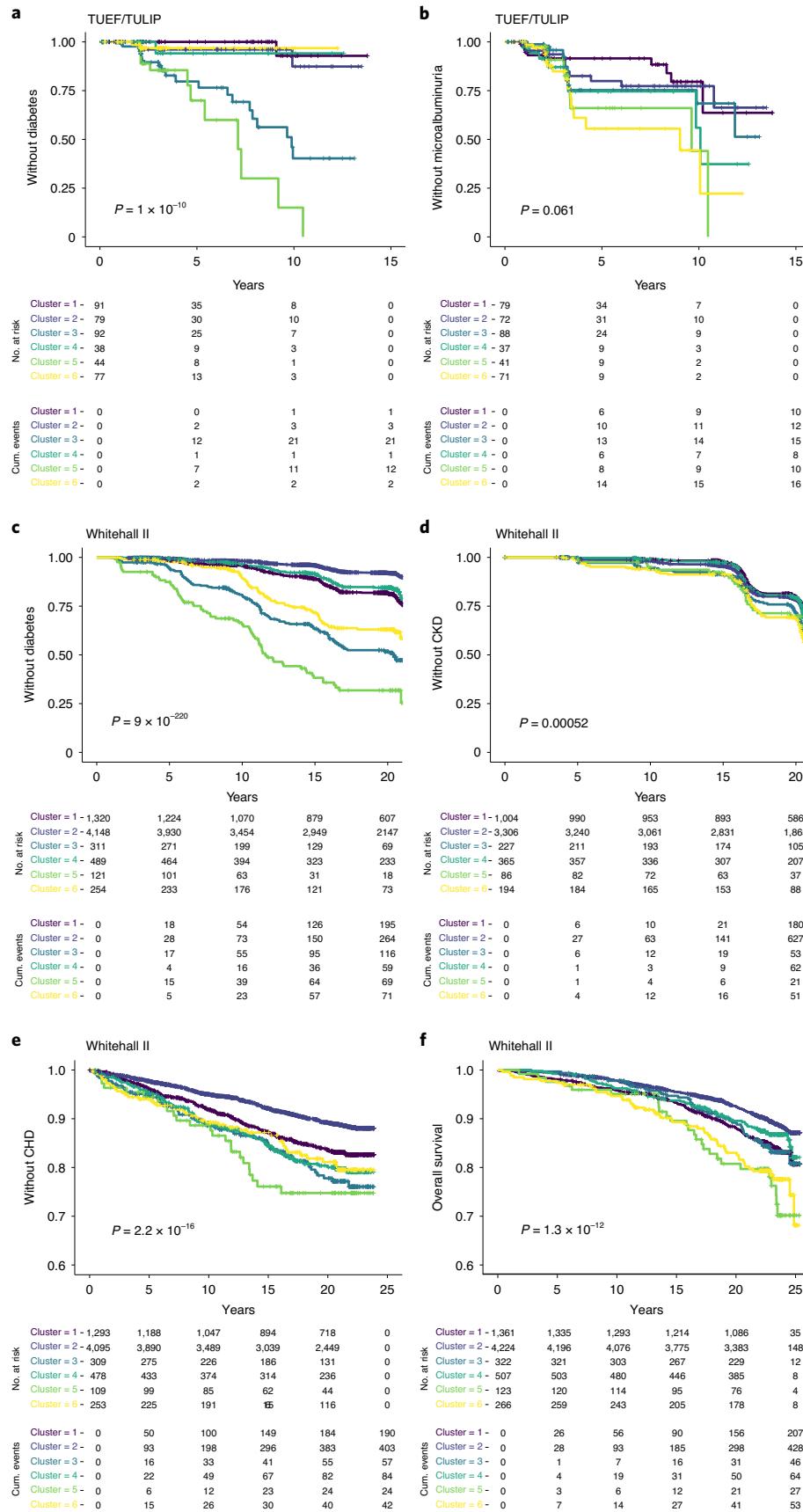
**Fig. 2 | Characteristics potentially contributing to cluster pathomechanism.** **a**, Mean pathway-specific genetic scores according to Udler et al.<sup>12</sup> across the six clusters of this work. Genetic scores ( $n=899$  risk scores of individuals in TUEF/TULIP for each of the 5 specific pathways) were transformed to z scores to eliminate differences in absolute levels due to the differing number of genetic variants in each genetic pathway. Boxes (hinges) denote the 25th and 75th percentiles with an additional horizontal line indicating the median. Whiskers show the highest and lowest data points excluding outliers (defined as at least 1.5 times the interquartile range below the lower or above the upper hinge). Outliers are shown as individual data points. Differences were tested with one-way ANOVA. **b**, Distribution of renal sinus fat (ratio of sinus fat to kidney area, mean of left and right) for  $n=520$  individuals with MR imaging-assessed renal sinus fat in TUEF/TULIP across clusters ( $P=1.25 \times 10^{-26}$  with one-way ANOVA). Pairwise tests for cluster 6 with Tukey's test yielded the following  $P$  values:  $P_{5-6}=0.02$ ,  $P_{3-6}=0.049$ ,  $P_{6-others}<1 \times 10^{-14}$ .

not dependent from baseline blood pressure. However, individuals in cluster 6 had elevated renal sinus fat, which could contribute to manifestation of nephropathy. We have previously shown an association between renal sinus fat and exercise-induced albuminuria in a cross-sectional cohort and an association of microalbuminuria with renal sinus fat in individuals with non-alcoholic fatty liver disease<sup>14,18</sup>. In renal sinus fat and renal cell coculture experiments, the combination of renal sinus fat and Fetalin-A-induced inflammation

indicate a combination of an insulin-resistant metabolic milieu and adverse fat accumulation as a likely cause of organ damage<sup>18</sup>. This finding is consistent with the phenotypes of insulin resistance, moderately high liver fat and high renal sinus fat in cluster 6. Cluster 6, in which participants had moderate or delayed risk of diabetes, showed a relatively low genetic risk for type 2 diabetes and a low abundance of genetic variants from the beta-cell class in the Udler classification<sup>12</sup>. This result implies that there is an effective compensation

of insulin resistance through excellent beta-cell function. We speculate that hyperinsulinemia associated with the combination of good beta-cell function and insulin resistance contributes to renal

disease and mortality<sup>19–21</sup>. Smoking was a risk factor both for diabetes and chronic kidney disease<sup>22–24</sup>, but did not explain the differences among clusters.



**Fig. 3 | Cluster-specific outcomes.** **a–d**, Kaplan–Meier curves showing cluster-specific probability of not developing diabetes (**a,c**) or nephropathy (**c,d**) in the TUEF/TULIP and Whitehall II cohorts, respectively. **e,f**, Cumulative probability of coronary heart disease (CHD, **e**) and overall mortality (**f**) are shown for the Whitehall II cohort. For diabetes incidence,  $n=421$ , mean follow-up 4.1 yr, number of diabetes events = 40 in TUEF/TULIP and  $n=6,643$ , mean follow-up 16.3 yr, number of diabetes events = 828 in Whitehall II. For microalbuminuria incidence:  $n=388$ , mean follow-up 4.3 yr, number of microalbuminuria events = 71 in TUEF/TULIP. In Whitehall II  $n=5,182$ , mean follow-up 18.2 yr with 1,387 stage 3 chronic kidney disease or worse (estimated glomerular filtration rate < 60 ml per min per  $1.73\text{m}^2$ ) incidences. For CHD,  $n=6,537$ , mean follow-up 17.2 yr, 800 events. For all-cause-mortality,  $n=6,803$ , mean follow-up 21.1 yr, 825 deaths. All P values were computed with two-sided log-rank tests.

In contrast to the three high-risk clusters 3, 5 and 6, cluster 4 comprises individuals with obesity but low glycemic deterioration. Phenotypic traits of individuals in this cluster are compatible with the concept of metabolically healthy obesity<sup>25</sup>. Cluster 4 was also associated with lower risk of type 2 diabetes, independent of sex, age and BMI. Individuals in this cluster had body fat predominantly stored in subcutaneous, rather than visceral, depots, a pattern known to be metabolically more favorable<sup>26</sup>.

In cluster 3, the partitioning identified a phenotype characterized by elevated genetic risk and low insulin secretion, which might explain the high diabetes incidence seen in this group. The moderately elevated visceral fat compartment correlates with pancreatic fat, which has been associated with disturbed insulin secretion in a prediabetic environment<sup>18,27,28</sup>. Cluster 3, with a disposition index as low as that of cluster 5 but higher insulin sensitivity, could correspond to beta-cell dysfunction subphenotypes identified in previous studies<sup>6,7,29</sup>. Cluster 3 had high IMT, independent from sex, age and BMI. Increased cardiovascular risk was not replicated for this cluster in Whitehall II, but individuals in this cluster had a moderately elevated risk of chronic kidney disease.

Our clustering approach is not designed to provide definitive subphenotypes for individual patients in a clinical setting; however, the approach can be helpful for characterizing the metabolic heterogeneity prior to clinical manifestation of type 2 diabetes. The identification of such subphenotypes suggests some potential therapeutic implications. Individuals in cluster 5 are at imminent risk for diabetes and could benefit from high-intensity dietary and/or lifestyle interventions aimed at weight loss and liver-fat reduction. Individuals with the characteristics of cluster 3 might benefit from a standard aerobic exercise and dietary caloric restriction via reduction of visceral fat. Although clusters 3 and 5 have elevated genetic risk as a non-modifiable risk factor, genetic predisposition might be protective against development of type 2 diabetes for individuals with a cluster 6 phenotype. This group could be easily overlooked when risk-stratification focuses on established diabetes-related glycemic cut-offs. Insulin resistance with or without prevalent prediabetes associates with renal disease and elevated mortality in cluster 6, which should motivate consideration of preventive measures even with low glycemic progression.

Our subphenotyping was performed in persons who did not yet suffer from diabetes, but who are at potentially increased risk, as demonstrated by the newly diagnosed cases in the follow-up period. The classification emerges partly from variables that require an OGTT. OGTT-derived glycemic traits can reasonably assess insulin sensitivity and secretion, particularly in the absence of diabetes. An elegant metabolic clustering of glycemic courses during OGTT has been proposed by Hulman et al<sup>13</sup>. We have applied an alternative approach with a broad set of variables in addition to OGTT. Our data complement other clustering approaches targeting the disentanglement of the heterogeneity of adult-onset diabetes<sup>6,7,12</sup>. We show that cluster 6 most strongly connects to the SIRD cluster of the Ahlqvist classification<sup>6,30</sup>. Cluster 6 and SIRD bear similarities, such as an elevated risk of nephropathy in the absence of marked glucose elevation. Thus, accumulating data indicate that the pathogenesis of kidney damage in type 2 diabetes appears to be different from that of type 1 diabetes, with only a minor contribution of glycemia in prediabetes and type 2 diabetes. Of note, by contrast

with the Ahlqvist classification, our work analyzed screen-detected diabetes cases as outcomes during the follow-up periods. These cases probably have milder phenotypes than do clinically detected type 2 diabetes cases.

Our results are demonstrated in two independent study groups: a cohort by design enriched in diabetes-prone persons and a UK occupational cohort. This most likely contributes to the observed differences between the Kaplan–Meier plots in the two cohorts, especially for diabetes incidence. Given the lack of ethnic diversity of the investigated populations leveraged in our study, our findings might only be applicable to populations of European descent. We also acknowledge the limitations of the partitioning approach: there is uncertainty with regard to variable selection, the optimal number of clusters and whether these approaches are inferior to conventional predictions from multivariable modeling<sup>29</sup>. Additional specific limitations of our work are the different feature variable set and the moderate reassignment rate (63%) of the original clusters to the feature set of Whitehall II. Given the sophisticated nature of the variables in TUEF/TULIP cohort, the clinical utility of these features for metabolic classification could be limited. Further, in the TUEF/TULIP cohort, about only half the population was available for follow-up visits. This high attrition rate could lead to a potential underestimation of the risk for diabetes and nephropathy in TUEF/TULIP cohort. A final limitation is that the nephropathy models in Whitehall II were not adjusted for baseline eGFR due to a lack of baseline measurements and the absolute risks being low.

In summary, we show the feasibility of multivariable subphenotyping in individuals without diabetes to disentangle metabolic heterogeneity prior to diagnosis of type 2 diabetes. The metabolic clusters identified here associate with future complications related to prediabetes, insulin resistance, future risk of type 2 diabetes and mortality. These subphenotypes likely reflect key pathologic features potentially underlying different fates of metabolic complications, but are not aimed at classifying single patients in clinical practice; however, with further development and validation, such approaches could guide prevention and treatment strategies for cardiovascular and renal disease as well as for type 2 diabetes.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-020-1116-9>.

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## Methods

**TUEF/TULIP cohort.** Prediabetes subphenotyping was initially performed on a complete cases subset of participants of the TUEF/TULIP cohort<sup>2,3</sup>, who had no missing values for the preselected phenotyping variables ( $n=899$ , baseline characteristics for this and the whole cohort are shown in the Supplementary Table 11). Participants were recruited in 2003–2018. Recruitment was mostly performed via newspaper announcements and e-mail bulletins. The studies were designed to phenotype individuals at increased risk of diabetes. Eligibility criteria for inclusion comprised either a history of prediabetes, a family history of diabetes, a BMI greater than  $27\text{ kg m}^{-2}$  or a history of gestational diabetes<sup>2</sup>. Participants underwent a frequently sampled OGTT and received MR-tomography-based measurement of body fat distribution and  $^1\text{H}$ -MR-spectroscopy-based measurements of hepatic fat content. Follow-up data were available for individuals who responded to invitations to follow-up appointments or participated in follow-up studies. The follow-up measurements were comparable to the initial assessments. Glycemic traits (fasting glucose, OGTT or HbA1c) were available for 421 participants, whereas urine sample during follow-up, for the determination of microalbuminuria, was available for 388 participants. The study protocol was approved by the Ethics Committee of the University of Tübingen (422/2002). All participants gave written informed consent.

**Whitehall II cohort.** Data from the occupational Whitehall II cohort were accessed by a data-sharing agreement. Details of the study have been described elsewhere<sup>4</sup>. In brief, the study was established to explore the relationship between socio-economic status, stress and cardiovascular disease. All London-based civil servants aged 33–55 years were invited in 1985–1988, and 10,308 (73%) participated. Since then, 5 further clinical examinations have taken place that are available for data sharing at approximately 5-yr intervals (phases 3, 5, 7, 9 and 11). The study was approved by the Joint UCL/UCLH Committees on the Ethics of Human Research (Committee Alpha). For the current analysis, the baseline was defined as the first available fasting OGTT ( $\geq 8\text{ h}$  of fasting for morning and  $\geq 5\text{ hours}$  of fasting after a light fat-free breakfast eaten before 8:00 for afternoon OGTTs). Participants with prevalent or incident diabetes at baseline and those of a non-white ethnicity were excluded. From the 6,916 available baseline OGTTs, 6,810 were complete cases in regards of the used clustering variables and underwent cluster assignments. The cohort characteristics are reported in Supplementary Table 12.

**Variable selection and de novo clustering in TUEF/TULIP.** We aimed to identify subphenotypes that reflect differences in pathophysiological processes in the natural history of type 2 diabetes. The main paradigm of type 2 diabetes pathogenesis is an insufficient compensatory increase of insulin secretion in response to insulin resistance<sup>5,6</sup>. Therefore, insulin sensitivity and insulin secretion are key variables<sup>6,7</sup>. We used OGTT-based indices of insulin sensitivity (Matsuda-index)<sup>32</sup> and insulin secretion ( $AUC_{0-30}\text{ C-peptide}/AUC_{0-30}\text{ glucose}$ ) that correlate well with gold-standard measures and are preferable to static measurements obtained in the fasting state<sup>33,34</sup>. Glycemia was quantified in the partitioning procedure as  $AUC_{0-120}\text{ glucose}$ . Furthermore, we aimed to capture diverse etiologies of insulin resistance by accounting for visceral and subcutaneous adipose tissue volume (VAT and SCAT), that have distinct metabolic characteristics<sup>35</sup>. We especially focused on elevated fat content in the liver, as it is strongly associated with insulin resistance<sup>8</sup>. HDL-cholesterol levels have long been known as explanatory variables of the metabolic syndrome and insulin resistance<sup>37</sup>. Moreover, causal inference from large genomic datasets provides evidence not only for a genetic correlation of HDL-cholesterol levels with type 2 diabetes, but also for a causal link between HDL-cholesterol levels and type 2 diabetes<sup>9</sup>. We also added a genome-wide polygenic risk score to the analysis to better differentiate between genetically determined beta-cell dysfunction and environmentally determined beta-cell dysfunction. The correlation of the clustering variables is reported in Supplementary Table 13.

For computation of the polygenic risk score, we used the LDpred algorithm of Vilhjalmsson et al.<sup>10</sup> on a combination of BMI-adjusted effect sizes and  $P$  values from a meta-analysis in ~900,000 European individuals and genotypes<sup>11</sup>. After quality control, exclusion of multiallelic and low-frequency variants, we combined 484,788 variants from the 2 datasets, yielding an estimated genome-wide single-nucleotide-polymorphism heritability of 0.069. Of the top 94 diabetes-related genetic variants shown in the latest large-scale genome-wide association study<sup>11</sup>, 63 were genotyped in TUEF/TULIP. The association of cluster assignment with the genotype was tested separately for each variant using ANOVA to analyze the enrichment of certain genotypes in clusters. A further genetic-pathophysiological classification of clusters was performed according to data from Udler et al.<sup>12</sup>. Here, we computed the genetic risk score for every individual and every genetic class (beta cell, proinsulin, obesity, lipodystrophy and liver/lipid), taking only weights  $\geq 0.75$  into account, as described in the original publication. The classification of glucose response curves according to Hulman et al.<sup>13</sup> (Hulman classes) was performed with the corresponding web-based calculator from five-point OGTT glucose values in the TUEF/TULIP study.

**Cluster assignment in the Whitehall II cohort.** To assign participants in the Whitehall II cohort to clusters established in TUEF/TULIP, we used proxy

variables. Because liver fat, visceral adipose tissue and subcutaneous adipose tissue were not available in the Whitehall II cohort, and only two-point OGTTs were performed, other anthropometric variables and analytes were employed instead of these variables. Variables were selected on the basis of statistical considerations (correlation) and pathophysiologic (theoretical) connections to the original trait (for example, for liver fat, the variables were fasting triglycerides, fasting insulin and waist circumference). Transaminase activity was not available during the early phases of the Whitehall II study. The final variable set was selected upon the highest agreement in reidentification of the original cluster assignments using the new proxy variables in TUEF. The variables used in Whitehall II comprised glycemia during glucose challenge, insulin sensitivity<sup>10</sup>, Stumvoll's first-phase insulin-secretion index using insulin and glucose levels at fasting and at 120 min during OGTT<sup>39</sup>, fasting insulin, fasting triglycerides, waist circumference, hip circumference, BMI and HDL cholesterol. The median values of these variables in TUEF/TULIP were used to assign participants to clusters in Whitehall II (Extended Data Fig. 1) by taking the nearest neighbors of the 6 cluster centers based on Euclidean distances. Because Whitehall II used a restricted CVD-focused genotyping platform with only 48,000 markers and the full-scale genotyping data were not readily available, we decided to omit the genetic-risk score from the reassignment procedure. Despite these limitations, successful reassignment of the clusters was achieved in 63% of the original TUEF cohort.

**OGTT and laboratory analysis.** All participants of TUEF/TULIP received a 75-g glucose solution (Accu-Chek Dextro, Roche) at 8:00 following an overnight fast. Venous blood was obtained through an indwelling venous catheter before and 30, 60, 90 and 120 min after glucose ingestion. In the Whitehall II cohort, the OGTT procedure has been described previously. In short, venous blood samples were collected after an overnight fast in the morning ( $\geq 8\text{ h}$  of fasting) or in the afternoon after no more than a light fat-free breakfast eaten before 8:00 ( $\geq 5\text{ h}$  of fasting), followed by a standard 75-g OGTT with a venous blood sample taken 2 h after ingestion of the glucose solution. Glucose was analyzed in the Whitehall II study using an YSI glucose analyzer (Yellow Springs Instruments). Glucose values were measured in TUEF/TULIP directly using a bedside glucose analyzer (YSI, Yellow Springs, or Biosen C-line, EKF Diagnostics). In TUEF/TULIP, all other obtained blood samples were put on ice, and the serum was centrifuged within 2 h. Plasma insulin and C-peptide were determined by an immunoassay with the ADVIA Centaur XP Immunoassay System, and HDL was measured using the ADVIA XPT clinical chemical analyzer (all from Siemens Healthineers), while triglycerides were measured with standard colorimetric methods using a Bayer analyzer. In Whitehall II, insulin was measured with an in-house human insulin RIA and later with a DAKO ELISA kit (DAKO Cytomation). Serum creatinine was measured using a kinetic colorimetric (Jaffe) method on a Roche 'P' Modular system (phase 9) and on a COBAS 8000 system (phase 11). Lipid measurements have been described previously<sup>40</sup>. HbA1c measurements were performed using Tosoh glycohemoglobin analyzers in both studies (Tosoh Bioscience).

**Body fat distribution, liver fat content and renal sinus fat.** Body fat distribution variables, that is, VAT and SCAT, were determined by whole-body T1-weighted MRI as described earlier<sup>41</sup>. Liver fat content was measured by volume-selective  $^1\text{H}$ -MR spectroscopy<sup>42</sup>. Renal sinus fat was measured with manual segmentation from MR image slices specifically in cluster 5 and 6 using a method described previously<sup>14</sup>. The operator performing the segmentation (J.M.) was not aware of the cluster assignments. The procedure could not be completed in 6 participants (2% missing) due to breathing artifacts in the images. Renal sinus fat data for clusters 1–4 were partly available from segmentations for previous projects (mean data availability 40% over clusters 1–4).

**Outcomes.** For detection of incident diabetes, either of the following was used: clinically ascertained diabetes (from patient history, or by the use of a diabetes medication), an elevated fasting glucose ( $\geq 7\text{ mmol l}^{-1}$ ), postchallenge glucose ( $\geq 11.1\text{ mmol l}^{-1}$ ) or HbA1c ( $48\text{ mmol mol}^{-1}$  or 6.5%) in both cohorts. To assess the Ahlqvist classification<sup>6</sup> for the subtypes of diabetes in Whitehall II, we used insulin-based HOMA2-indices, because C-peptide was not measured. GAD measurements were not available. HbA1c assessment had been introduced beginning with phase 7. Cluster assignment was performed using the lowest Euclidean distances from the published cluster centers in the All New Diabetes in Scania (ANDIS) cohort after scaling the variables for the means and SDs of the ANDIS cohort. Microalbuminuria was assessed in TUEF/TULIP upon the first occurrence from morning spot urine using the albumin-to-creatinine ratio (ACR). Measurements with excessive leukocyturia (175 measurements out of 3,218) were excluded from this analysis. Microalbuminuria was established with an  $ACR \geq 30\text{ mg g}^{-1}$  creatinine. IMT, which is associated with future cardiovascular and cerebrovascular events<sup>43</sup>, was determined by a high-resolution ultrasound of the left and right common carotid artery. A trained physician who was unaware of the clinical and laboratory variables of the participants performed B-mode ultrasound imaging using a linear ultrasound transducer (10–13 MHz; AU5 Harmonic, Esaote Biomedica). IMT was specified according to the European Mannheim IMT consensus criteria<sup>44</sup>. To ascertain renal disease, we used estimated glomerular filtration rate calculated using the CKD-EPI creatinine equation<sup>45</sup>.

Serum creatinine was available from phase 9. Only participants with at least one eGFR value went into these analyses. Stages of chronic kidney disease were ascertained with the Kidney Disease: Improving Global Outcomes (KDIGO) classification<sup>46</sup>. Ascertainment of coronary heart disease and mortality in Whitehall II has been described earlier<sup>47</sup>. In brief, incident CHD was defined as CHD death, nonfatal CHD and typical angina ascertained from clinical records, without self-reported cases from the Rose angina questionnaire. The cases were ascertained from participants' general practitioners, information extracted from hospital medical records by study nurses or data from the NHS Hospital Episode Statistics (HES) and death-register databases obtained after linking the participants' unique NHS identification numbers to this national database. Mortality data to June 2015 was drawn from the British National Mortality Register (National Health Service (NHS) Central Register) using each participants' NHS identification number.

**Statistical analysis.** Statistical analyses were performed using R version 3.4.3 (ref. <sup>48</sup>). In the clustering analysis, distances were computed as Gower distances using standardized variables (scaled to a mean of 0 and s.d. of 1). Participants with outlier variables (absolute standardized levels  $\geq 5$ ) were excluded from the clustering procedure. To find the optimal cluster count, we evaluated the dendrogram and silhouette-widths. The clustering procedure was performed with the partitioning around medoids (pam) method in the R-package 'cluster', which is a more robust version of k-means clustering<sup>49</sup>. Using repeated subsetting with the clusterboot function from the fpc package, the mean Jaccard-similarity measure was 0.74 across all clusters<sup>50</sup>. To further validate the stability of clusters, we iterated the clustering procedure for each of the 429 participants who had repeated measurements comprising all clustering variables (mean number of measurements  $2.6 \pm 0.9$ , follow-up duration  $4.2 \pm 3.6$  yr, also see Extended Data Fig. 8). We assessed the per-participant agreement of the generated 1,112 cluster assignments using interrater reliabilities. The intraclass correlation coefficient (two-way random, absolute agreements, ICC(2,k)) for cluster agreement was 0.72 (CI 0.68–0.76). Detailed reports on means and s.d. values of the clustering variables in both cohorts and the cluster medians are provided in Supplementary Tables 14 and 15.

Cluster means were compared using ANOVA. Specific outcomes were compared using analysis of covariance, adjusting for covariates such as sex, age and BMI. Post-hoc comparisons were performed using Tukey's honest significant differences procedure.

Endpoints related to diabetes complications were analyzed in the follow-up data of both cohorts using survival analysis and proportional-hazard models. Differences in cumulative risks for reaching endpoints were tested with log-rank tests. When not indicated otherwise, the uncorrected *P* value of a specific cluster's risk relative to cluster 1 is provided in the proportional-hazard analysis. Given the relatively low number of outcomes in TUEF/TULIP (40 for diabetes and 71 for microalbuminuria), assessment of proportional hazards adjusted for potential confounders was performed in the Whitehall II cohort only. Proportional-hazards assumptions were tested by visualization of the Schoenfeld residuals. The performed statistical tests were two-sided.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

For TUEF/TULIP data, all requests for data and materials will be promptly reviewed by the Data Access Steering Committee of the Institute of Diabetes and Metabolic Research, Tübingen, to verify whether the request is subject to any intellectual property or confidentiality obligations. Individual-level data may be subject to confidentiality. Any data and materials that can be shared will be released via a Material Transfer Agreement. Access to individual-level data of the Whitehall II study is subject to a separate data-sharing agreement according to the data-sharing policy of Whitehall II. This policy conforms to the MRC Policy on Research Data Sharing. More details can be found on the Whitehall II webpage: <https://www.ucl.ac.uk/epidemiology-health-care/research/epidemiology-and-public-health/research/whitehall-ii/data-sharing>.

## Code availability

The R code used to generate all results of this manuscript is available upon request. Requests will be reviewed by the Data Access Steering Committee of the Institute of Diabetes and Metabolic Research, Tübingen.

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## Author contributions

R.W. analyzed the data and wrote the manuscript. M.H., A.G.T., J.M., F.S., E.R. and A.F. contributed to data acquisition and the interpretation of data, and edited the manuscript. M.H.d.A., A.P. A.L.B. and N.S. contributed to the interpretation of data and edited the manuscript. H.-U.H. and A.F. contributed to the concept of the work and edited the manuscript. All authors have reviewed the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

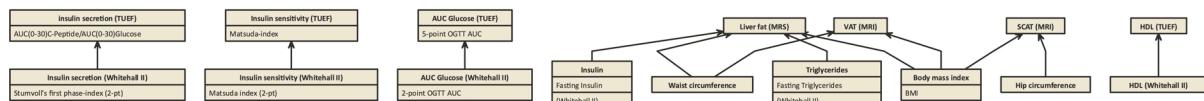
Extended data is available for this paper at <https://doi.org/10.1038/s41591-020-1116-9>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41591-020-1116-9>.

Correspondence and requests for materials should be addressed to R.W.

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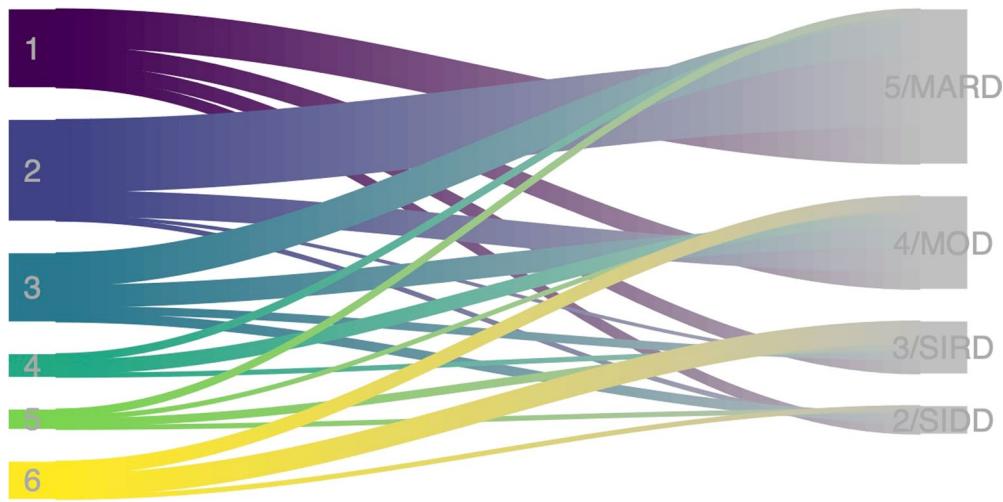
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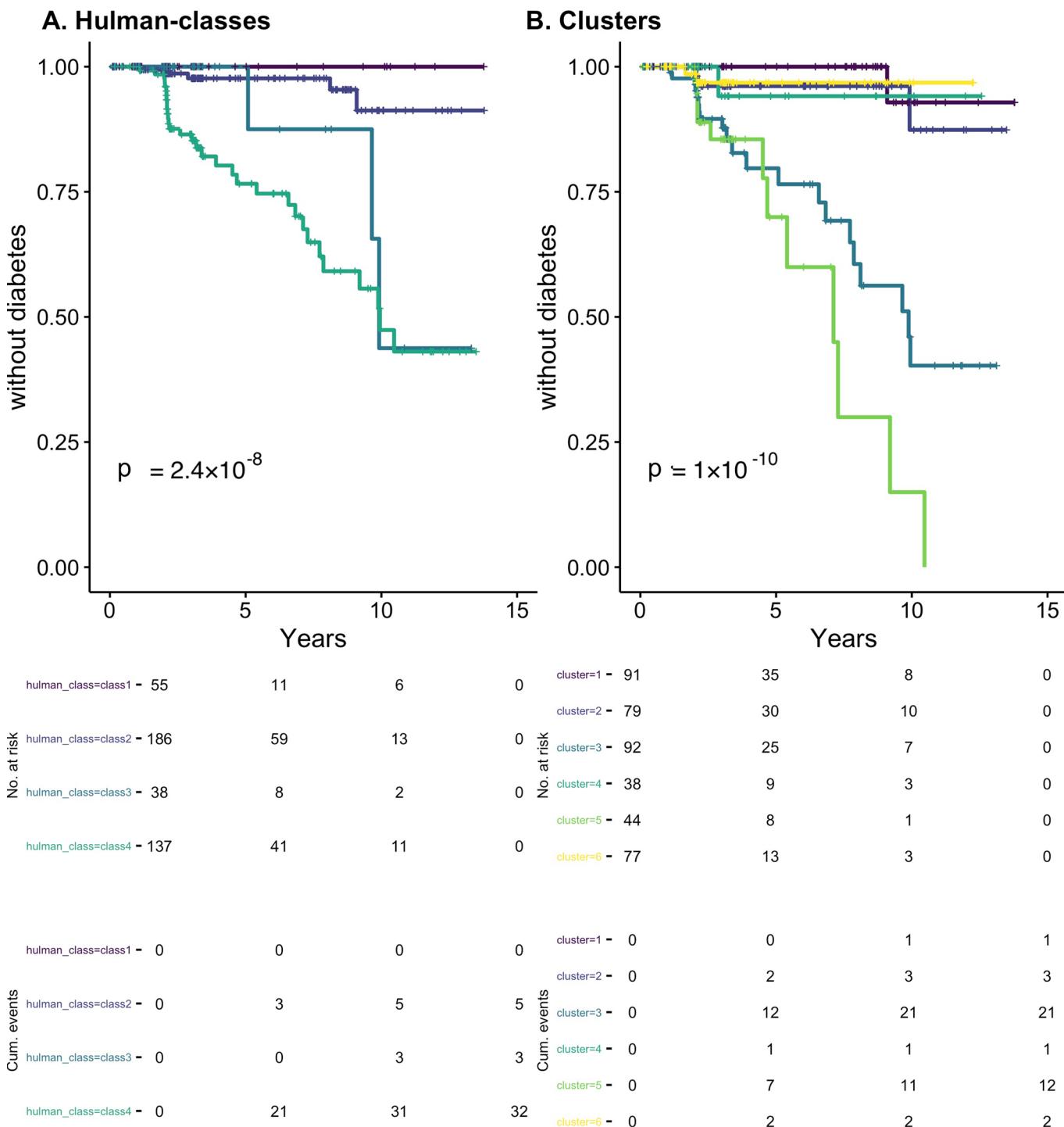
**Extended Data Fig. 1 | Assignment of proxy variables from the Whitehall II cohort to the original clustering variables.** Assignment of proxy variables from the Whitehall II cohort (variables that were both assessed in Whitehall-II and the original clustering cohort TUEF/TULIP) to the original clustering variables. Clusters were identified in Whitehall II using the Euclidean distances of the subjects computed from these variables to the cluster medians in TUEF/TULIP. The upper row shows the original clustering variables available in TUEF/TULIP, the lower row the variables in Whitehall-II. Arrows show the physiological connection between the variables.

Cluster	Main feature	Obesity and fat distribution	Insulin sensitivity	Insulin secretion	Glycemia	Other specific features
1	Low risk	Overweight	Average	Adequate	Mostly NGT	
2	Very low risk	Normal	Good	Adequate	Mostly NGT	
3	Beta cell failure	Overweight/Obese	Moderately low	Low	Mostly prediabetes	Increased genetic T2D risk
4	Low risk obese	Obese	Good	Adequate	Mostly NGT	
5	High risk insulin resistant fatty liver	Obese	Very low	Low	Mostly prediabetes (most of the latter IGT with or without IFG)	Above average genetic T2D risk, very high liver fat
6	High risk visceral fat nephropathy	Obese	Low	Moderately low	NGT and prediabetes (most of the latter IFG)	Low genetic T2D risk, high visceral fat, high renal sinus fat

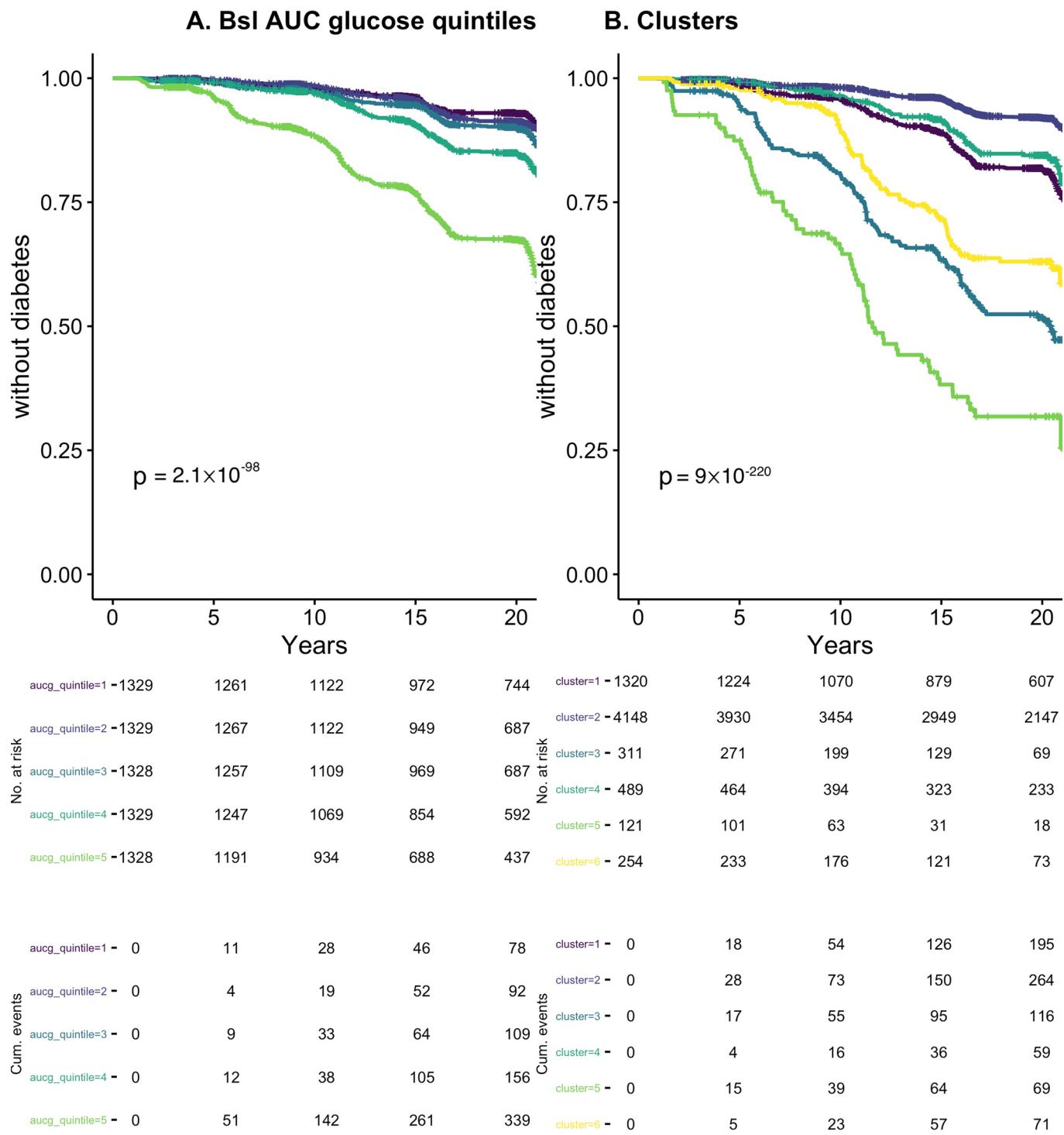
**Extended Data Fig. 2 | Key features of the clusters.** NGT: normal glucose tolerance, IFG: impaired fasting glucose, IGT: impaired glucose tolerance, T2D: type 2 diabetes.



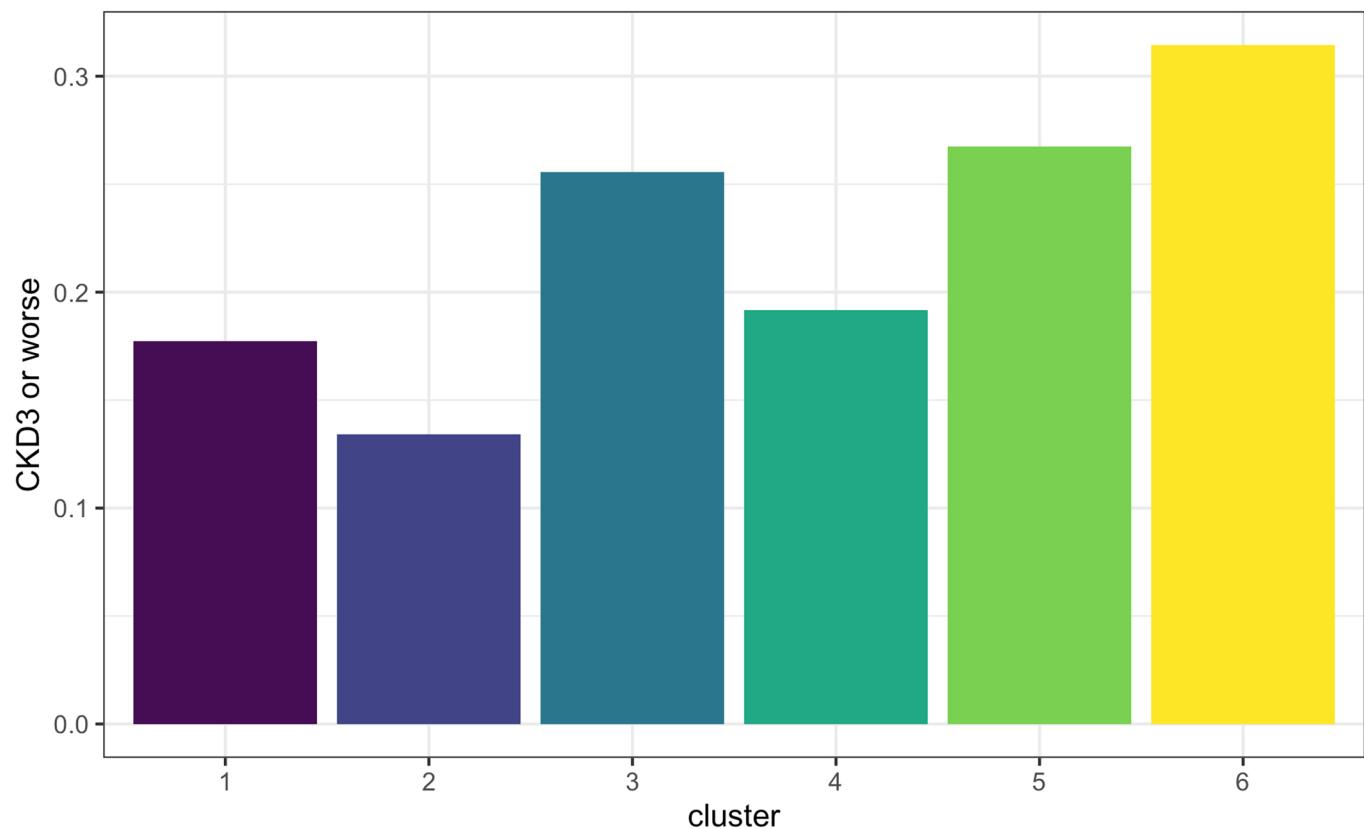
**Extended Data Fig. 3 | Transitions into Ahlqvist-diabetes-classes for subjects who were assigned to clusters in the Whitehall II study and developed diabetes during follow-up.** Transitions into Ahlqvist-diabetes-classes (right hand side) for subjects who were assigned to clusters in the Whitehall II study and developed diabetes during follow-up (left hand side, N = 201).



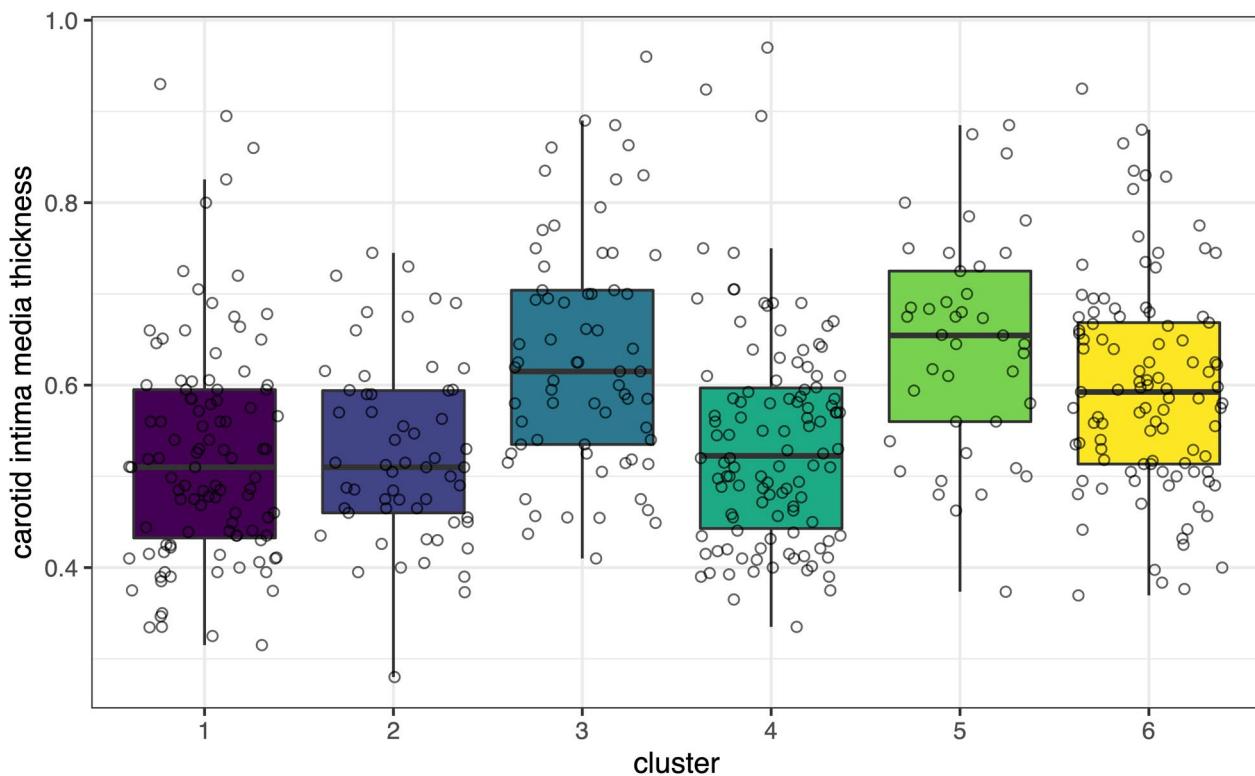
**Extended Data Fig. 4 | Kaplan-Meier curves to compare the risk discrimination between Hulman-classes and clusters.** Kaplan-Meier curves to compare the risk discrimination between Hulman-classes (A, n = 416 individuals with follow-up) and clusters (B, n = 421 individuals with follow-up) showing probabilities of remaining diabetes free in the TUEF/TULIP cohort. P-values indicate two-sided log-rank tests.


**Extended Data Fig. 5 | Kaplan-Meier curves to compare the risk discrimination between quintiles of baseline glucose AUC levels and clusters.**

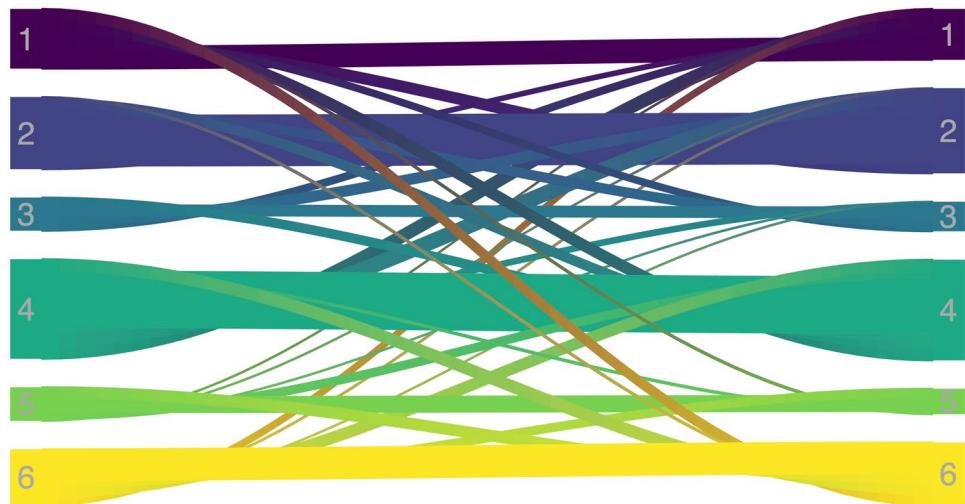
Kaplan-Meier curves to compare the risk discrimination between quintiles of baseline glucose AUC levels (A, n=6643 individuals with follow-up) and clusters (B, n=6643 individuals with follow-up) for diabetes development in the Whitehall II study. P-values indicate two-sided log-rank tests.



**Extended Data Fig. 6 | Cumulative incidence of chronic kidney disease stage 3 or worse.** Cumulative incidence of chronic kidney disease stage 3 or worse in the Whitehall-II study (N=5182).



**Extended Data Fig. 7 | Cluster-stratified carotid intima media thickness.** Cluster-stratified carotid intima media thickness (IMT) in the subset with ultrasound measurements ( $N = 479$ ) in the TUEF/TULIP study. IMT was measured in 60%, 37%, 46%, 72%, 45% and 55% of the participants of cluster 1 through 6, respectively. Boxes (hinges) denote the 25<sup>th</sup> and 75<sup>th</sup> percentiles with an additional horizontal line indicating the median. Whiskers show the highest and lowest data points excluding outliers (defined as at least 1.5×interquartile range below the lower or above the upper hinge).



**Extended Data Fig. 8 | Cluster stability plot.** Cluster stability plot showing all consecutive cluster transitions in the iterative re-clustering of repeated measurements in TUEF/TULIP (N = 429 individuals with repeated measurements).

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### Software and code

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Data collection

data collection into Microsoft Access and proprietary tools of the Whitehall II cohort; data export as xlsx or csv; subsequent data processing in R

Data analysis

Rstats V3.4.3 with the following packages: tidyverse (including ggplot), fpc, cluster, mclust, class, sjlabelled, sjPlot, viridis, riverplot, survminer, tableone, rms, emmeans

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Sample size	all available records with a full set of the clustering variables in TUEF/TULIP and Whitehall II were used
Data exclusions	complete cases set of the available variables from TUEF/TULIP, exclusions described in methods (outliers with more than 5 SD from mean); in Whitehall II: full set of clustering variables in first oGTT, non-fasting participants and participants with non-white ethnicity were excluded
Replication	replication using a different set of variables in the Whitehall II study, see methods
Randomization	no randomization performed
Blinding	no blinding performed

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## Human research participants

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Population characteristics	TUEF/TULIP: N=899, gender = 346 males, age = 44.6 +- 13.35, BMI = 29.84 +- 5.7, HbA1c = 5.57 +- 0.43 Whitehall II: N=6810, gender = 4860 males, age = 51 +- 6.7, BMI = 25.3 +- 3.58, fasting glucose = 5.21 +- 0.47
Recruitment	TUEF/TULIP: subjects with elevated risk for type 2 diabetes via bulletin boards, e-mail bulletins, newspaper advertisements. Whitehall-II: occupational cohort - London-based civil servants were invited (see references)
Ethics oversight	TUEF/TULIP: Ethics Committee of the University of Tübingen (422/2002) Whitehall II: Joint UCL/UCLH Committees on the Ethics of Human Research (Committee Alpha)

Note that full information on the approval of the study protocol must also be provided in the manuscript.