Supplementary information

Metabolic Biomarkers for Prognostic Prediction of Pre-diabetic Patients: results from a longitudinal cohort study

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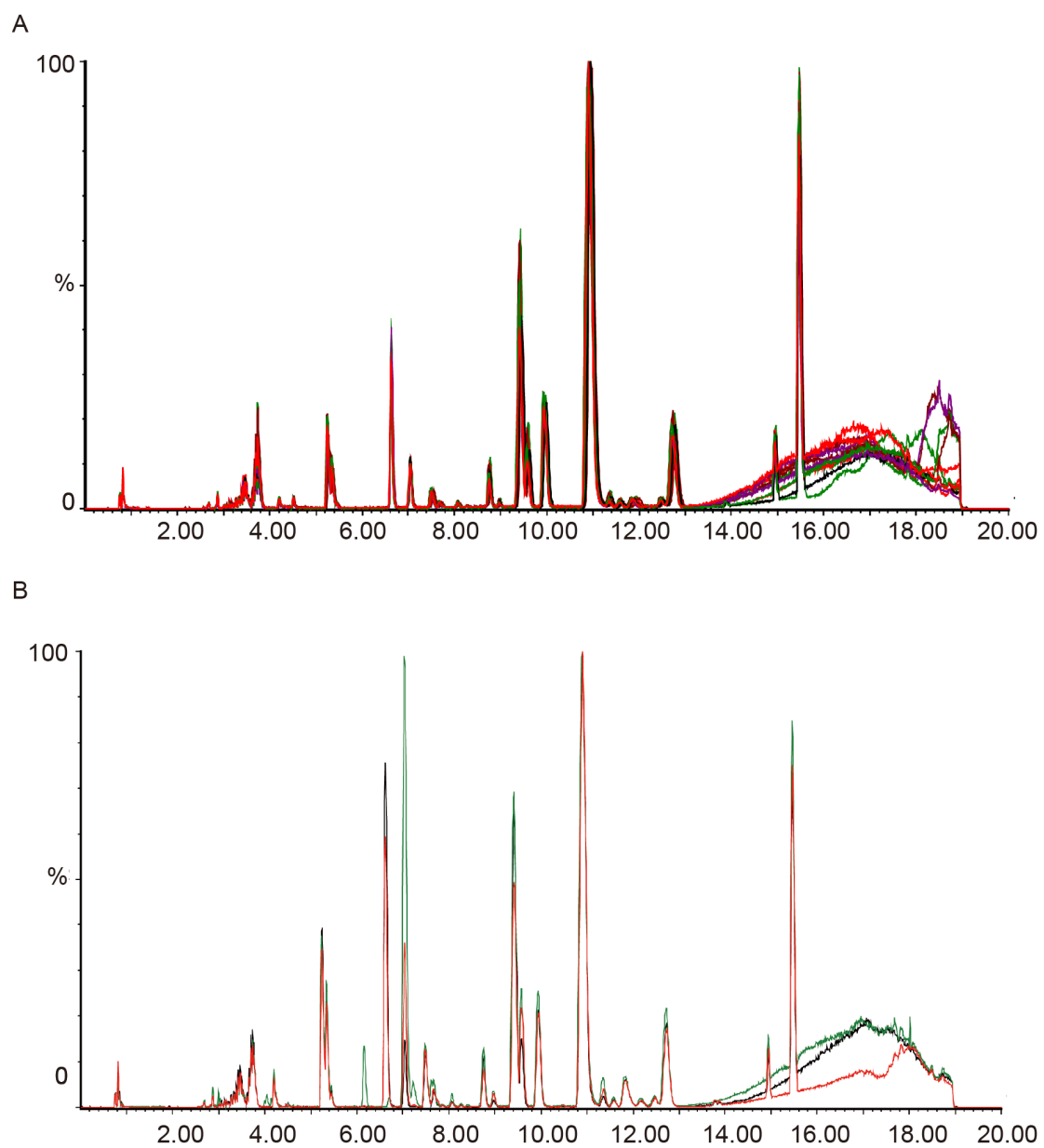
A

B

Fig. S1. A. Total ion current chromatograms of the QC samples. B. Typical UPLC-QTOF/MS base peak intensity (BPI) chromatograms for 1 NGR, 1 pre-DM and 1 DM sample in the positive mode. The Y axis shows relative abundance (%), and the X axis shows retention time in minutes, which was used to identify individual metabolites.
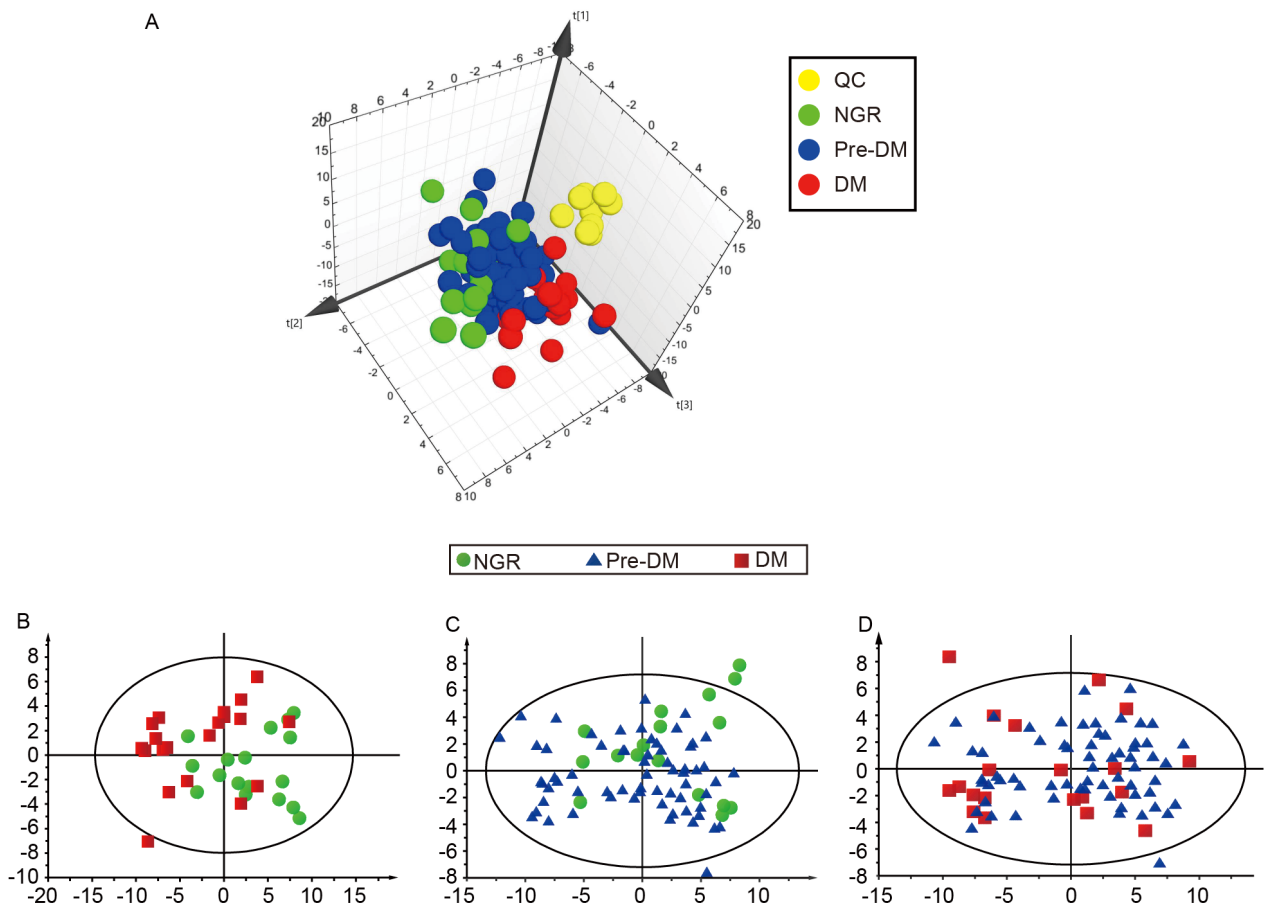
Fig. S2. PCA score plots of different groups based on plasma spectral data of UPLC-QTOF-MS positive ion mode. One point stands for one subject. A. PCA score plot of the NGR vs pre-DM vs DM groups and QC samples. B. PCA score plot of the the NGR vs DM. C. PCA score plot of the the NGR vs pre-DM. D. PCA score plot of the the pre-DM vs DM.
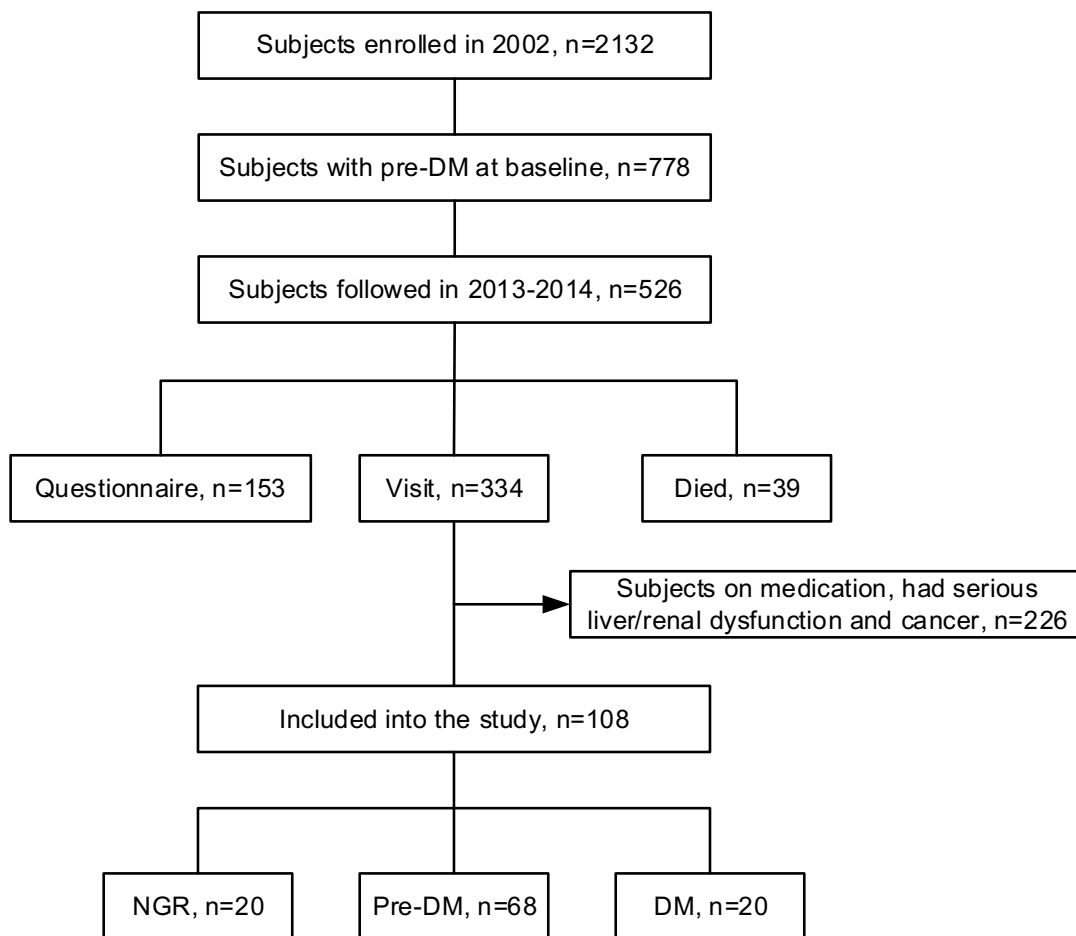
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                    │  Subjects enrolled in 2002, n=2132      │
                    └─────────────────────────────────────────┘
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                    │  Subjects with pre-DM at baseline, n=778 │
                    └─────────────────────────────────────────┘
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                    │  Subjects followed in 2013-2014, n=526  │
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             ┌────────────────────────┼────────────────────────┐
   ┌──────────────────────┐  ┌──────────────────┐  ┌──────────────────┐
   │ Questionnaire, n=153 │  │  Visit, n=334    │  │  Died, n=39      │
   └──────────────────────┘  └──────────────────┘  └──────────────────┘
                                      │
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                                      │       │ Subjects on medication, had serious│
                                      │       │ liver/renal dysfunction and cancer,│
                                      │       │ n=226                              │
                                      │       └────────────────────────────────────┘
                    ┌─────────────────────────────────────────┐
                    │  Included into the study, n=108          │
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   │  NGR, n=20       │    │  Pre-DM, n=68    │    │  DM, n=20        │
   └──────────────────┘    └──────────────────┘    └──────────────────┘
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Fig. S3. Details on the study population.

	NGR(n=20)	Pre-DM(n=68)	DM(n=20)
Body mass index, kg/m^2	25.7±2.8	25.6±2.7	26.1±3.5
Waist circumference, cm	80.9±8.5	82.3±7.1	84.0±10.8
Waist-hip ratio	0.84±0.05	0.84±0.05	0.86±0.06
Hypertension, %	55.0	54.4	70.0
SBP, mmHg	134.8±16.9	134.5±16.6	135.3±22.7
DBP, mmHg	84.3±9.5	84.0±12.1	83.0±11.7
Fasting glucose, mmol/L	5.9±0.3	5.9±0.4	6.1±0.3
2-h glucose, mmol/L	5.7±0.8	5.9±1.3	6.0±1.5
HDL, mmol/L	1.4±0.3	1.4±0.3	1.5±0.4
LDL, mmol/L	3.0±0.8	2.9±0.7	3.1±0.9
TC, mmol/L	5.1±0.9	5.1±0.9	5.1±1.2
TG, mmol/L	1.4±0.8	1.7±1.1	1.8±1.1

Table S1. Characteristics of the study participants at baseline. Values are mean ± SD or %. Abbreviations are the same as Table 1.

Metabolites	AUC	Significance	95% CI		CV% in QC samples
			Lower	Upper	
20-Hydroxy-leukotriene E4	0.749	0.001	0.637	0.862	13.9
Delta 8,14 -Sterol	0.640	0.063	0.489	0.791	14.8
Pantetheine	0.743	0.001	0.606	0.879	18.5
Caprylic acid	0.670	0.024	0.538	0.802	10.6
N(6)-(octanoyl)lysine	0.665	0.028	0.515	0.816	11.3
Lysyl-Tyrosine	0.642	0.060	0.490	0.793	8.7
Pc(18:3/20:3)	0.639	0.065	0.487	0.792	14.8
LysoPE(20:5/0:0)	0.651	0.045	0.494	0.808	13.7
Lysopc(20:4)	0.429	0.349	0.262	0.597	14.2
2,3-Epoxymenaquinone	0.764	<0.001	0.654	0.873	11.1
Lysopc(18:3)	0.332	0.026	0.204	0.460	10.9
cis-13,16-Docosadienoic acid	0.724	0.003	0.582	0.866	11.0
S-(hydroxymethyl)glutathione	0.716	0.004	0.579	0.854	12.8
3-Phenylbutyric acid	0.661	0.033	0.509	0.813	12.6
1-Stearoylglycerophosphoglycerol	0.725	0.003	0.595	0.855	12.4
5-methoxytryptamine	0.717	0.004	0.575	0.858	10.6
Lysopc(20:5)	0.366	0.075	0.241	0.490	13.1
Endomorphin-1	0.727	0.003	0.596	0.859	11.8
L-palmitoylcarnitine	0.715	0.004	0.584	0.837	7.9
N6-Acetyl-L-lysine	0.644	0.056	0.491	0.798	4.1
Pc(14:1/16:1)	0.683	0.015	0.564	0.803	9.4
LysoPC(20:3)	0.381	0.115	0.253	0.509	9.1
3-Ethylphenol	0.699	0.008	0.571	0.827	6.8

Table S2. AUC of single biomarkers identified for transition to NGR, significance of ROC analysis, 95% CI and CV% values in QC samples.

| Metabolites | AUC | Significance | 95% CI | | CV% in |
			Lower	Upper	QC samples
2-ketobutyric acid	0.234	<0.001	0.107	0.362	14.6
Iso-valeraldehyde	0.183	<0.001	0.076	0.289	9.9
Betaine	0.246	0.001	0.118	0.373	13.3
Pantetheine	0.343	0.033	0.199	0.486	14.2
Uric acid	0.331	0.022	0.214	0.447	15.2
L-carnitine	0.243	0.001	0.117	0.368	13.6
L-threonine	0.204	<0.001	0.081	0.328	14.8
Lysope(16:0/0:0)	0.348	<0.040	0.224	0.472	10.0
Linoleic acid	0.671	0.021	0.536	0.806	7.7
Lysopc(18:1)	0.325	0.018	0.193	0.457	10.3
3-dehydroxycarnitine	0.703	0.006	0.575	0.832	5.7
Palmitic amide	0.367	0.073	0.234	0.500	10.9
3,5-dihydroxybenzoic acid	0.256	0.001	0.125	0.388	12.7
L-lysine	0.276	0.002	0.167	0.385	12.8
2-Pyrroloylglycine	0.292	0.005	0.160	0.424	10.0
Kynuramine	0.784	<0.001	0.661	0.908	16.1
Dityrosine	0.687	0.012	0.551	0.822	13.2
5-hydroxy-2-oxo-4-ureido-2,5-dihydro-1h-imidazole-5-carboxylate	0.225	<0.001	0.107	0.343	11.8
Lysopc(18:0)	0.376	0.094	0.238	0.514	13.3
Pc(18:0/18:2)	0.432	0.356	0.298	0.566	18.1
1,3,7-trimethyluric acid	0.699	0.007	0.579	0.820	11.4
Pc(16:0/14:0)	0.347	0.039	0.211	0.483	18.6

Table S3. AUC of single biomarkers identified for transition to DM, significance of ROC analysis, 95% CI and CV% in QC samples.