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URINARY PROTEOMICS IN DIABETES AND CHRONIC RENAL DISEASE

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ABSTRACT

We identified and validated urinary biomarkers for diabetes mellitus and one of its common complications, renal damage. We also identified biomarkers differentiating patients with diabetic nephropathy from those with other proteinuric renal diseases. For 305 individuals, these biomarkers were defined and validated in blinded datasets using high-resolution capillary electrophoresis coupled with electrospray-ionization mass spectrometry. A panel of 40 biomarkers distinguished patients with diabetes mellitus from healthy non-diabetic individuals (94 blinded samples) with 89% sensitivity and 91% specificity. One-hundred-two urinary biomarkers differed significantly between diabetic patients with normoalbuminuria and nephropathy. A model based on 65 of them correctly indicated diabetic nephropathy with 97% sensitivity and specificity (70 blinded samples). Furthermore, this biomarker panel identified microalbuminuric diabetic patients who progressed towards overt diabetic nephropathy over three years. Differentiation between diabetic nephropathy and other chronic renal diseases (masked dataset of 64 samples) reached 81% sensitivity and 91% specificity. Many of the biomarkers were fragments of collagen type I, and quantities were reduced in patients with diabetes or diabetic nephropathy. In conclusion, our study shows that analysis of the urinary proteome is a powerful tool in the detection of diabetes-associated renal damage, and may be a valuable asset in its early diagnosis and prognosis.

Nephropathy is a serious and common complication of diabetes mellitus and has become the most prevalent cause of end-stage renal disease. Over the years there has been an ongoing quest to find biomarkers early in the clinical course to better identify and treat individuals at high risk for diabetic nephropathy. Knowledge of the complex molecular and pathophysiologic mechanisms leading to renal disease remains limited, in part because conventional research tools have hampered investigators by restricting their focus to a single or relatively few risk markers at a time. Recent advances in proteomics enable screening a vast array of proteins simultaneously, aiding assessment of their potential role in the development and progression of disease.¹ Urine is well suited for proteomic analysis to identify predictive biomarkers and to unravel the pathogenetic mechanisms of chronic renal disease. The online combination of capillary electrophoresis (CE) and electrospray mass spectrometry (MS) was developed for the rapid (~45 minutes/sample), sensitive, and automated approach for such an analysis.²

The aim of our study was to examine whether CE-MS can detect differences in the urinary proteome between normo-, micro-, and macroalbuminuric patients with type 1 diabetes mellitus. Furthermore, we sought to evaluate if CE-MS-defined patterns derived from urinary polypeptides of patients with diabetic nephropathy differ from those of patients with other chronic renal diseases.

Healthy non-diabetic subjects and diabetic patients were matched with respect to age and gender to establish urinary polypeptide patterns characteristic for diabetes and diabetic nephropathy (**Table 1**). The three groups of diabetic patients (persistent normoalbuminuria, microalbuminuria, and diabetic nephropathy) had comparable duration of diabetes, severity of diabetic retinopathy, blood pressure, and serum cholesterol levels. Hemoglobin A1c was lower in normoalbuminuric patients and serum creatinine was significantly higher in patients with diabetic nephropathy. Urine samples from all subjects were analyzed using CE-MS and the data were normalized using internal standards³ and deposited in a database. Only polypeptides present at a frequency >50% in any of the groups were evaluated. The compiled data for the four groups are shown in **Figure 1A**. While the urinary peptide profiles in samples from the healthy individuals

(STENOcontrol), as well as diabetic normalalbuminuric (STENOnormo) and microalbuminuric (STENOmico) patients appeared quite similar, the urinary polypeptide patterns of patients with diabetic nephropathy (STENOmaco) markedly differed.

To identify potential biomarkers for diabetes, urinary polypeptides of healthy individuals were compared to those of diabetic patients with persistent normoalbuminuria. This analysis identified 40 peptides of statistical significance ($p < 0.05$ in maxT testing, **Supplementary Table 1**). A support vector machine-based model (SVM-BM) with these 40 biomarkers discriminated healthy individuals from normoalbuminuric diabetics with 97% sensitivity and specificity at crossvalidation. The distribution of the polypeptides in the two groups is shown in **Figure 1B**. The validity of the diabetes biomarkers was further evaluated in a test-set cohort of STENOmaco, STENOmico, and diabetic patients with normoalbuminuria (HANNOVERcontrol): 53 of 59 diabetic patients and 32 of 35 healthy controls were correctly classified, resulting in 89% sensitivity and 91% specificity.

To define biomarkers for diabetic renal disease, urine samples from diabetic patients with persistent normoalbuminuria (STENOnormo) were compared to those from patients with diabetic nephropathy (STENOmaco). This analysis identified 102 biomarkers of statistical significance ($p < 0.05$ in maxT testing adjusted for multiple tests, 24 of these biomarkers have been sequenced; **Supplementary Table 2**). To reduce the number of variables, a “take-one-out” procedure was used, decreasing the number of biomarkers to 65 (**Figure 1C**) without losing performance in the classification. A SVM-BM with these 65 polypeptides performed with 93% sensitivity and 97% specificity at crossvalidation.

Because SVM-BMs do not assign probability, we investigated whether a linear combination of biomarkers may improve disease severity classification. Transformed and calibrated logarithmic amplitudes of all biomarkers were combined for each patient.⁴ This approach resulted in 100% sensitivity and 93% specificity.

The diabetic renal disease model was validated in an external dataset of 35 macroalbuminuric diabetic patients (HANNOVERmacro) and 35 healthy individuals

(HANNOVERcontrol).⁵ Using SVM-BM, 34 of 35 controls and all patients were classified correctly. When applying the linear model, the same control and one of the patients were misclassified, resulting in 97% sensitivity and 97% specificity.

When applying the diabetic renal disease models to the matched 30 microalbuminuric diabetic patients in the study (STENOmicro), 21 scored positive for diabetic nephropathy when using the linear model (6 were only marginally positive). Based on the same biomarkers in the SVM-BM, 17 of 30 patients scored positive. Eight microalbuminuric patients later showed an increase in albuminuria of > 25% or progressed to macroalbuminuria during a 3-year follow-up interval. Each scored positive in the linear model and 7 scored positive in the SVM-BM. These data indicate the utility of the biomarkers for not only detection of overt nephropathy, but also prediction of its development in microalbuminuric diabetic patients with statistical significance ($p = 0.0359$).

Several peptides were sequenced using MS/MS.⁶ The sequenced biomarkers that differentiated diabetic patients from healthy controls included fragments of collagen type I (alpha 1 chain) and uromodulin (**Supplementary Table 1**). For differentiation of patients with and without diabetic nephropathy, the sequences were more diverse, consisting of various collagen types as well as uromodulin fragments (**Supplementary Table 2**).

We tested the specificity of the diabetic renal disease pattern using urine samples from patients with other chronic renal diseases: biopsy-proven IgA nephropathy (IgAN; N=57), focal segmental glomerulosclerosis (FSGS; N=30), membranous glomerulonephritis (MNGN; N=35), and minimal-change disease (MCD; N=25). These patients and datasets have been described previously.^{5;7;8} The compiled polypeptides of the different chronic renal diseases are shown in **Figure 2A**. As expected, most (N=104) of these 147 samples scored positive for “diabetic renal disease”, only 43 scored as normoalbuminuria, indicating that the “diabetic renal disease” pattern reflects chronic renal damage.

To investigate if we could define biomarkers to distinguish diabetic nephropathy from the other chronic renal diseases, 104 samples from patients with non-diabetic nephropathy were randomly selected and compared to those from 70% of the patients with diabetic nephropathy in (STENOmacro and HANNOVERmacro, N=44). This process identified 37 biomarkers of statistical significance ($p < 0.05$ in maxT testing, **Table 2** and **Figure 2B**).

Using a “take-one-out” procedure, the number of biomarkers decreased from 37 to 17. A SVM-BM based on these 17 biomarkers distinguished diabetic renal disease from the 4 other chronic kidney diseases. This model correctly classified 42 of 44 patients with diabetic nephropathy, and 98 of 104 patients with other chronic renal diseases. Crossvalidation of this training set showed 91% sensitivity and 89% specificity. To further validate these biomarkers, the 30% of initially recruited patients and controls who had been omitted were analyzed. Classification of this masked dataset showed 81% sensitivity and 91% specificity (**Figure 2C**).

We sequenced only some biomarkers, because the procedure is quite challenging.^{1;9} The most striking observation was the decreased excretion of specific collagen fragments in patients with diabetes compared to healthy controls; several additional collagen fragments were less common in macroalbuminuric diabetic patients compared to the normoalbuminuric patients. This observation supports previous reports,¹⁰ and extends earlier findings by using external datasets, CE-MS with ultrafiltration to remove larger proteins, and a new generation of mass spectrometers with better sensitivity, mass accuracy, and resolution.

Collagen increases in renal tissue of patients with diabetic nephropathy,^{11,12} and the decreased proteolysis due to increased synthesis of protease inhibitors diminishes excretion of collagen fragments.^{13;14} Furthermore, advanced-glycation endproducts cross-link collagen and thus increase its resistance against proteases.^{15;16} A similar principle was shown for tubulointerstitial elastin deposition in experimental diabetic nephropathy: decreased activity of elastase IIIB and increased production of elastase inhibitor diminished elastin turnover and increased its quantity.¹⁷

Urine contains a plethora of collagen fragments. In a study of >3000 individuals, 231 of 353 sequences were from collagen (Coon et al., submitted). Several fragments were found in >90% of all samples, with similar amplitudes. Other collagen fragments showed significant differences between controls and patients. These fragments are likely products of specific proteases, and may serve as indicators of the activity of these enzymes.

Patients with diabetic nephropathy showed decreased levels of collagen type I and uromodulin fragments, while albumin fragments were highly up-regulated. Assessment of these peptides by immunological technologies, such as Western blot, is difficult due to their small size. Alternative approaches, such as ELISA, appear inappropriate, as several peptides sharing an antigenic epitope cannot be easily distinguished immunologically.¹⁸

We demonstrated that the urinary proteome differentiates healthy individuals from diabetic patients with persistent normoalbuminuria, low-grade albuminuria, or nephropathy. Furthermore, it also distinguishes patients with diabetic nephropathy from patients with other chronic renal diseases. This finding could have great clinical value among patients with type 2 diabetes and nephropathy as they are known to have increased albuminuria on a very heterogenic background when proteinuria occurs in the absence of diabetic retinopathy.¹⁹ The data from the blinded prospective evaluation of the microalbuminuric patients indicated that urinary proteome analysis identified microalbuminuric patients at greater risk for diabetic nephropathy. Based on sequencing of some biomarkers, we speculate that changes in the collagen metabolism are closely linked with renal damage in diabetic patients.

METHODS

Patients, Procedures and Demographics

This study included an internal dataset used to identify biomarkers of diabetes and diabetic nephropathy by proteomic analysis. The internal dataset consisted of subjects examined at the Steno Diabetes Center in 2004 and was comprised of 30 Caucasian healthy individuals (“STENOcontrol”) and 3 groups of Caucasian patients with type 1 diabetes attending the Steno Diabetes Center: 1) 30 patients with persistent normoalbuminuria [“STENOnormo”, <30 mg/24 hr, and long-standing diabetes (>15 years)], 2) 29 patients with persistent microalbuminuria (“STENOmico”, ≥ 30 but < 300 mg per 24 hr in at least two of three consecutive samples), and 3) 30 patients with diabetic nephropathy [persistent macroalbuminuria (“STENOmico”, ≥ 300 mg/24 hr), and co-existence of diabetic retinopathy or a renal biopsy showing diabetic glomerulosclerosis]. The groups were matched for age, gender, and duration of diabetes. The external datasets consisted of 35 healthy, non-diabetic individuals (“HANNOVERcontrol”) and 35 patients with diabetic nephropathy, most with type II diabetes (“HANNOVERmacro”). Furthermore, biomarkers of diabetic nephropathy were evaluated in patients with biopsy-verified IgA nephropathy (IgAN; N=57), focal segmental glomerulosclerosis (FSGS; N=30), membranous glomerulonephritis (MNGN; N=35), and minimal-change disease (MCD; N=25). Subjects in the external datasets had been evaluated in other hospitals.^{5;8;20;21} The local ethics committees approved the study, and all subjects gave informed consent. The study was performed in accordance with the Helsinki Declaration.

Sample preparation and CE-MS analysis

All analyses and data processing were performed in accordance with the recently published MIAPE guidelines,²² and in agreement with the suggested guidelines for clinical proteome analysis.²³ All urine samples for CE-MS analyses were from spontaneously voided urine and stored at -80°C until analysis. Sample preparation and CE-MS analysis were performed.³ Briefly, a 0.7-ml sample was prepared and resuspended in 10 – 100 μ l H₂O (depending on the peptide concentration) to a concentration of 1-5 μ g/ μ l. Between 100 and 500 nl of sample was injected hydrodynamically, aiming for a total amount of 0.5 μ g peptide per analysis, corresponding

to 0.7 -14 μ l of the original sample. The data on all CE-MS analyses of all patients in the study are available in **Supplementary Table 3** (downloadable at http://www.mosaiques-diagnostics.de/GenexPivot_12092007.exe password:99Mischak123). A total of 5616 different polypeptides were tentatively identified (annotated) and, on average, 1632 polypeptides were found in each urine sample; on average, 1185 of these were annotated.

Data processing and analysis

Mass spectral ion peaks representing identical molecules at different charge states were deconvoluted into a single mass using MosaiquesVisu software.³ In addition, the migration time and ion signal intensity (amplitude) were normalized using internal polypeptide standards as described in detail.²⁴ This approach results in superior comparability of the datasets as compared to normalization of the signal intensity to urinary creatinine or total protein content, as especially the latter will give poor results in patients with proteinuria. Each polypeptide is characterized by its molecular mass [kDa], normalized migration time [min], and normalized signal intensity as the measure for relative abundance. All detected polypeptides were deposited, matched, and annotated in a Microsoft SQL database, allowing further analysis and comparison of multiple samples (patient groups). Polypeptides within different samples were considered identical if the mass deviation was less than 50 ppm and the migration time deviation was less than 1 min. CE-MS data of all individual samples can be accessed in **Supplementary Table 3** (downloadable at http://www.mosaiques-diagnostics.de/GenexPivot_12092007.exe password:99Mischak123).

Definition of biomarkers and sample classification

The unadjusted p-values were calculated using the arcsinh-transformed intensities and the Gaussian approximation to the t-distribution. Bonferroni adjustments were made by applying the standard Bonferroni criterion to markers that passed the frequency threshold of 70%. MaxT p-values were calculated using the Westfall and Young maxT-procedure.²⁵

Estimates of sensitivity and specificity were calculated by tabulating the number of correctly classified samples. Confidence intervals (95% CI) were based on exact binomial calculations and using MedCalc version 8.1.1.0 (MedCalc Software; Mariakerke, Belgium; <http://www.medcalc.be>).

The Receiver Operating Characteristic (ROC) plot was generated by plotting all sensitivity values (true-positive fraction) on the y-axis against their equivalent (1-specificity) values (false-positive fraction) for all available thresholds on the x-axis (MedCalc Software).²⁶

Disease-specific polypeptide patterns based on pre-defined polypeptides were generated using the support-vector-machine-based MosaCluster software.²⁷

For linear combination, all normalized signal intensity values of biomarkers were log-transformed. Values below 1 were substituted with a value of 1 to avoid negative values. The average signal intensity for a specific biomarker over all cases was compared to the average intensity for the same biomarker over all controls. To avoid artificial weighting of specific biomarkers in the set due to the difference in observed signal intensities for case and control, the distance between the two averages (case and control) was set relative to a value of 2. The relative distance of signal intensities between the disease and control samples was provided using the

formula: $(A_k^i - mean_{averages}) \frac{2}{|\bar{x}_{case} - \bar{x}_{control}|}$, where A_k^i is the log-transformed signal intensity of the i^{th}

biomarker in the k^{th} sample in either the test set or the blinded set, $mean_{averages}$ is the average of the mean intensity of all possible markers for test set samples, \bar{x}_{case} represents the mean observed signal intensity of the possible biomarker from all case samples and $\bar{x}_{control}$ represents the mean signal intensity of the possible biomarker from the control samples.

Crossvalidation was performed and defined by the take-one-out procedure.²⁸ To reduce the number of biomarkers in a model, a “take-one-out” procedure was used.³ Briefly, models were generated that were each based on n-1 biomarkers. These were evaluated using complete crossvalidation and compared to the classification results of the model based on n biomarkers. A single biomarker that apparently did not improve classification was removed.

Sequencing of polypeptides

Candidate biomarkers were sequenced using LC-MS/MS analysis (on a Q-TOF instrument).⁶ Further analysis was performed using instruments with electron transfer dissociation (ETD) capability.^{4;29;30}

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Competing financial interests

H. Mischak is founder and co-owner of Mosaiques Diagnostics, which developed the CE-MS technology and the MosaiquesVisu software. M. Dakna and P. Züribig are employees of Mosaiques Diagnostics.

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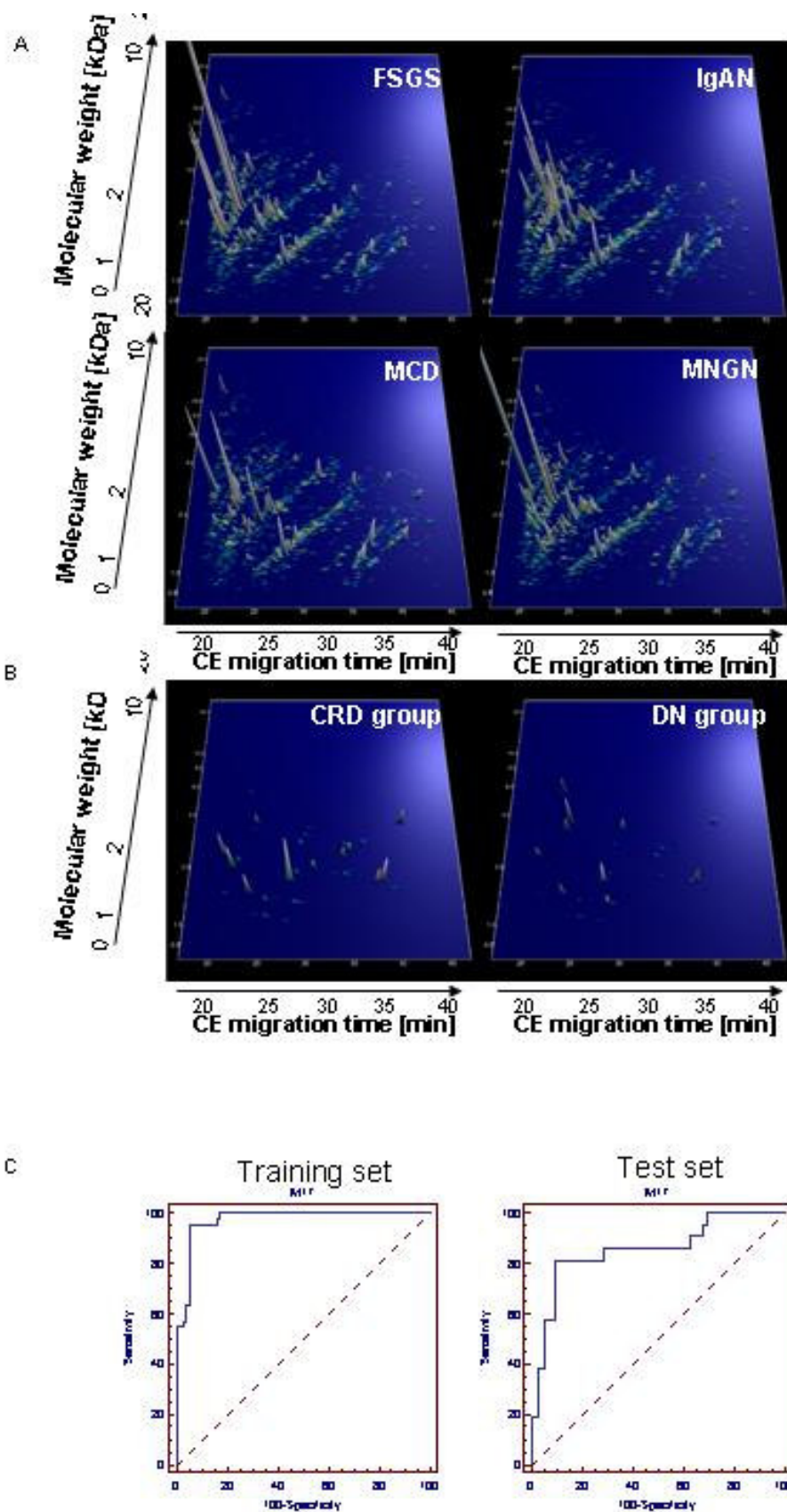
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FIGURE LEGENDS

Figure 1 A: Protein patterns of the diabetic patients and controls examined in this study. Shown are compiled patterns consisting of all samples from each of the four groups. The molecular mass (0.7 – 25 kDa, on a logarithmic scale) is plotted against normalized migration time (17-47 min). Signal intensity is encoded by peak height and color. **1B:** Distribution of potential differential-diagnostic biomarkers for diabetes in the normoalbuminuric patients and healthy controls. All statistically significant biomarkers from **Supplementary Table 1** are shown in the figure. **1C:** Distribution of potential differential-diagnostic biomarkers for diabetic nephropathy in the different groups of patients and healthy controls. Shown are all statistically significant biomarkers listed in **Supplementary Table 2**.

Figure 2A: Protein patterns of the patients with chronic renal disease. Shown are compiled patterns consisting of samples from patients with focal segmental glomerulosclerosis (FSGS, N=35) IgA nephropathy (IgAN, N=57), minimal-change disease (MCD, N=25) and membranous glomerulonephritis (MNGN, N=29). In comparison to **Figure 1A**, these compiled data show a much higher degree of similarity to the macroalbuminuric patients than to any other group with diabetes mellitus. The molecular mass (0.7-25 kDa, on a logarithmic scale) is plotted against normalized migration time (17-47 min). Signal intensity is encoded by peak height and color. **2B:** Distribution of potential differential-diagnostic biomarkers for diabetic nephropathy in all macroalbuminuric patients (DN group) and all “chronic renal disease controls” (CRD group) used in the study. All 37 statistically significant biomarkers from **Table 2** are shown in the figure. **2C:** ROC analysis of the performance of the “differential diagnostic biomarker pattern” for diabetic nephropathy. The left panel shows the data from the training set of 44 cases and 104 controls (sensitivity of 95.5% and specificity of 94.2%, AUC was 0.971). The right panel shows the data from validating the masked test set consisting of 64 samples (81.0% sensitivity and 90.7% specificity, AUC was 0.856).



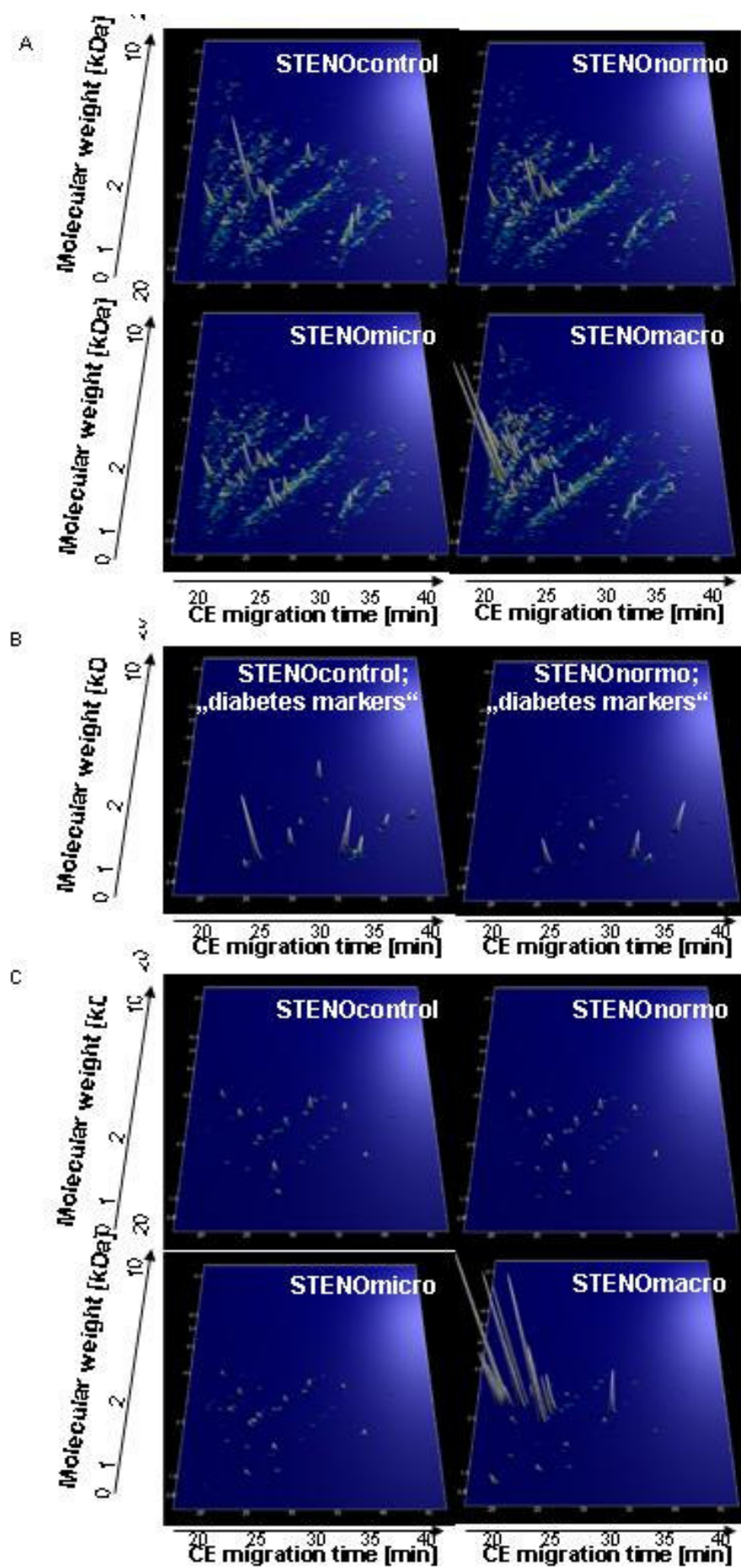


TABLE LEGENDS

Table 1: Characteristics of all subjects recruited at the STENO Diabetes Center.

Table 2: The table lists the 37 urinary biomarkers that statistically differentiated 104 patients randomly selected from the group of 147 patients with nondiabetic chronic renal diseases from a cohort comprised of 70% of the patients with clinically confirmed diabetic nephropathy. Shown are the protein/peptide identification number in the dataset (ID), mass (in Da) and normalized migration time (CE-Time), the p-values, (unadjusted, adjusted using maxT and Bonferroni) and the frequency and mean amplitude in the two groups, diabetic nephropathy (DN) and “other chronic renal disease” (CRD). Those highlighted in bold were subsequently used in the classification model. Where available, sequence and original protein are shown.

Supplementary Table 1: Panel of biomarkers used to discriminate between healthy, non-diabetic patients and those with diabetes (without clinical presentation of diabetic nephropathy). Shown are the protein/peptide identification number in the dataset (ID), mass (in Da) and normalized migration time (CE-Time), the p-values, (unadjusted, adjusted using maxT and Bonferroni) and the frequency and mean amplitude in the two groups, STENOcontrol and STENOnormo. Where available, sequence and original protein are shown.

Supplementary Table 2: Sequenced polypeptides that displayed a statistically significant difference between normo- and macroalbuminuric diabetic patients. Shown are the protein/peptide identification number in the dataset (ID), mass (in Da) and normalized migration time (CE-Time), the p-values, (unadjusted, adjusted using maxT and Bonferroni) and the frequency and mean amplitude in the two groups, STENOm macro and STENOnormo. Identifications highlighted in bold are those that were subsequently used in the classification model. Where available, sequence and original protein are shown.

Supplementary Table 3:

(downloadable at http://www.mosaiques-diagnostics.de/GenexPivot_12092007.exe password:99Mischak123).

Pivot Table consisting of 4 worksheets which includes all CE-MS data for every sample in the study. The worksheet Protein assignment shown the migration time and mass (with standard deviation) of peptides assigned to a certain ID. Sample assignment indicates the respective group and original analysis file assigned to the Sample IDs. Worksheets T1 and T2 list the amplitudes of each polypeptide in the individual samples.

Table 1.

| | Healthy controls (n=30) | Normoalbuminuria (n=30) | Microalbuminuria (n=29) | Diabetic Nephropathy (n=30) | p-value (normo vs. macro) | p-value (all groups) |
|---|----------------------------|----------------------------|----------------------------|--------------------------------|------------------------------|-------------------------|
| Age | 51 (1.9) | 54 (2.0) | 56 (1.8) | 50 (1.6) | 0.12 | 0.07 |
| Gender (female/male) | 7/23 | 7/23 | 7/22 | 7/23 | 0.99 | 0.99 |
| Diabetes duration (yrs) | - | 34 (1.9) | 35 (2.0) | 34 (2.0) | - | - |
| Diabetic retinopathy (nil/simple/proliferative) | - | 2/12/16 | 1/9/19 | 2/7/21 | 0.23 | 0.99 |
| Urinary albumin/creatinine* | - | 5 (4 to 6) | 45 (32 to 63) | 765 (564 to 1036) | - | - |
| Systolic blood pressure (mm Hg) | 133 (3) | 136 (3) | 148 (4) | 142 (4) | 0.28 | 0.02 |
| Diastolic blood pressure (mm Hg) | 80 (2) | 77 (2) | 75 (2) | 77 (2) | 0.98 | 0.24 |
| Cholesterol (mmol/l) | 5.6 (0.1) | 4.6 (0.2) | 4.9 (0.2) | 4.6 (0.2) | 0.37 | <0.001 |
| Creatinine (micromol/l) | 97 (2) | 94 (2) | 94 (3) | 171 (15) | <0.001 | <0.001 |
| Hemoglobin A1c (%) | 5.4 (0.1) | 8.0 (0.2) | 8.9 (0.3) | 8.8 (0.2) | 0.015 | <0.001 |

Values are mean (SE) *geometric mean (95%CI)

Table 2.

| Polypeptide | | | p-value | | | Diabetic Nephropathy | | Non-diabetic chronic renal disease | | Sequence | Protein |
|-------------|----------|--------------|-------------------|--------|----------|----------------------|----------------|------------------------------------|----------------|---------------------------------|--|
| ID | Mass[Da] | CE-Time[Min] | unaj _i | maxT | Bonf- | Frequency | Mean Amplitude | Frequency | Mean Amplitude | | |
| 175343 | 5574,25 | 23,2 | 8,77E-06 | 0.0213 | 5,01E-03 | 0,11 | 0,55 | 0,43 | 1,06 | | |
| 140401 | 3669,67 | 24,17 | 1,90E-07 | 0.0017 | 1,10E-04 | 0,34 | 0,82 | 0,48 | 1,26 | | |
| 140112 | 3657,67 | 40,71 | 1,07E-05 | 0.0175 | 6,23E-03 | -0,4 | 0,32 | 0,72 | 1,95 | | |
| 130855 | 3363,54 | 30,22 | 8,03E-08 | 0.0006 | 4,66E-05 | 0,37 | 0,8 | 0,42 | 0,94 | | |
| 129940 | 3333,72 | 23,83 | 3,34E-06 | 0.0456 | 1,91E-03 | 0,26 | 0,55 | 0,29 | 0,67 | | |
| 121444 | 3081,42 | 29,83 | 1,69E-05 | 0.0301 | 9,82E-03 | -0,29 | 0,23 | 0,52 | 1,24 | | |
| 110175 | 2802,82 | 36,34 | 3,94E-14 | 0.0001 | 2,29E-11 | -0,49 | 0,07 | 0,56 | 1,31 | | |
| 107858 | 2751,34 | 29,23 | 1,14E-06 | 0.0043 | 6,62E-04 | -0,4 | 0,2 | 0,61 | 1,52 | | |
| 107763 | 2748,79 | 36,38 | 8,12E-15 | 0.0001 | 4,72E-12 | -0,51 | 0,09 | 0,6 | 1,18 | | |
| 107016 | 2733,78 | 34,16 | 8,38E-12 | 0.0001 | 4,87E-09 | -0,45 | 0,07 | 0,52 | 1,13 | | |
| 99251 | 2574,01 | 32,81 | 3,86E-07 | 0.0027 | 2,24E-04 | -0,37 | 0,16 | 0,53 | 1,04 | | |
| 97736 | 2551,15 | 34,72 | 8,65E-06 | 0.0315 | 4,94E-03 | -0,27 | 0,57 | 0,84 | 2,09 | | |
| 90344 | 2377,1 | 20,8 | 1,29E-05 | 0.0185 | 7,49E-03 | -0,31 | 0,45 | 0,77 | 2,29 | GKNGDDGEAGKhPGRPhGERGPP hGPQ | Collagen alpha-1 (I) chain [227-250] [Homo sapiens] |
| 88282 | 2339 | 34,01 | 3,74E-05 | 0.0438 | 2,18E-02 | -0,29 | 0,52 | 0,82 | 2,21 | | |
| 80012 | 2191,99 | 22,39 | 6,40E-12 | 0.0001 | 3,72E-09 | -0,49 | 0,14 | 0,63 | 1,62 | NGDDGEAGkPGRpGERGpGPQ | Collagen alpha-1 (I) chain |
| 72521 | 2046,81 | 30,72 | 1,89E-05 | 0.0351 | 1,10E-02 | -0,28 | 0,23 | 0,51 | 1,26 | | |
| 72161 | 2039,13 | 21,78 | 1,92E-13 | 0.0001 | 1,11E-10 | -0,47 | 0,14 | 0,61 | 1,83 | SGSVIDQSRVNLNGPITRK | Uromodulin [589-607] [Homo sapiens] |
| 67382 | 1936,87 | 34,75 | 1,53E-05 | 0.0275 | 8,89E-03 | -0,32 | 0,2 | 0,53 | 1,13 | | |
| 64905 | 1893,03 | 28,86 | 3,02E-05 | 0.0429 | 1,75E-02 | -0,36 | 0,27 | 0,63 | 1,57 | GEKGPSEAGTAGPPGTPGPQG | Collagen alpha-2 (I) chain [844-865] [Homo sapiens] |
| 64431 | 1885,65 | 38,82 | 1,73E-05 | 0.0276 | 1,01E-02 | -0,38 | 0,23 | 0,61 | 1,29 | | |
| 63427 | 1865,81 | 32,98 | 2,73E-09 | 0.0003 | 1,59E-06 | -0,43 | 0,18 | 0,62 | 1,31 | DAGPAGPKGEPHGSPHGENGAPH G | Collagen alpha-1 (I) chain [279-299] [Homo sapiens] |
| 62387 | 1844,48 | 34,27 | 3,82E-07 | 0.0025 | 2,22E-04 | -0,4 | 0,18 | 0,58 | 1,39 | | |
| 57265 | 1732,77 | 28,18 | 1,08E-06 | 0.0023 | 6,30E-04 | -0,39 | 0,55 | 0,93 | 3,04 | | |
| 56884 | 1725,59 | 38,32 | 5,12E-05 | 0.0494 | 2,97E-02 | -0,36 | 0,52 | 0,88 | 2,62 | | |
| 56514 | 1716,77 | 28 | 3,09E-06 | 0.0060 | 1,79E-03 | -0,33 | 0,45 | 0,79 | 2,07 | | |
| 55637 | 1698,57 | 37,73 | 3,60E-06 | 0.0082 | 2,09E-03 | -0,35 | 0,27 | 0,63 | 1,79 | | |
| 45863 | 1549,7 | 39,49 | 2,28E-05 | 0.0366 | 1,32E-02 | -0,35 | 0,18 | 0,53 | 1,21 | | |
| 44802 | 1526,69 | 23,92 | 2,62E-06 | 0.0065 | 1,52E-03 | -0,39 | 0,23 | 0,62 | 1,31 | | |
| 42378 | 1486,68 | 21,15 | 1,39E-09 | 0.0002 | 8,09E-07 | -0,47 | 0,11 | 0,59 | 1,35 | | |
| 42304 | 1485,67 | 23,77 | 7,18E-06 | 0.0072 | 4,17E-03 | -0,31 | 0,64 | 0,94 | 2,81 | DGQPhGAKGEPHG DAGAK | Collagen alpha-1 (I) chain [820-835] [Homo sapiens] |
| 36350 | 1396,62 | 26,67 | 1,04E-05 | 0.0194 | 6,01E-03 | -0,35 | 0,18 | 0,53 | 1,07 | | |
| 33973 | 1353,66 | 25,63 | 2,10E-05 | 0.0241 | 1,22E-02 | -0,25 | 0,61 | 0,87 | 2,16 | | |
| 32470 | 1326,55 | 29,2 | 1,18E-06 | 0.0044 | 6,86E-04 | -0,39 | 0,23 | 0,62 | 1,36 | SpGGpGSDGkPpGPpG | Collagen type 3 alpha1 |
| 32343 | 1324,59 | 28,7 | 7,45E-07 | 0.0032 | 4,33E-04 | 0,37 | 0,77 | 0,4 | 0,95 | TGPGDKGDTGPpGP | Collagen alpha-1 (III) chain |
| 20750 | 1141,52 | 24,51 | 1,89E-07 | 0.0017 | 1,10E-04 | -0,38 | 0,2 | 0,59 | 1,29 | | |
| 19791 | 1128,49 | 25,65 | 1,48E-08 | 0.0003 | 8,59E-06 | -0,47 | 0,34 | 0,81 | 1,93 | | |
| 16497 | 1078,47 | 27,76 | 1,01E-05 | 0.0195 | 5,89E-03 | -0,3 | 0,25 | 0,55 | 1,2 | | |

Supplementary table 1

| ID | Polypeptide | | p-value | | | Healthy Non-diabetic subjects (STENOcontrol, n=30) | | Diabetic patients with normoalbuminuria (STENOnormo, n=30) | | sequence | protein |
|--------|-------------|--------------|----------|----------|----------|---|-----------|---|-----------|---------------------------------|---|
| | Mass[Da] | CE-Time[Min] | unaj. | maxT | Bonf. | Frequency | Amplitude | Frequency | Amplitude | | |
| | 126982 | 3256.53 | 33,03 | 2,52E-05 | 0.028 | 2,20E-02 | 0,83 | 2,58 | 0,48 | | |
| 110175 | 2802.82 | 36,34 | 1,68E-05 | 0.007 | 1,46E-02 | 0,53 | 1,01 | 0,03 | 0,03 | | |
| 109328 | 2779.86 | 36,57 | 8,95E-06 | 0.007 | 7,80E-03 | 0,6 | 1,28 | 0,1 | 0,17 | | |
| 101081 | 2614.17 | 29,68 | 1,28E-07 | 0.001 | 1,12E-04 | 0,07 | 0,12 | 0,69 | 1,24 | | |
| 100991 | 2612.21 | 34,91 | 5,41E-05 | 0.044 | 4,71E-02 | 0,4 | 0,91 | 0,83 | 2,12 | | |
| 89857 | 2367.06 | 27,63 | 1,21E-05 | 0.007 | 1,05E-02 | 0,07 | 0,11 | 0,55 | 1,17 | | |
| 87272 | 2319.07 | 33,82 | 5,87E-06 | 0.007 | 5,12E-03 | 0,87 | 2,47 | 0,31 | 0,93 | | |
| 76839 | 2128.98 | 26,97 | 5,60E-08 | 0.001 | 4,88E-05 | 0,9 | 1,89 | 0,34 | 0,62 | DGKTGPhPGPAGQDG RPGPPhGPhPhG | Collagen alpha-1 (I) chain [550-572] [Homo sapiens] |
| 68415 | 1962.88 | 31,81 | 4,67E-05 | 0.042 | 4,06E-02 | 0,3 | 0,67 | 0,76 | 1,88 | | |
| 64170 | 1880.9 | 43,91 | 4,38E-05 | 0.038 | 3,81E-02 | 0,9 | 2,59 | 0,52 | 1,3 | | |
| 62135 | 1838.82 | 27,06 | 1,22E-05 | 0.008 | 1,07E-02 | 0,07 | 0,15 | 0,62 | 1,23 | | |
| 61446 | 1822.83 | 27,00 | 8,22E-06 | 0.007 | 7,16E-03 | 0,1 | 0,21 | 0,62 | 1,35 | | |
| 61429 | 1822.73 | 30,87 | 8,99E-06 | 0.009 | 7,83E-03 | 0,83 | 2,03 | 0,28 | 0,71 | | |
| 61087 | 1814.72 | 37,17 | 2,51E-05 | 0.020 | 2,19E-02 | 0,63 | 1,38 | 0,17 | 0,29 | | |
| 55582 | 1697.74 | 30,88 | 1,36E-06 | 0.002 | 1,19E-03 | 1 | 2,84 | 1 | 3,1 | NGAPGNDGAKGDAGA PGAPG | Collagen alpha-1 (I) chain |
| 53035 | 1651.79 | 40,66 | 1,03E-06 | 0.002 | 8,95E-04 | 1 | 3,24 | 1 | 3,55 | | |
| 46184 | 1556.74 | 40,03 | 1,04E-05 | 0.007 | 9,08E-03 | 1 | 2,45 | 0,55 | 1,31 | | |
| 38011 | 1426.64 | 22,42 | 3,83E-05 | 0.036 | 3,33E-02 | 0,67 | 1,48 | 0,17 | 0,37 | PPhGKNGDDGEAGKP hG | Collagen alpha-1 (I) chain [225-239] [Homo sapiens] |
| 31480 | 1312.55 | 29,77 | 3,05E-10 | 0.001 | 2,66E-07 | 1 | 3,37 | 1 | 2,9 | | |
| 29279 | 1276.4 | 35,92 | 2,68E-06 | 0.006 | 2,33E-03 | 1 | 3,7 | 1 | 3,43 | | |
| 26488 | 1232.53 | 38,04 | 5,41E-05 | 0.044 | 4,71E-02 | 0,8 | 1,75 | 0,34 | 0,69 | | |
| 24990 | 1210.39 | 36,48 | 8,97E-07 | 0.001 | 7,81E-04 | 0,87 | 2,11 | 0,28 | 0,63 | | |
| 24952 | 1209.53 | 39,65 | 6,77E-05 | 0.047 | 5,90E-02 | 0,63 | 1,2 | 0,17 | 0,28 | DGPAGAPGTPGPQG | Collagen alpha-1 (I) chain [940-953] [Homo sapiens] |
| 23518 | 1182.55 | 28,27 | 2,79E-05 | 0.031 | 2,43E-02 | 0,8 | 1,64 | 0,31 | 0,61 | | |
| 22835 | 1173.53 | 37,49 | 8,47E-07 | 0.001 | 7,38E-04 | 0,93 | 2,46 | 0,48 | 1,07 | | |
| 21972 | 1160.36 | 35,60 | 3,98E-08 | 0.001 | 3,47E-05 | 1 | 3,47 | 1 | 3,15 | | |

| | | | | | | | | | | | |
|-------|---------|-------|----------|-------|----------|------|------|------|------|------------------|---|
| 21747 | 1157.54 | 37,44 | 6,08E-05 | 0.022 | 5,29E-02 | 1 | 3,38 | 0,79 | 2,3 | GPPGPhPhGPhPGPPS | Collagen alpha-1 (I) chain [1178-1190] [Homo sapiens] |
| 20862 | 1143.52 | 36,97 | 2,35E-05 | 0.007 | 2,04E-02 | 1 | 2,78 | 0,72 | 1,71 | GPPhGPhPGPPGPPS | Collagen alpha-1 (I) chain [1181-1193] [Homo sapiens] |
| 20756 | 1141.54 | 37,33 | 5,53E-05 | 0.042 | 4,82E-02 | 0,9 | 2,39 | 0,52 | 1,22 | | |
| 20204 | 1137.51 | 26,43 | 2,19E-05 | 0.025 | 1,91E-02 | 0,8 | 1,7 | 0,38 | 0,65 | | |
| 19137 | 1118.49 | 37,76 | 7,27E-06 | 0.007 | 6,33E-03 | 0,7 | 1,51 | 0,21 | 0,35 | | |
| 18936 | 1114.47 | 36,77 | 8,71E-05 | 0.049 | 7,58E-02 | 0,53 | 1,1 | 0,07 | 0,15 | | |
| 18395 | 1107.51 | 28,87 | 3,46E-05 | 0.029 | 3,01E-02 | 0,13 | 0,2 | 0,59 | 1,11 | | |
| 18029 | 1100.5 | 37,04 | 3,11E-05 | 0.028 | 2,71E-02 | 0,93 | 2,3 | 0,59 | 1,22 | | |
| 17694 | 1096.48 | 26,08 | 1,56E-04 | 0.049 | 1,35E-01 | 1 | 3,89 | 0,72 | 2,59 | APhGDRGEPHGPPh | Collagen alpha-1 (I) chain [798-808] [Homo sapiens] |
| 16910 | 1083.46 | 26,78 | 1,08E-06 | 0.001 | 9,45E-04 | 0,73 | 1,47 | 0,21 | 0,32 | SPhGPDGKTGPPh | Collagen alpha-1 (I) chain [546-556] [Homo sapiens] |
| 15954 | 1070.49 | 36,49 | 1,09E-06 | 0.002 | 9,45E-04 | 0,8 | 1,9 | 0,28 | 0,55 | | |
| 14478 | 1040.47 | 39,23 | 7,38E-05 | 0.029 | 6,43E-02 | 1 | 2,47 | 0,55 | 1,38 | | |
| 13793 | 1026.44 | 35,41 | 2,11E-05 | 0.011 | 1,84E-02 | 0,57 | 1,04 | 0,1 | 0,12 | VLNLGPITR | Uromodulin [598-606] [Homo sapiens] |
| 11413 | 981.58 | 24,80 | 8,24E-05 | 0.034 | 7,18E-02 | 1 | 3 | 0,62 | 1,82 | | |

Supplementary table 2.

| Polypeptide | | | p-value | | | Diabetic Nephropathy (STENomacro, n=30) | | Normoalbuminuria (STENOnormo, n=30) | | Sequence | Protein | Regulation |
|---------------|----------------|---------------|-----------------|---------------|-----------------|---|-------------|-------------------------------------|-------------|---|---|-------------|
| ID | Mass [Da] | CE-Time [Min] | Unadj. | maxT | Bonf | Frequen cy | Amplitud e | Frequen cy | Amplitude | | | |
| 169424 | 4863,16 | 26,74 | 7,26E-06 | 0,0054 | 4,93E-03 | 0,13 | 0,32 | 0,69 | 1,81 | | | |
| 162644 | 4538,09 | 26,27 | 4,40E-06 | 0,0018 | 2,99E-03 | 0,03 | 0,07 | 0,59 | 1,27 | | | |
| 149872 | 4047,92 | 25,45 | 1,16E-05 | 0,0093 | 7,88E-03 | 0,27 | 0,57 | 0,76 | 1,86 | | | |
| 148717 | 4008,81 | 23,42 | 8,56E-07 | 0,0004 | 5,81E-04 | 0,6 | 2,12 | 0,03 | 0,06 | | | |
| 147541 | 3968,60 | 21,09 | 1,44E-05 | 0,0094 | 9,76E-03 | 0,47 | 1,27 | 0,9 | 2,83 | | | |
| 146936 | 3943,83 | 33,63 | 2,46E-06 | 0,0028 | 1,67E-03 | 0,27 | 0,47 | 0,79 | 1,75 | | | |
| 146624 | 3927,82 | 33,60 | 7,68E-06 | 0,0065 | 5,22E-03 | 0,13 | 0,33 | 0,72 | 1,59 | | | |
| 145793 | 3886,83 | 33,54 | 2,46E-05 | 0,0142 | 1,67E-02 | 0,1 | 0,2 | 0,62 | 1,3 | | | |
| 145456 | 3870,81 | 33,49 | 3,49E-06 | 0,0008 | 2,37E-03 | 0 | 0 | 0,55 | 1,15 | | | |
| 145238 | 3858,84 | 25,85 | 1,24E-05 | 0,0087 | 8,44E-03 | 0,43 | 1,23 | 0,9 | 2,8 | | | |
| 143652 | 3788,82 | 25,19 | 3,81E-05 | 0,023 | 2,59E-02 | 0,13 | 0,29 | 0,62 | 1,42 | | | |
| 142080 | 3734,72 | 32,50 | 3,59E-05 | 0,0247 | 2,44E-02 | 0,47 | 1,02 | 0,86 | 2,24 | | | |
| 135412 | 3510,60 | 40,24 | 1,37E-05 | 0,0093 | 9,29E-03 | 0,13 | 0,29 | 0,66 | 1,44 | | | |
| 132950 | 3425,60 | 31,27 | 2,53E-05 | 0,0058 | 1,72E-02 | 0,7 | 2,05 | 1 | 3,35 | | | |
| 132504 | 3409,62 | 31,93 | 7,63E-07 | 0,0012 | 5,18E-04 | 0,3 | 0,63 | 0,83 | 2,03 | DLPETGVWPPEPRTDPP QPPRPDDPWAGP | Psoriasis susceptibility 1 candidate gene 2 protein [75-105] [Homo sapiens] | down |
| 132057 | 3401,66 | 23,49 | 3,33E-05 | 0,0181 | 2,26E-02 | 0,5 | 1,39 | 0,93 | 2,79 | | | |
| 131294 | 3375,57 | 31,92 | 1,19E-05 | 0,0058 | 8,05E-03 | 0,67 | 1,65 | 0,97 | 2,94 | | | |
| 130747 | 3359,58 | 31,90 | 2,52E-05 | 0,0155 | 1,71E-02 | 0,63 | 1,59 | 0,93 | 2,92 | | | |
| 130243 | 3343,57 | 31,85 | 4,26E-05 | 0,0283 | 2,89E-02 | 0,27 | 0,54 | 0,69 | 1,8 | PhGADGQPGAKGEpGDA GAKGDAGpPGPAGPAGP PGPIG | Collagen alpha-1 (I) chain [819-854] [Homo sapiens] | down |
| 127899 | 3281,43 | 36,09 | 3,09E-05 | 0,0129 | 2,10E-02 | 0,57 | 1,62 | 0,97 | 2,99 | | | |
| 127351 | 3264,56 | 25,75 | 1,24E-06 | 0,0017 | 8,42E-04 | 0,33 | 0,92 | 0,86 | 2,61 | | | |
| 124172 | 3165,46 | 31,32 | 2,67E-05 | 0,02 | 1,82E-02 | 0,23 | 0,54 | 0,76 | 1,91 | | | |
| 122400 | 3108,45 | 31,28 | 1,20E-06 | 0,0012 | 8,14E-04 | 0,43 | 1,03 | 0,93 | 2,48 | ADGQPhGAKGEPHGDAG AKGDAGPhPGPAGPAGP PGPhIG | Collagen alpha-1 (I) chain [819-854] [Homo sapiens] | down |
| 121775 | 3092,46 | 31,25 | 2,11E-07 | 0,0003 | 1,43E-04 | 0,47 | 1,14 | 0,97 | 2,74 | | | |
| 121772 | 3092,44 | 36,30 | 3,12E-08 | 0,0003 | 2,12E-05 | 0,33 | 0,65 | 0,9 | 2,12 | | | |
| 119292 | 3035,19 | 42,02 | 9,65E-06 | 0,0082 | 6,55E-03 | 0,17 | 0,43 | 0,76 | 1,83 | | | |

| | | | | | | | | | | | | |
|---------------|----------------|--------------|-----------------|---------------|-----------------|-------------|-------------|-------------|-------------|---|--|-------------|
| 118788 | 3025,40 | 29,86 | 2,45E-05 | 0,0175 | 1,67E-02 | 0,2 | 0,43 | 0,72 | 1,72 | | | |
| 118597 | 3021,35 | 23,42 | 1,70E-05 | 0,0094 | 1,16E-02 | 0,57 | 1,57 | 0,93 | 3,07 | DGVSGGEGKGGSDGGG SHRKEGEEADAPGVIPG | | |
| 114823 | 2926,30 | 22,22 | 2,84E-06 | 0,0022 | 1,93E-03 | 0,1 | 0,22 | 0,66 | 1,58 | ESGREGAPGAEGSPPhGR DGSPPhGAKGDRGETGP | Collagen alpha-1 (I) chain [1011-1041] [Homo sapiens] | down |
| 111863 | 2849,27 | 23,34 | 1,30E-05 | 0,0073 | 8,83E-03 | 0,1 | 0,19 | 0,59 | 1,3 | | | |
| 111001 | 2825,27 | 24,49 | 8,04E-05 | 0,0441 | 5,46E-02 | 1 | 3,97 | 1 | 4,25 | ERGEAGIPhGVPhGAKGE DGKDGSPPhGEPPhGANG | Collagen alpha-1 (III) chain [448-477] [Homo sapiens] | down |
| 109752 | 2791,37 | 27,78 | 1,94E-06 | 0,0006 | 1,32E-03 | 0,57 | 1,52 | 0 | 0 | | | |
| 108021 | 2754,27 | 29,68 | 1,98E-06 | 0,0024 | 1,35E-03 | 0,47 | 0,99 | 0,9 | 2,35 | | | |
| 107571 | 2744,13 | 35,10 | 4,32E-06 | 0,0019 | 2,93E-03 | 0,03 | 0,07 | 0,59 | 1,19 | | | |
| 107460 | 2742,25 | 28,98 | 2,60E-07 | 0,0003 | 1,76E-04 | 0,57 | 1,48 | 1 | 3,16 | KNGETGPQGPPPhGPTGP GGDKGDTGPPPhGPQG | Collagen alpha-1 (III) chain [610-639] [Homo sapiens] | down |
| 106558 | 2724,27 | 23,72 | 1,94E-05 | 0,0052 | 1,31E-02 | 0,53 | 1,67 | 0,03 | 0,06 | | | |
| 106195 | 2716,36 | 20,19 | 1,06E-05 | 0,0042 | 7,23E-03 | 0,57 | 2,2 | 0,07 | 0,19 | LLKNGERIEKVEHSDLF SKDWS | Beta-2-microglobulin | up |
| 106067 | 2713,23 | 29,22 | 7,16E-06 | 0,0059 | 4,86E-03 | 0,17 | 0,35 | 0,69 | 1,52 | | | |
| 105105 | 2687,22 | 28,99 | 5,15E-05 | 0,0294 | 3,50E-02 | 0,17 | 0,31 | 0,62 | 1,32 | | | |
| 102392 | 2639,32 | 19,78 | 2,79E-06 | 0,0007 | 1,89E-03 | 0,57 | 2,04 | 0 | 0 | DAHKSEVAHRFKDLGEE NFKALV | Serum albumin; N-term. | up |
| 102164 | 2636,20 | 24,39 | 2,04E-07 | 0,0004 | 1,39E-04 | 0,1 | 0,19 | 0,72 | 1,57 | | | |
| 101081 | 2614,17 | 29,67 | 2,92E-05 | 0,0209 | 1,98E-02 | 0,17 | 0,32 | 0,69 | 1,24 | | | |
| 98485 | 2560,12 | 34,15 | 5,06E-06 | 0,0043 | 3,44E-03 | 0,17 | 0,3 | 0,72 | 1,51 | | | |
| 97349 | 2541,19 | 27,90 | 9,28E-05 | 0,0426 | 6,30E-02 | 0,07 | 0,14 | 0,55 | 1,08 | | | |
| 97301 | 2540,26 | 19,68 | 1,86E-05 | 0,0093 | 1,26E-02 | 0,63 | 2,37 | 0,17 | 0,45 | DAHKSEVAHRFKDLGEE NFKAL | Serum albumin; N-term. | up |
| 96370 | 2518,31 | 22,79 | 3,29E-06 | 0,0017 | 2,24E-03 | 0,6 | 2,22 | 0,07 | 0,2 | LmIEQNTKSPLFMGKVVN PTQK | Alpha-1-antitrypsin; C-term. | up |
| 95552 | 2502,31 | 22,73 | 3,58E-07 | 0,0003 | 2,43E-04 | 0,63 | 2,53 | 0,03 | 0,09 | | | |
| 94948 | 2487,13 | 28,27 | 2,76E-05 | 0,0158 | 1,87E-02 | 0,5 | 1,25 | 0,93 | 2,51 | | | |
| 94308 | 2471,16 | 34,77 | 1,60E-06 | 0,0017 | 1,09E-03 | 0,47 | 1 | 0,93 | 2,34 | TGPIGPPPhGPAGAPhGDK GESGSPGPAGPTG | Collagen alpha-1 (I) chain [766-794] [Homo sapiens] | down |
| 92841 | 2430,10 | 28,33 | 7,13E-05 | 0,0358 | 4,84E-02 | 0,57 | 1,3 | 0,93 | 2,4 | ADGQPGAKGEPPhGDAGA KGDAGPPPhGPAGP | Collagen alpha-1 (I) chain [819-846] [Homo sapiens] | down |
| 92698 | 2427,18 | 19,58 | 5,14E-06 | 0,0022 | 3,49E-03 | 0,63 | 2,28 | 0,14 | 0,26 | DAHKSEVAHRFKDLGEE NFKA | Serum albumin [25-45; N-term.] [Homo sapiens] | up |
| 92456 | 2423,32 | 21,08 | 2,18E-05 | 0,008 | 1,48E-02 | 0,53 | 1,91 | 0,07 | 0,16 | | | |
| 92410 | 2423,09 | 27,67 | 6,59E-06 | 0,0049 | 4,47E-03 | 0,57 | 1,23 | 0,93 | 2,51 | LDGAKGDAGPAGPKGEP GSPGENGAPG | Collagen alpha-1 (I) chain [273-299] [Homo sapiens] | down |
| 91542 | 2407,09 | 27,67 | 7,44E-06 | 0,005 | 5,05E-03 | 0,53 | 1,32 | 0,93 | 2,72 | | | |

| | | | | | | | | | | | | | |
|-------|---------|-------|----------|--------|----------|------|------|------|------|-------------------------------------|--|------|--|
| 91421 | 2405,22 | 22,47 | 1,24E-07 | 0,0003 | 8,42E-05 | 0,7 | 2,57 | 0,14 | 0,26 | | | | |
| 90840 | 2389,24 | 22,40 | 1,16E-08 | 0,0001 | 7,86E-06 | 0,73 | 3,01 | 0,07 | 0,17 | MIEQNTKSPLFMGKVVNP TQK | Alpha-1-antitrypsin [398-418; C-terminus] [Homo sapiens] | up | |
| 87223 | 2318,21 | 26,30 | 6,65E-05 | 0,0268 | 4,51E-02 | 0,53 | 1,81 | 0,1 | 0,24 | | | | |
| 86201 | 2302,20 | 26,13 | 1,13E-07 | 0,0002 | 7,64E-05 | 0,67 | 2,48 | 0,03 | 0,1 | | | | |
| 85627 | 2289,04 | 33,59 | 7,63E-05 | 0,0344 | 5,18E-02 | 0,07 | 0,13 | 0,55 | 1,06 | | | | |
| 85315 | 2281,98 | 33,93 | 1,53E-05 | 0,0101 | 1,04E-02 | 1 | 3,56 | 1 | 3,92 | ANGAPhGNDGAKGDAGA PhGAPhGSQGAPhG | Collagen alpha-1 (I) chain [699-725] [Homo sapiens] | down | |
| 84192 | 2258,19 | 22,09 | 2,48E-08 | 0,0001 | 1,69E-05 | 0,7 | 2,34 | 0,03 | 0,06 | IEQNTKSPLFMGKVVNP QK | serpin peptidase inhibitor, clade A | up | |
| 83823 | 2249,98 | 27,20 | 3,69E-05 | 0,0201 | 2,50E-02 | 0,6 | 1,61 | 0,14 | 0,3 | | | | |
| 81457 | 2216,03 | 33,83 | 7,07E-07 | 0,0006 | 4,80E-04 | 0,5 | 0,97 | 0,97 | 2,23 | | | | |
| 81424 | 2215,11 | 33,18 | 5,89E-06 | 0,0012 | 4,00E-03 | 0,53 | 1,96 | 0 | 0 | LRTLNPDSQLQLTTGNG LF | Alpha-1-antitrypsin | up | |
| 79816 | 2189,13 | 25,68 | 5,68E-07 | 0,0003 | 3,86E-04 | 0,6 | 2,29 | 0 | 0 | | | | |
| 79626 | 2185,98 | 25,88 | 5,41E-07 | 0,0003 | 3,68E-04 | 0,47 | 1,38 | 1 | 3,17 | | | | |
| 79590 | 2184,96 | 33,09 | 4,08E-05 | 0,0264 | 2,77E-02 | 0,5 | 1,04 | 0,93 | 2,23 | | | | |
| 76423 | 2117,96 | 27,71 | 4,18E-05 | 0,0175 | 2,84E-02 | 0,57 | 1,5 | 0,97 | 2,77 | | | | |
| 76415 | 2117,93 | 32,97 | 3,19E-06 | 0,0022 | 2,16E-03 | 0,1 | 0,19 | 0,66 | 1,54 | SNGNPhGPhPGPSGSPG KDGPPhGPhAG | Collagen alpha-1 (III) chain [886-909] [Homo sapiens] | down | |
| 72896 | 2055,94 | 25,44 | 1,06E-05 | 0,0028 | 7,20E-03 | 0,57 | 1,5 | 1 | 2,85 | | | | |
| 72343 | 2042,07 | 25,14 | 9,27E-07 | 0,0005 | 6,29E-04 | 0,63 | 2,34 | 0,07 | 0,14 | | | | |
| 72161 | 2039,13 | 21,78 | 2,61E-06 | 0,0032 | 1,77E-03 | 0,3 | 0,75 | 0,83 | 2,33 | SGSVIDQSRVNLGPITRK | Uromodulin [589-607] [Homo sapiens] | down | |
| 71029 | 2022,89 | 33,37 | 1,24E-06 | 0,0008 | 8,41E-04 | 0,07 | 0,12 | 0,66 | 1,36 | | | | |
| 70633 | 2013,89 | 31,76 | 1,03E-06 | 0,0012 | 7,00E-04 | 0,1 | 0,18 | 0,69 | 1,4 | AGPhGPPPGPhPhGTSG HPhGSPHGSPhG | Collagen alpha-1 (III) chain [176-198] [Homo sapiens] | down | |
| 69681 | 1989,88 | 32,44 | 2,24E-07 | 0,0004 | 1,52E-04 | 0,57 | 1,12 | 0,97 | 2,48 | | | | |
| 68670 | 1968,96 | 32,35 | 8,09E-06 | 0,0028 | 5,49E-03 | 0,57 | 1,59 | 0,07 | 0,1 | LLSPYSYSTTAVVTNPKE | Transthyretin (Prealbumin); C-term. | up | |
| 68316 | 1959,01 | 25,02 | 1,09E-05 | 0,0041 | 7,39E-03 | 0,57 | 1,96 | 0,07 | 0,15 | | | | |
| 68036 | 1952,91 | 32,22 | 4,57E-05 | 0,018 | 3,10E-02 | 0,1 | 0,13 | 0,55 | 1,17 | GEKGPPhSGEAGTAGPPhG TPhGPQG | Collagen alpha-2 (I) chain [844-865] [Homo sapiens] | down | |
| 67696 | 1945,00 | 33,71 | 1,55E-05 | 0,0096 | 1,05E-02 | 0,43 | 0,94 | 0,9 | 2,14 | | | | |
| 67632 | 1943,01 | 24,94 | 4,51E-05 | 0,0188 | 3,06E-02 | 0,57 | 2,12 | 0,1 | 0,29 | EAIPMSIPPEVKFNKPF | Alpha-1-antitrypsin [378-394] [Homo sapiens] | up | |
| 67386 | 1936,88 | 32,24 | 3,32E-06 | 0,0028 | 2,25E-03 | 0,53 | 1,16 | 0,93 | 2,47 | GEKGPSGEAGTAGPPhG TPhGPQG | Collagen alpha-2 (I) chain [844-865] [Homo sapiens] | down | |

| | | | | | | | | | | | | |
|--------------|----------------|--------------|-----------------|---------------|-----------------|-------------|-------------|-------------|-------------|-------------------------------------|---|-------------|
| 66483 | 1923,97 | 21,60 | 1,06E-05 | 0,0033 | 7,19E-03 | 0,53 | 1,67 | 0,03 | 0,07 | MGVVS LGSPSGEVSHPR KT | Alpha-2-HS-glycoprotein | up |
| 62135 | 1838,82 | 27,06 | 2,49E-06 | 0,0012 | 1,69E-03 | 0,03 | 0,08 | 0,62 | 1,23 | | | |
| 61446 | 1822,83 | 27,00 | 6,68E-06 | 0,0044 | 4,54E-03 | 0,1 | 0,2 | 0,62 | 1,35 | | | |
| 60871 | 1809,88 | 32,30 | 1,03E-05 | 0,0087 | 6,97E-03 | 0,27 | 0,52 | 0,79 | 1,79 | | | |
| 60126 | 1793,88 | 32,37 | 6,52E-06 | 0,0058 | 4,43E-03 | 0,4 | 0,84 | 0,86 | 2,12 | EEAPSLRPAPPPISGGGY | Fibronogen beta chain [54-71] [Homo sapiens] | down |
| 59745 | 1782,84 | 25,91 | 1,04E-06 | 0,0017 | 7,09E-04 | 0,2 | 0,5 | 0,76 | 2,04 | | | |
| 57531 | 1737,78 | 23,73 | 1,27E-04 | 0,0325 | 8,63E-02 | 0,93 | 3,18 | 0,97 | 3,68 | TGSPHGSPHGPDGKTGPP GPhAG | Collagen alpha-1 (I) chain [541- 560] [Homo sapiens] | down |
| 56884 | 1725,59 | 38,32 | 1,44E-07 | 0,0002 | 9,75E-05 | 0,57 | 1,48 | 1 | 3,23 | | | |
| 49958 | 1608,73 | 30,93 | 4,88E-06 | 0,0042 | 3,31E-03 | 0,23 | 0,46 | 0,72 | 1,9 | SGDSDDEPPPLPRL | Membrane associated progesterone receptor component 1 [53-67] [Homo sapiens] | down |
| 49948 | 1608,68 | 22,35 | 1,37E-06 | 0,0012 | 9,30E-04 | 0,07 | 0,13 | 0,62 | 1,35 | | | |
| 48751 | 1592,70 | 22,18 | 1,41E-05 | 0,0093 | 9,56E-03 | 0,17 | 0,37 | 0,66 | 1,66 | | | |
| 42833 | 1495,68 | 23,36 | 1,23E-05 | 0,0042 | 8,36E-03 | 0,03 | 0,06 | 0,52 | 1,09 | | | |
| 41514 | 1467,81 | 24,68 | 1,23E-05 | 0,0094 | 8,34E-03 | 0,3 | 0,7 | 0,79 | 2,32 | DQSRVLNLGPITR | Uromodulin [594-606] [Homo sapiens] | down |
| 38798 | 1438,67 | 27,88 | 8,29E-05 | 0,0466 | 5,63E-02 | 0,53 | 1,48 | 0,9 | 2,95 | GLPhGTGGPhPGENGKP hG | Collagen alpha-1 (III) chain [642-657] [Homo sapiens] | down |
| 33209 | 1339,60 | 27,49 | 3,88E-07 | 0,0006 | 2,64E-04 | 0,33 | 0,74 | 0,93 | 2,07 | | | |
| 27797 | 1252,63 | 21,55 | 3,60E-05 | 0,0163 | 2,44E-02 | 0,57 | 1,6 | 0,1 | 0,23 | | | |
| 18395 | 1107,51 | 28,87 | 3,90E-07 | 0,0003 | 2,65E-04 | 0 | 0 | 0,59 | 1,11 | | | |
| 17968 | 1099,49 | 28,24 | 7,81E-05 | 0,023 | 5,30E-02 | 0,7 | 1,71 | 1 | 2,74 | | | |
| 15216 | 1058,48 | 24,89 | 3,60E-05 | 0,0373 | 2,44E-02 | 0,77 | 2,42 | 0,41 | 1,19 | | | |
| 5913 | 912,52 | 20,06 | 6,04E-06 | 0,0017 | 4,10E-03 | 0,57 | 1,56 | 0,03 | 0,05 | | | |
| 4356 | 892,27 | 35,21 | 6,52E-05 | 0,041 | 4,42E-02 | 0,6 | 1,15 | 0,86 | 2,33 | | | |