Differentiating primary and secondary FSGS using non-invasive urine biomarkers

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BACKGROUND:

Focal segmental glomerulosclerosis (FSGS) is a descriptive renal histologic lesion with diverse causes and pathogenicities. FSGS includes primary (p) and secondary (s) forms. The subclasses differ in management and prognosis with differentiation often being challenging. We aimed to identify specific urine proteins/peptides significantly associated with pFSGS, distinguishing it from sFSGS, other chronic kidney disease (CKD) etiologies, and normal controls, and combining these using machine learning algorithm into a classifier.

METHODS:

Urine samples were collected in two different centers in Germany from CKD patients at the time of biopsy. Among these, 19 pFSGS and 44 sFSGS were identified based on biopsy assessment and clinical presentation. The urine samples were analysed using capillary electrophoresis coupled to mass spectrometry (CE-MS, **Figure 1**). For biomarker definition, urine samples of patients with other CKD etiologies from the above collection (CKD, n=100) were analysed. In addition, datasets of age/sex-matched normal controls with preserved kidney function (NC, n=98) were extracted from the urinary proteome database[1]. The characterization of the cohort used for biomarker discovery is showed in **Table 1**. Biomarker definition was performed in three steps as shown in **Figure 2**.

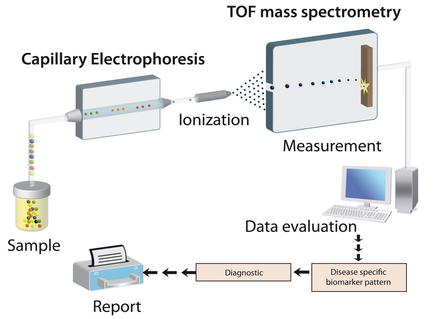

Figure 1: Schematic depiction of capillary electrophoresis coupled to mass spectrometer platform used for the analysis of peptides in urine. After electrophoretic separation, the peptides are ionized by application of high voltage and analyzed in the mass spectrometer.

Table 1: Characteristics of patients used for biomarker definition.

	primary FSGS n=19	secondary FSGS n=44	p-value	normal control n=98	p-value	CKD n=100	p-value
Sex, n male (%)	13 (68.4)	30 (68.2)	0.7824	73 (74.5)	0.7913	73 (73.0)	0.8972
Age (years)	47. [33.1-30.3]	57.5 [50.0-69.9]	0.044	45 [42.3-49.2]	0.6735	46.1 [42.3-49.4]	0.8052
BMI (kg/m2)	31.0 [27.2-33.3]	28.7 [27.1-30.4]	0.1493	na	na	na	na
BP syst. (mmHg)	140 [134-145]	140 [128-144]	0.7549	na	na	na	na
BP diast. (mmHg)	85 [78-90]	50 [75-85]	0.6499	na	na	na	na
eGFR (CKD-EPI) ml/min/1,73m²	56.0 [40.1-89.9]	31.1 [23.8-37.0]	0.0008	88.7 [77.5-107.4]	0.0054	40.7 [34.1-49.3]	0.0414
Uprot g/g Crea	8.03 [6.00-10.28]	2.56 [1.63-3.30]	<0.0001	0.012 [0.009-0.015]	<0.0001	2.00 [1.37-2.88]	<0.0001
IFTA (%)	7.5 [4.5-21.1]	21.3 [17.6-35.0]	0.0007	na	na	10.0 [5.0-15.0]	0.9469
No. Antihypertensives	3 [1-4]	3 [2-3]	0.5597	na	na	na	na
Diabetes, yes (%)	4 (21)	9 (20)	0.7754	24 (24)	0.978	15 (15)	0.75

RESULTS:

Biomarker definition and generation of the classifier

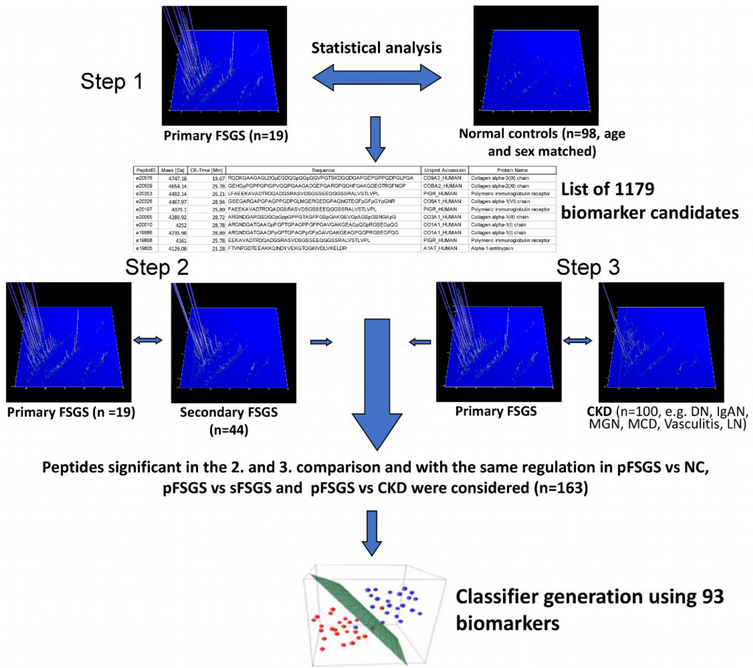

Figure 2: Definition of pFSGS specific biomarkers. pFSGS specific biomarkers were defined in 3 steps. In the first step, the CE-MS data of pFSGS were compared to NC. For further analysis only peptides with a p-value <0.05 (adjusted for multiple testing) were considered (n=1179). These potential biomarkers were investigated for significant differences and identical directional change (up- or downregulated) in two additional comparisons: pFSGS versus sFSGS, and pFSGS versus other CKD etiologies. This resulted in a final list of 163 pFSGS specific peptide biomarkers that were combined into a high-dimensional classifier using support vector machine. For training of the classifier pFSGS vs. sFSGS data were used. The classifier was optimized using a take-one-out procedure which resulted in exclusion of 70 peptides. The final classifier, pFSGS93, consisted of 93 peptides.

The statistical analysis performed in three steps (**Figure 2**) resulted in 163 biomarkers candidates. The generation of the classifier resulted in further reduction of the number of biomarkers to 93. These peptides were combined in the FSGS93 classifier. Defined biomarkers are at large fragments of different collagens (49%). Identified were also fragments of alpha-1-antitrypsin, apolipoprotein, complement C3, polymeric immunoglobulin receptor, uromodulin etc.

Classifier validation

Total cross validation of the pFSGS93 classifier resulted in discrimination between the pFSGS and sFSGS groups in an area under the curve (AUC) of the receiving operating characteristic (ROC) of 0.95 (**Figure 3A**). The diagnosis threshold of -0.001 defined by Youden index resulted in sensitivity of 84.2% and specificity of 100%.

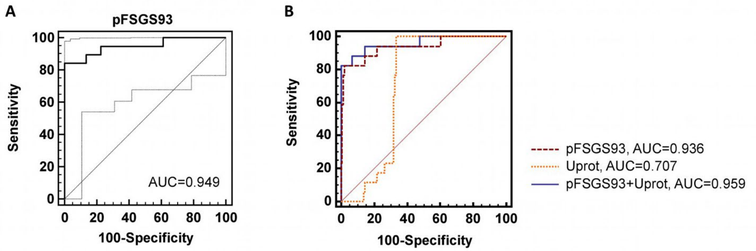

Figure 3: ROC-analysis of the cross validated training data (pFSGS vs. sFSGS) is shown in Figure A. Comparison of the ROC based on the FSGS93, proteinuria (Uprot) and nomogram of FGSG93 and proteinuria of the training data together with the 100 additional CKD patients.

Analysis of covariables and nomogram generation

Multiple regression was used to estimate whether additional parameters are associated with the diagnosis of pFSGS. Used were the data of pFSGS, sFSGS and additional 100 CKD patients. The following parameters were analysed: FSGS93, sex, age, proteinuria (Uprot), eGFR and IFTA. Only FSGS93 and Uprot remained significant. These two parameters were combined in a nomogram. The comparison of the ROC analysis is shown in **Figure 3B**. The pFSGS93 resulted in significant higher AUC than Uprot. The combination of pFSGS93 and Uprot resulted in significant highest AUC.

Specificity analysis in independent cohort

Independent specificity assessment was performed in additional data of NC (n=110) and CKD (n=170). For this purpose, data were extracted from the human urinary database[1]. Using the before defined cut-off of -0.001 only nine of the patients with other CKD etiologies (spec. 94.7%) and one of the NC (spec. 99.1%) were not correctly classified as no pFSGS.

CONCLUSIONS:

A urine peptide-based classifier that selectively detects pFSGS could be developed. Specificity of 95-99% could be assessed in independent samples. The data indicate that differentiation of pFSGS can be facilitated by urinary peptide analysis and our classifier can provide helpful information for therapeutic decisions where biopsy findings and clinical presentation are inconclusive.

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Disclosures

BH, IP, MW: Employees, Travere Therapeutics, Inc. **HM:** Founder and co-owner of Mosaiques Diagnostics GmbH; **JS:** Employee of Mosaiques Diagnostics GmbH; **RW, JB, LC, HR:** Nothing to disclose

References

1. Latosinska A et al, *Electrophoresis*. 2019 Sep;40(18-19):2294-2308.

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