The complexity of kidney disease and diagnosing it - Cystatin C, selective glomerular hypofiltration syndromes and proteome regulation

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Abstract

Estimation of kidney function is often part of daily clinical practice, mostly done by using the endogenous GFR-markers creatinine or cystatin C. A recommendation to use both markers in parallel in 2010 has resulted in new knowledge concerning the pathophysiology of kidney disorders by identification of a new set of kidney disorders, *selective glomerular hypofiltration syndromes*. These syndromes, connected to strong increases in mortality and morbidity, are characterised by a selective reduction in the glomerular filtration of 5-30 kDa molecules, such as cystatin C, compared to the filtration of small molecules < 1kDa dominating the glomerular filtrate e.g., water, urea, creatinine. At least two types of such disorders, shrunken or elongated pore syndrome, are possible according to the pore model for glomerular filtration. Selective glomerular hypofiltration syndromes are prevalent in investigated populations, and patients with these syndromes often display normal measured GFR or creatinine-based GFR-estimates. The syndromes are characterised by proteomic changes promoting the development of atherosclerosis, indicating antibodies and specific receptor-blocking substances as possible new treatment modalities. Presently, the KDIGO guidelines for diagnosing kidney disorders do not recommend cystatin C as a general marker of kidney function and will therefore not allow the identification of a considerable number of patients with selective glomerular hypofiltration syndromes. Furthermore, as cystatin C is uninfluenced by muscle mass, diet or variations in tubular secretion and cystatin C-based GFR-estimation equations do not require controversial race or sex terms, it is obvious that cystatin C should be a part of future KDIGO guidelines.

Keywords: Kidney disease, Proteomics

Introduction

Knowledge of kidney function, expressed as glomerular filtration rate (GFR), is pivotal in most clinical situations. Measuring GFR involves invasive and cumbersome procedures, and estimations of GFR (eGFR) based on the plasma or serum level of endogenous marker molecules are therefore widely applied in clinical practice. While creatinine has been used for this purpose since the late nineteen fifties, it was not until 1979 that studies of the plasma concentration of cystatin C, then called γ-trace, indicated that this protein might serve as an alternative to creatinine for estimation of GFR [1]. Early investigations of cystatin C as a marker of GFR, suggested that a raised plasma level, not only indicated a reduction in GFR, but also significant changes in the human proteome in patients with kidney disease [2]. Further studies of cystatin C have shown that the protein in many respects is superior to creatinine as a marker of GFR. In addition, recent investigations of cystatin C and kidney

disease have demonstrated that *mere* GFR-determination is insufficient to diagnose a significant part of kidney disorders and must be supplemented with measurements of the quality of the glomerular filtrate. These observations strongly urge the necessity to complement present recommendations for detecting kidney diseases (i.e. measurement, or estimation, of GFR, and determining the presence and level of albuminuria) with analysis of cystatin C to identify defects in the glomerular filtration process and the subsequent proteome changes in kidney disorders. This review describes those observations in the studies of cystatin C and kidney disorders, which prompt these conclusions.

Measuring and estimating glomerular filtration rate

The first proposal on how to measure GFR was by Rehberg, who almost a century ago, in 1926, suggested that endogenous creatinine clearance could be used for this purpose [3]. The plasma or serum level of creatinine has based upon this suggestion, been used as a marker of GFR since 1959 [4,5]. However, the use of creatinine clearance as a measure of GFR has its drawbacks, as shown by Shannon, who in 1935 demonstrated that a substantial part, generally about 30 %, of the creatinine excretion by the kidneys, occurs via tubular secretion [6]. Furthermore, the tubular secretion of creatinine may significantly and proportionally increase with a decrease in GFR, in addition to being strongly influenced by various medications. The limitation of creatinine clearance as a measure of GFR caused by the varying tubular secretion of creatinine is, of course, also valid for creatinine as a marker of GFR [7,8].

Thus, to develop a method for measuring GFR, uninfluenced by tubular function, Smith and Shannon used inulin [9,10], a mixture of fructose polymers with a heterogeneous molecular mass spectrum with molecules of about 5 kDa dominating the spectrum [11,12]. Inulin is excreted only by glomerular filtration and is not subject to tubular secretion or reabsorption [9,10]. When inulin is injected or infused intravenously, and its plasma concentration is measured in parallel with its excretion in urine, the calculation of GFR uninfluenced by kidney tubular function is possible [9,10]. The inulin clearance procedure for measuring GFR has become acknowledged as the “gold standard” for measuring GFR [7]. However, as the determination of inulin is complicated and inulin availability limited, several other substances supposedly not subjected to tubular secretion or reabsorption have been used for the determination of GFR, *e.g.,* ^{51}Cr-EDTA, ^{125}I-iothalamate, iohexol, and ^{99m}Tc-DTPA [7] with molecular masses between 0.344 and 0.821 kDa. Like inulin, they must be administered intravenously to measure GFR [7].

Since all procedures used to determine GFR are invasive, expensive, and time-consuming, in addition to conferring a certain risk for the patient, estimations of GFR based on endogenous markers of GFR are generally used in the clinical routine. Of these endogenous markers, creatinine and cystatin C dominate. Since their introduction as GFR-markers in 1959 [4] and 1985 [2] respectively, several thousand articles and reviews have been published to describe their usefulness in estimating GFR. The plasma or serum concentration of creatinine or cystatin C are often used as terms in creatinine- or cystatin C-based GFR-estimating equations resulting in GFR estimates designated $eGFR_{creatinine}$ or $eGFR_{cystatin\ C}$. To evaluate the diagnostic performance of these markers, estimated GFR is compared with measured GFR using intravenous injections of the substances described above. However, to interpret such comparisons correctly, one must observe that the recommended “gold standard”

procedures for measuring GFR differ in some important respects. For example, several of them use radioactive substances and are therefore not suitable for children and potentially pregnant women. Furthermore, one of the most used substances, iothalamate, is described to be partially excreted by tubular secretion [13,14]. To counteract these drawbacks, plasma clearance of iohexol was suggested as a simple way of measuring GFR in 1984 [15]. Since iohexol is not radioactive and not subjected to tubular excretion, it is suitable for measurement in both children and adults, including pregnant females, and has therefore recently been suggested as the method of choice for measuring GFR [16-18].

The mathematical basis for using the plasma or serum concentration, *c,* of a solute species to estimate GFR relies on the assumptions (i) that the solute is eliminated mainly via glomerular filtration and (ii) that its generation rate can be viewed as being constant during steady state, as follows

$$\frac{\mathrm{d}c}{\mathrm{dt}} = -\frac{\mathrm{GFR}}{\mathrm{V_D}}c + \frac{\mathrm{G}}{\mathrm{V_D}} = 0$$

Here G is the generation rate (mmol/min) and V_D is the distribution volume (l) of the solute species with concentration *c* (mmol/l). Re-arranging this equation gives the expression for GFR (ml/min) as a function of solute concentration as follows

$$\mathrm{GFR} = 1000 \cdot \frac{\mathrm{G}}{c}$$

Since the numerator, G, is constant, GFR can be estimated from *c*, with a higher concentration resulting in a lower GFR and *vice versa*.

Non-renal factors influencing the levels of creatinine or cystatin C

Several factors, unrelated to the GFR of a person, are known to influence the levels of creatinine and cystatin C, thus impairing their use for estimating GFR. This is also true for the different GFR-estimating equations based on creatinine or cystatin C and other parameters.

Muscle mass

In contrast to the cystatin C level, the creatinine level is strongly influenced by an individual's muscle mass, rendering creatinine, but not cystatin C, unsuitable for estimating GFR in patients with severe sarcopenia e.g., in paralytic or anorexic patients, or older patients with low muscle mass due to immobility [19-22]. The creatinine level in an individual with normal kidney function has been described to correlate with muscle mass, but not with the GFR of the individual, whereas the opposite is true for cystatin C [23]. As a matter of fact, the creatinine/cystatin C-ratio is useful for describing the muscle mass of different patient groups to identify sarcopenia and frailty associated with sarcopenia [24-26].

Dietary intake and renal reserve

Cooked meat with its high content of creatinine will increase the plasma level of creatinine, limiting the use of postprandial creatinine values for the estimation of GFR [27]. Ingestion of meat increases GFR in the healthy kidney, and a protein load is one way of determining kidney functional reserve, i.e. renal reserve [28]. Although the kidney functional reserve has been shown to be useful in the clinical assessment of the severity of kidney disorders [29], it has not been extensively used because of the requirement to measure GFR by invasive methods after a protein load [30]. Cooked meat does not contain any undenatured cystatin C and the increase in kidney function after such a meal is reflected in the cystatin C level, which will be reduced. This observation has been used to devise a simple way of estimating kidney functional reserve by measuring this reduction in the cystatin C level after a protein load [30,31].

Glucocorticoid treatment and hyper- or hypothyroidism

The cystatin C level is increased if patients are treated with moderate or high glucocorticoid doses due to increased production of the protein [32]. Hyper- and hypothyroidism both affect the levels of cystatin C and creatinine, but in opposite directions [33].

Inflammation

Contrary to previous suggestions, inflammation **does not** affect cystatin C level, as shown in studies of elective surgery in which a strong inflammatory reaction does not cause an increase in the cystatin C level [34-36]. The correlation between cystatin C and CRP seen in large populations [37] is not caused by inflammation *per se*, but plausibly because inflammation promotes the development of atherosclerosis producing a decline in GFR [38].

Cystatin C-based GFR-estimating equations do not require race- or sex-coefficients in contrast to creatinine-based equations

The strong influence of muscle mass on the level of creatinine and the variation in average muscle mass between males and females, and between some ethnic groups ("races"), has initiated the use of sex- and, since 1999 of race-coefficients in creatinine-based GFR-estimating equations [39]. More than ten different "race"-coefficients are now used in creatinine-based equations [40] even though "race" cannot be determined by biological testing and generally relies upon self-reporting, often associated with discomfort of the individual asked about his/her race [40]. Furthermore, race-adjusted formulas have often demonstrated falsely higher eGFR values for Afro-Americans than eGFR formulas for Caucasians resulting in misclassification of CKD (chronic kidney disease) and delayed care [41]. Self-reporting of sex is generally also used in health care and, since usually only two alternatives are allowed; some patients will experience discomfort when asked to self-report their sex [42,43]. This is, of course, connected to the recent acknowledgment of more than two genders in national legislation and issues pertaining to the LGBTQIA+-spectrum [41-43]. Both these sources of discomfort to patients can be avoided by the use of cystatin C-based GFR estimating equations since they do not require any race- or sex-coefficient [40,44,45].

Optimal estimation of GFR and prediction of morbidity and mortality

Since both cystatin C and creatinine are markers of GFR, several GFR-estimating equations containing *both* these parameters have been suggested and their diagnostic performances concerning the estimation of GFR are generally superior to those of equations based upon either marker alone [44,46-49]. However, since the non-renal factors influencing cystatin C or creatinine differ, the estimation of GFR is even better if $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$ are calculated separately and the average value used as an estimate of GFR after considering the potential non-renal influence of $eGFR_{creatinine}$ or $eGFR_{cystatin\ C}$ separately [50-57]. If a condition is identified in which either $eGFR_{creatinine}$ or $eGFR_{cystatin\ C}$ is disturbed by non-renal factors, the other estimation is used rather than the average value [50-53]. Although the ability of creatinine- or cystatin C-based GFR-estimating equations to estimate GFR is similar for most populations, the performance of the equations to predict morbidity and mortality differs significantly; with cystatin C-based estimations being much superior to creatinine-based estimations [58-66]. Naturally, substantial efforts have been made to identify the cause of this superiority. One proposal has been that cystatin C, or $eGFR_{cystatin\ C}$, is superior to creatinine, or $eGFR_{creatinine}$, in the estimation of measured GFR by invasive methods. However, recent careful studies have rejected this hypothesis [67]. Another proposal of why cystatin C or $eGFR_{cystatin\ C}$ is superior to creatinine and $eGFR_{creatinine}$ in predicting morbidity and mortality has been that inflammation leads to a raised level of cystatin C [37]. However, as described above, this hypothesis has been proven wrong [34-36]. Thus, we need to search elsewhere for an explanation of the association between cystatin C and morbidity and mortality (see below). It should also be noted that careful genetic studies have proven that the cystatin C protein itself does not promote the development of cardiovascular disease [68-70].

The missing piece of the puzzle – shrunken or elongated pore syndrome representing selective glomerular hypofiltration syndromes

As stated above the best way of estimating GFR is to calculate separate values for $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$ and then, in the absence of non-renal factors influencing the estimates, use the average value as the best GFR estimate [50-57]. This procedure has been used in different laboratories since 2010 [50] and it has then been observed that a significant number of patients display discordant values of $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$, although no non-renal cause for the discrepancies are found. In virtually all these cases, $eGFR_{cystatin\ C}$ was lower than $eGFR_{creatinine}$. These discrepancies soon led to the hypothesis of a set of kidney disorders in which the glomerular filtration of 5-30 kDa molecular mass is selectively decreased compared to that of the low molecular mass substances dominating in urine like water, 0.018 kDa, urea, 0.060 kDa, or creatinine, 0.113 kDa. Such kidney disorders could thus tentatively be labelled selective glomerular hypofiltration syndromes.

This mechanism was first suggested in an article published in 2015 showing that in patients with an $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio significantly below 1, and thus an increased cystatin C/creatinine-ratio, the ratios between several other 11 – 29 kDa proteins and creatinine were also increased [71]. According to the pore model for glomerular filtration [72,73], these observations could be explained by a shrinking (or elongation) of the pores [71]. Almost immediately after this observation the presence of shrunken pore syndrome and/or an $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio significantly below 1, was found to be strongly correlated to mortality, morbidity, heart failure and end-stage renal disease [63, 74-88]. Similar observations between an abnormally low $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio and morbidity have recently been described in an American study, although it used the difference between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ to identify a specific reduction in the excretion of 5-30 kDa molecules [89,90].

It should be noted that, according to the pore model for glomerular filtration [71-73],

a selective decrease in filtration of 5-30 kDa molecules will occur not only by a shrinking of the pores but also by elongation of the pores and this has been confirmed by structural studies of kidneys in patients with diabetic kidney disease [91]. In this investigation, the thickness of the glomerular basement membrane was inversely correlated with the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio, which is the expected result according to the pore model for glomerular filtration when the pore length is increased [91]. In line with these findings, previous experimental studies in a rat model of diabetic kidney disease also showed a lower diffusion capacity for polysucrose [92], indicating possible elongation of pores. However, in a similar more recent study, diabetic rats were shown to exhibit a smaller mean small-pore radius compared to control animals indicating shrunken pores [93].

Figure 1 illustrates how the identification of selective glomerular hypofiltration syndromes, e.g. shrunken or elongated pore syndromes, results in the recognition that the normal glomerular filtration process might be impaired in different ways.

Diagnosing selective glomerular hypofiltration syndromes

To diagnose selective glomerular hypofiltration syndromes resulting in a selective decrease in filtration of 5-30 kDa molecules, the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio is used [71]. Although no absolute cut-off in the ratio of $eGFR_{cystatin\ C}/eGFR_{creatinine}$ to diagnose selective glomerular hypofiltration

syndromes has been identified, the first-ever study of such a syndrome used a ratio <0.60 [71]. However, later investigations have shown that the mortality of patients with abnormally low $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio will start to increase already at a ratio of 0.90 and further increase as the ratio decreases [63,74,79]. To date, however, most studies have used an $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio of 0.70 or 0.60 to diagnose hypofiltration syndromes [63,71,74,75,77,87]. Possibly, no general cut-off should be used, so that the clinical setting at hand can be used to identify a suitable cut-off.

(Suggested site for FIGURE 1 here)

The risk of relying exclusively on the present KDIGO guidelines for diagnosing kidney disease

According to the international KDIGO guidelines [94], the main criteria for diagnosing chronic kidney disease are a decrease in measured or estimated GFR (stages G1-G5) and/or the presence and degree of albuminuria (stages A1-A3). However, several studies in healthy individuals [74,76], or patients without kidney disorders according to the KDIGO criteria, showed an increase in mortality and/or morbidity when the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio is significantly below 1.0, indicating a selective decrease in filtration of 5-30 kDa molecules in selective glomerular hypofiltration syndromes [63,74-76,78]. The prevalence of selective glomerular hypofiltration syndromes in different populations varies with the cut-off of the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio used for diagnosing the syndrome, but if a ratio <0.70 is used, the prevalence varies from 0.3 to 36% [63,71,74,76,87]. In population-based studies, there is a variation between 8 and 17% at an eGFRcystatin C/eGFRcreatinine-ratio <0.7 [74,87]. This means that if only creatinine-based GFR-estimating equations are used, a significant number of patients with severe kidney disorders will be missed, which may infer serious health implications.

Selective glomerular hypofiltration syndromes and the plasma proteome

The glomerular filtration of molecules of various sizes is characterized by a specific sieving coefficient for each molecule, describing the ratio of the concentration of the molecule in blood plasma and the primary urine (Fig. 2). Small molecules like water, urea, and creatinine have sieving coefficients of about 1.0 whereas bigger molecules like IgG (~160 kDa) have a sieving coefficient of about 0.00004 in humans [95]. The sieving coefficient will generally be lower the bigger the molecule. For example, the sieving coefficients for protein molecules of about 11 kDa are about 0.9 and for proteins of about 30 kDa about 0.08 [95]. Although a sieving coefficient of 0.08 is markedly lower than those for small molecules, the high production of primary urine, 150-200 litres per day, means that proteins up to 30-40 kDa will still be mainly eliminated by glomerular filtration and subsequent degradation in the tubules. For molecules above 40 kDa, the role of glomerular filtration as a significant clearance route will be smaller and successively diminish with the size of the molecule.

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About 36% of the proteins of the total human proteome have a molecular mass below 30 kDa [98,99], and a similar value has been described for the plasma proteome [100]. This means that the glomerular filtration process plays a decisive role in regulating the plasma proteome. It should be observed that the glomerular filtration process will not only play a role in regulating the levels of the regular plasma proteins below 30 kDa but also of the levels of the proteins below 30 kDa leaking from damaged tissues and used for diagnostic purposes, e.g. as markers for acute myocardial infarction.

The proposal that an $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio below 1.0 might signify a selective decrease in the filtration of 5-30 kDa molecules is supported by three previous invasive studies in humans between 1950 and 2001 [101-104] and one in rats from 2006 [105]. These studies show that the glomerular filtration of 5–30 kDa molecules can be decreased, although the filtration of low molecular mass substances (<0.9 kDa) like those used to measure GFR, is normal. Thus, selective glomerular hypofiltration syndromes, e.g. shrunken or elongated pore syndrome, characterized by a decrease in glomerular filtration of 5-30 kDa molecules, will have significant implications for a large part of the humane proteome and the recognition of such a syndrome opens for new and exciting treatment modalities.

Changes in glomerular filtration quality must be identified to characterise the proteome changes involved in the pathophysiology of kidney disorders

The plasma proteome known today comprises 5877 proteins (4395 canonical and 1482 additional nonredundant proteins) [106], which is a major part of the total human proteome of 18357 proteins, representing 92.8% the 19778 proteins predicted from the human genome [107]. If about 36% of the human plasma proteome is eliminated by glomerular filtration and subsequent tubular degradation, changes in the glomerular filtration process, e.g., a selective reduction of the filtration of 5-30 kDa proteins, will evidently cause major changes in the plasma proteome. The change in the proteome is therefore dependent upon the spectrum of substances filtered by the kidneys and this spectrum thus must be characterised by evaluation of the glomerular filtration quality [108]. The spectrum of substances filtered by healthy kidneys represents a normal and optimal filtration quality [108].

The fact that a decrease in the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio is associated with increased mortality and morbidity in patients with normal measured GFR and the absence of non-renal influences on $eGFR_{cystatin\ C}$ or $eGFR_{creatinine}$, shows that a panel of markers is required to characterise the glomerular filtration quality of the patients [63,71,76,77,82,109]. Although the parallel use since 2010 [50] of creatinine and cystatin C to characterise kidney disorders has demonstrated the requirement to characterise the glomerular filtration quality, it is obvious that an improved characterisation of the filtration quality will require the use of an increased number of marker substances. It would, e.g., be interesting to study the clinical gain by use of an expanded panel of markers with some substances of differing molecular size, in addition to cystatin C and creatinine, and some substances of equal size but with different isoelectric points to identify aberrations in the charge selective properties of the glomerular capillary wall [110,111].

What do GFR, mGFR, $eGFR_{creatinine}$, $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}/eGFR_{cystatin\ C}$ ratio represent?

GFR is defined as the volume of primary urine produced per unit of time e.g., ml/min [9]. It is a theoretical concept as, at present, it is impossible to measure directly (e.g. by cannulation of all of the about 2 million nephrons in the kidneys). However, since small molecules can freely pass through the renal filter, the clearance of a small substance — that is, the volume of plasma cleared from the substance per unit time (ml/min) — is approximately equivalent to GFR.

A practical, operational definition of GFR is the plasma-to-urine clearance of a marker that can be (i) accurately measured in both plasma and urine and is (ii) freely filtered across the glomerular filter, (iii) neither reabsorbed nor secreted by the tubules and (iv) does not itself affect GFR. Herein, GFR quantified according to this definition will be referred to as measured GFR (mGFR) [9]. Already in 1935, Shannon & Smith proposed the use of renal clearance of inulin to measure GFR [10]. Inulin is a mixture of polymers of fructose units with a heterogeneous molecular mass spectrum with molecules of about 5 kDa dominating the spectrum [11,12].

$eGFR_{creatinine}$ represents the mGFR estimated from the plasma level of creatinine with a molecular mass of 0.113 kDa. As this mass is close to that of the dominating constituent of primary urine, water, 0.018kDa, its clearance across the kidney filter should be close to the theoretical GFR of both the healthy kidney represented by **A** in figure 1 and the three types of filtration defects (**B**-**D**) of figure 1 with **C** and **D** representing selective glomerular hypofiltration syndromes, e.g. shrunken or elongated pore syndrome, with selectively reduced filtration of 5-30 kDa molecules. $eGFR_{creatinine}$ cannot differentiate between the three types of filtration defects in **B**-**D**, and neither can it identify selective glomerular hypofiltration syndromes if mGFR is normal. This means that selectively glomerular hypofiltration syndromes cannot be detected by the use of creatinine alone.

$eGFR_{cystatin\ C}$ represents the estimation of mGFR based on the plasma level of cystatin C having a molecular mass of 13.3 kDa. When the sieving curve of the patient is normal (Fig. 2) e.g. in **A** and **B** of figure 1, $eGFR_{cystatin\ C}$ will be close to the theoretical GFR, but not in selective glomerular hypofiltration syndromes (**C** and **D** of figure 1) with the abnormal filtration curves of figure 2. In these cases, $eGFR_{cystatin\ C}$ will represent the functional glomerular filtration rate of 5-30 kDa molecules.

The $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio will represent the relative decrease of the functional glomerular filtration rate of 5-30 kDa molecules compared to the (decrease of) glomerular filtration of small molecules e.g. water. The ratio will therefore be close to 1 in the **A** and **B** cases of figure 1, for which the filtration curves are normal, whereas the ratio will be significantly decreased in cases **C** and **D** of figure 1, in which the filtration curves are abnormal in the ways shown in figure 2.

The pathophysiological role of proteome changes in selective glomerular hypofiltration syndromes

Until today, relatively few investigations of the complex link between glomerular filtration and the plasma proteome have been undertaken. A study of how invasively measured GFR influences the human plasma proteome comprised 2893 proteins and showed that the levels of 678 of them correlated negatively with measured GFR with the level of cystatin C showing the strongest correlation [109]. So, in this investigation at least 23% of the studied part of the plasma proteome was affected by the decrease in GFR [109], which is compatible with the suggestion that up to 36% of the proteins in the plasma proteome are influenced by the glomerular filtration process [98,99]. Another study, using creatinine-based GFR-estimation equations, showed that the concentrations of 57 of 993 investigated proteins correlated with eGFR, again with cystatin C showing the highest correlation [112]. Although this study was unable to identify a significant number of patients with decreased measured GFR and none of the patients with shrunken or elongated pore syndrome with normal GFR, it nevertheless showed that at least 6% of the proteome is affected by the glomerular filtration process. Moreover, in a previous study, the plasma levels of 177 proteins were measured in four groups of patients with measured GFR [78], the proteomes of patients with normal GFR with or without shrunken pore syndrome and patients with reduced GFR with or without shrunken pore syndrome were studied. Raised levels of 28 proteins were specific for patients with shrunken pore syndrome and normal GFR, and 17 of these proteins concerned proteins described as promoting, or being associated with, atherosclerosis [78]. For the 28 proteins specific for shrunken pore syndrome a correlation between protein size and increased levels was observed, with smaller proteins being associated with higher levels probably because of their higher sieving coefficients [78]. Furthermore, another recent proteomic study of a population of 300 patients with heart failure investigated the plasma levels of 92 proteins as well as the presence of shrunken pore syndrome [82]. Increased levels of 6 proteins were specific for shrunken pore syndrome and 5 of them have been described to be linked to the development of atherosclerosis [82]. Taken together, a total of 66 proteins have been associated with shrunken pore syndrome, with or without reduced GFR, and 32 of them are associated with the development of atherosclerosis [78,82] (Table 1). These findings may be one explanation for the close relationship between CKD and cardiovascular disease, usually referred to as a cardiorenal syndrome. Figure 3 shows protein-protein associations between proteins so far known to be associated with selective glomerular hypofiltration syndromes. Of note, however, is that the two studies investigating proteomic changes in shrunken pore syndrome used different biomarker panels and only three of the investigated proteins overlapped in the different panels. Therefore, caution is needed when interpreting these results. While selective glomerular hypofiltration syndromes undoubtedly are associated with proteomic changes, it should also be noted that reduced GFR without selective glomerular hypofiltration syndromes is likewise associated with major changes in the plasma proteome [2,78,109]. Conventional renal replacement therapy cannot generally be used to correct the proteome deviations in most kidney disorders affecting GFR [113,114] as the elimination of larger molecules is very limited.

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Selective glomerular hypofiltration syndromes in pregnancy and childhood

Investigations of 5–30 kDa proteins and small molecules <1 kDa, including creatinine and urea, in pregnant women, demonstrated, about 13 years before selective glomerular hypofiltration

syndromes were identified, that a selective decrease in the glomerular elimination of 5–30 kDa proteins occurred in the last trimester of all pregnancies [115,116]. It was also noted that the decrease in the elimination of 5–30 kDa proteins was significantly greater in pre-eclampsia than in normal pregnancy and that this observation could be used to diagnose the condition as well as for optimal timing of delivery in patients with pre-eclampsia [117-120]. About 2 months after delivery, the elimination of 5–30 kDa proteins returned to normal, with normal plasma levels of such proteins, indicating that the pathophysiological process of shrunken pore syndrome in this instance is reversible [121]. However, the long-term effect of a severe decrease in the elimination of 5–30 kDa proteins during pregnancy is yet to be investigated. Correspondingly, the constellation of a decreased $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio in association with higher levels of beta-trace protein (23 kDa) has been demonstrated in paediatric populations, fitting with shrunken or elongated pore syndrome [122,123]. So far, no reports about an increased child- or adulthood morbidity or mortality as a consequence of the syndrome have been published.

Future treatment options

Identification of selective glomerular hypofiltration syndromes and elucidation of the resulting major changes in the human plasma proteome opens new types of treatments in kidney disorders. The so far identified proteome changes in selective glomerular hypofiltration syndromes concern raised concentrations of many peptides or proteins, some of which promote the development of atherosclerosis and cardiovascular disorders [78,82] (Table 1). Similarly, patients with reduced GFR, without selective glomerular hypofiltration syndromes, also show specific proteomic changes compared to persons with normal GFR and some of these changes may contribute to the increase in cardiovascular disorders generally associated with reduced GFR ("cardiorenal syndrome") [78]. This is an interesting field for future studies, and if further studies will identify the signalling proteins and peptides most pivotal for the development of, for example, cardiovascular disorders, the levels of these could be reduced by the use of monoclonal antibodies or their effects inhibited by the use of suitable receptor antagonists.

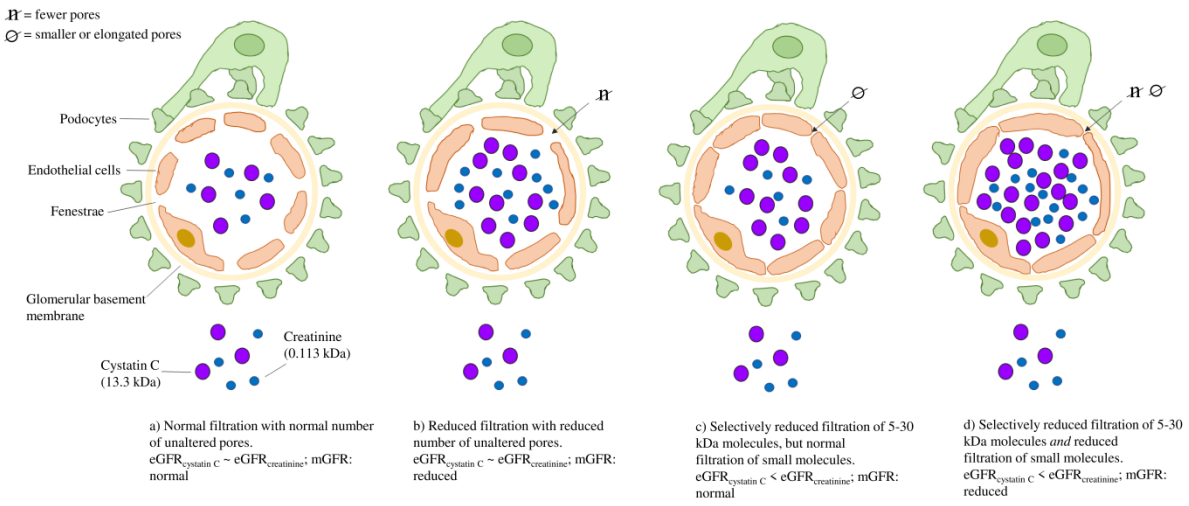
Figure legends

Figure 1

Three different types of glomerular filtration defects are based on the pore model. The discovery of selective glomerular hypofiltration syndromes with selectively reduced filtration of 5-30 kDa molecules, *e.g.* shrunken or elongated pore syndrome, means that different classes of filtration defects can be defined as schematically illustrated above. **A** represents normal filtration. **B** represents reduced filtration caused by the loss of unaltered pores. **C** represents selectively reduced

filtration of 5-30 kDa molecules in *e.g.* shrunken or elongated pore syndrome, but normal filtration of small molecules. **D** represents reduced filtration of all types of molecules, but a more severe reduction of the filtration of 5-30 kDa molecules. **C** and **D** represent selective glomerular hypofiltration syndromes, which are associated with higher morbidity and mortality than the type of filtration defect described in **B** in which no selective reduction of filtration of 5-30 kDa molecules occurs.

Variations in the endothelial fenestrae are used to illustrate the variation in the filtration process in the pore model *e.g.* representing shrunken or elongated pores (selective glomerular hypofiltration syndromes).



mGFR = Measured GFR; $eGFR_{cystatin\ C}$ = Cystatin C-based estimation of GFR; $eGFR_{creatinine}$ = Creatinine-based estimation of GFR

Figure 2

Glomerular sieving coefficients (θ) versus Stokes-Einstein radii for a few proteins and other substances (data from [95] and [96]) θ for insulin and cystatin C have been estimated from theory [73] and [97]. The solid blue line represents a theoretically predicted sieving curve for proteins [73]. The red solid line and yellow dashed line are simulated scenarios with elongated or shrunken pores, respectively. A longer pore has little effect on proteins larger than 3 nm (> 40 kDa). In contrast, a

smaller (shrunken) pore would have a significant impact on proteins larger than 3 nm (but smaller than the pore size of 3.7 nm/70 kDa). For a more detailed analysis see [90]. HRP - horse radish peroxidase; FLC - human myeloma-free light chain.

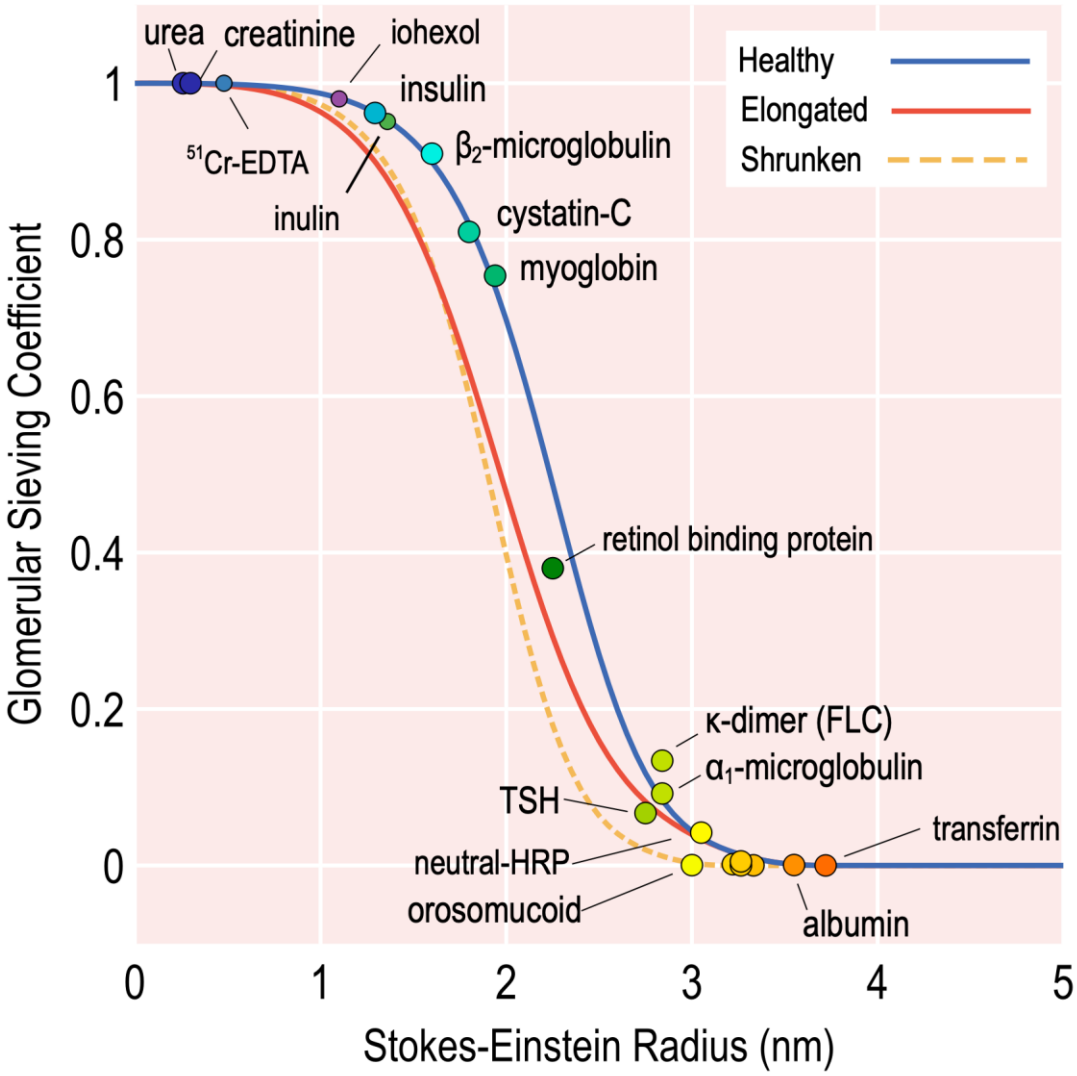


Figure 3

Protein-protein associations between currently identified proteins are known to be associated with selective glomerular hypofiltration syndromes.

Nodes represent proteins

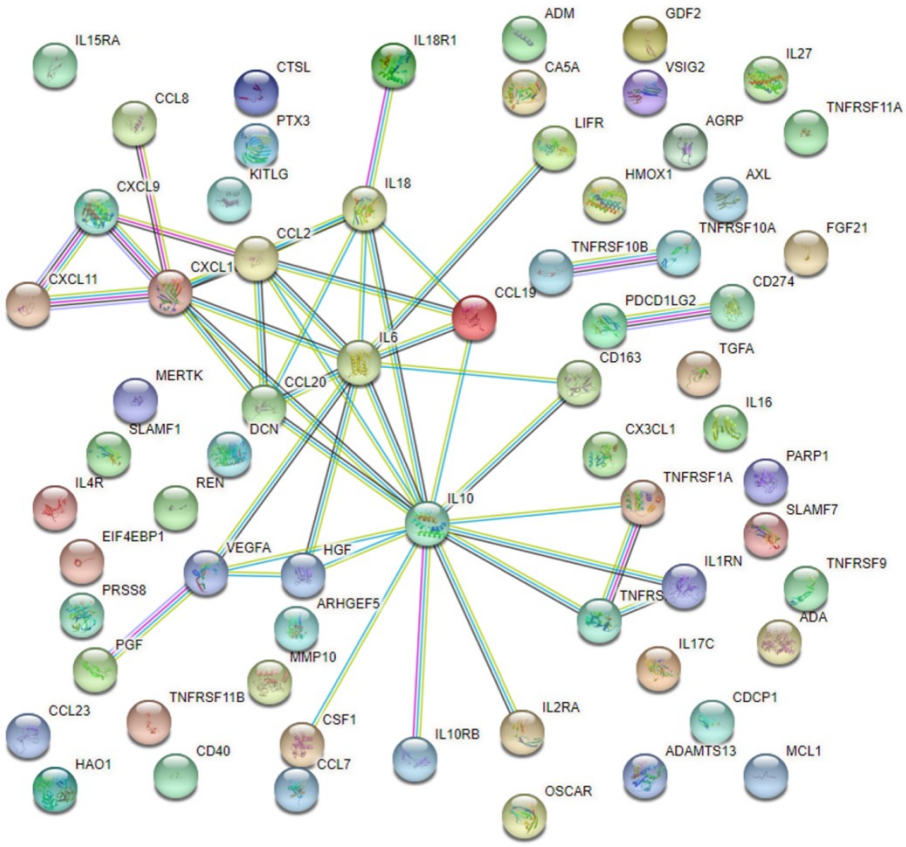
Lines represent protein-protein interactions

→ Known interactions: Light blue: from curated databases, Purple: experimentally determined

→ Predicted interactions: Green: gene neighbourhood, Red: gene fusion, Dark blue: gene co-occurrence

→ Others: Yellow: text mining, Black: co-expression, Blue: protein homology

Pathway analysis was conducted using STRING: functional protein association networks (string-db.org). Accessed 2022-08-09



Conflict of interest statement

No conflicts of interest were declared.

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Table 1. Proteins known to be associated with shrunken or elongated pore syndrome

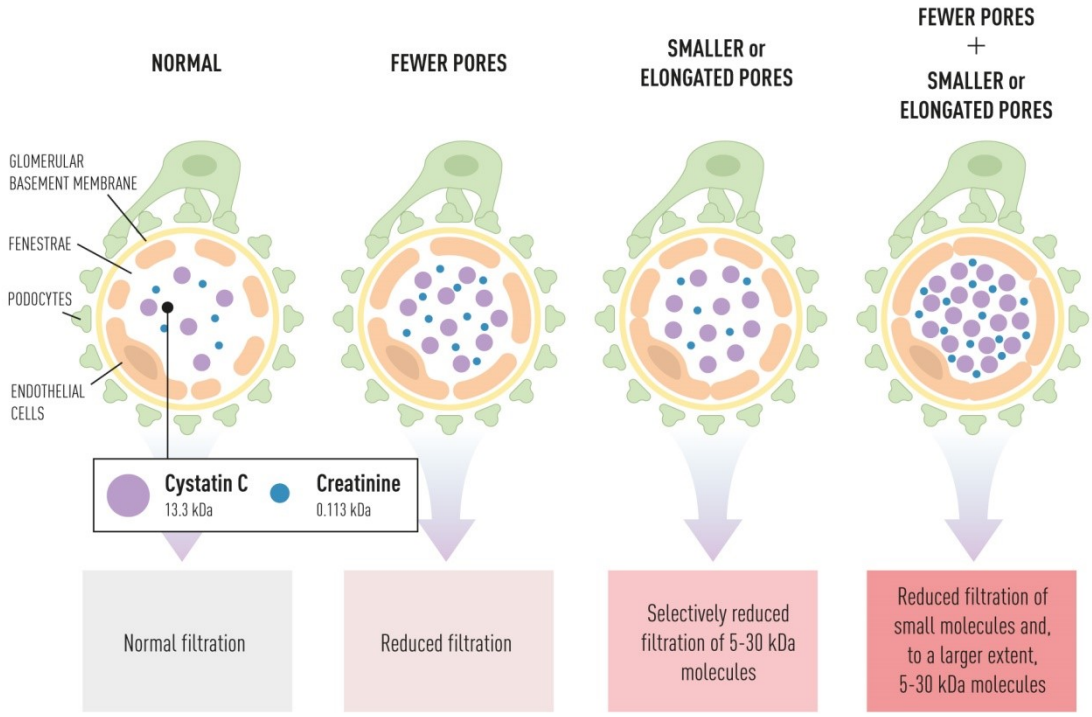
	Protein	**Full protein name**
1	CD163*	Scavenger receptor cysteine-rich type 1 protein M130
2	TNFRSF1A*	Tumor necrosis factor receptor superfamily member 1A
3	TNFRSF1B*	Tumor necrosis factor receptor superfamily member 1B
4	OPG*	Tumor necrosis factor receptor superfamily member 11B
5	IL2RA*	Interleukin-2 receptor subunit alpha
6	AXL	Tyrosine-protein kinase receptor UFO
7	MCP-3*	Monocyte chemotactic protein–3
8	CDCP1	CUB domain-containing protein 1
9	ADAM-TS13*	A disintegrin and metalloproteinase with thrombospondin motifs 13
10	IL-4RA	Interleukin-4 receptor subunit a
11	IL-1ra*	Interleukin-1 receptor antagonist protein
12	IL-6*	Interleukin-6
13	IL-17C*	Interleukin-17C
14	MCP-1*	Monocyte chemoattractant protein–1

15	CXCL11*	C-X-C motif chemokine 11
16	IL-18*	Interleukin-18
17	FGF-21	Fibroblast growth factor 21
18	TGFA	Protransforming growth factor a
19	CCL19*	C-C motif chemokine 19
20	IL-18R1*	Interleukin-18 receptor 1
21	PD-L1*	Programmed cell death 1 ligand 1
22	HGF*	Hepatocyte growth factor
23	HO-1	Heme oxygenase 1
24	IL-10	Interleukin-10
25	PTX3*	Pentraxin 3
26	CXCL10*	C-X-C motif chemokine 10
27	4E-BP1*	Eukaryotic translation initiation factor 4E-binding protein 1
28	GDF-2	Growth/differentiation factor 2
29	MCP-2	C-C motif chemokine 8
30	CTSL1*	Cathepsin L1
31	CA5A	Carbonic anhydrase 5A, mitochondrial
32	CCL20*	C-C motif chemokine 20
33	ADA	Adenosine deaminase
34	PARP-1	Poly [ADP-ribose] polymerase 1
35	HAOX1	Hydroxyacid oxidase 1
36	VEGF-A	Vascular endothelial growth factor A
37	ADM*	Adrenomedullin
38	PlGF*	Placenta growth factor
39	TNFRSF10A	Tumor necrosis factor receptor superfamily member 10A
40	TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A
41	TRAIL-R2*	Tumor necrosis factor-related apoptosis-inducing ligand receptor 2
42	CXCL9	C-X-C motif chemokine 9
43	IL27*	Interleukin 27
44	SCF	Kit ligand
45	SLAMF1	Signaling lymphocytic activation molecule
46	LIF-R	Leukemia inhibitory factor receptor
47	IL-15RA	Interleukin-15 receptor subunit a

48	IL-10RB	Interleukin-10 receptor subunit b
49	REN*	Renin
50	MERTK	Tyrosine-protein kinase Mer
51	TIM	Hepatitis A virus cellular receptor 1
52	TM*	Thrombomodulin
53	VSIG2	V-set and Ig domain-containing protein 2
54	IL16	Pro-interleukin-16
55	MMP-10*	Matrix metalloproteinase 10
56	CCL23*	C-C motif chemokine 23
57	PRSS8	Prostasin
58	AGRP	Agouti-related protein
59	CD40*	Tumor necrosis factor receptor superfamily member 5
60	PD-L2	Programmed cell death 1 ligand 2
61	CX3CL1*	Fractalkine
62	hOSCAR*	Osteoclast-associated Ig-like receptor
63	TNFRSF9*	Tumor necrosis factor receptor superfamily member 9
64	CSF-1	Macrophage colony-stimulating factor 1
65	DCN	Decorin
66	SLAMF7	SLAM family member 7

*Proteins previously associated with arteriosclerosis, as reported in the studies by Sällman-Almén et al (73) and Xhakollari et al (77), have been marked with an asterisk.

The complexity of kidney disease and diagnosing it - Cystatin C, selective glomerular hypofiltration syndromes and proteome regulation



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