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Gene expression profiling in the remnant kidney model of wild type and kinin B1 and B2 receptor knockout mice

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Angiotensin-converting enzyme inhibitors are the most efficient pharmacologic agents to delay the development of end-stage renal disease (ESRD). This is a multipharmacologic approach that inhibits angiotensin II formation while increasing kinin concentrations. Considerable attention has been focused on the role of decreased angiotensin II levels; however, the role of increased kinin levels is gaining in interest. Kinins affect cellular physiology by interacting with one of two receptors being the more inducible B1 and the more constitutive B2 receptors. This study utilizes the mouse remnant kidney of 20 weeks duration as a model of ESRD. Whole mouse genome microarrays were used to evaluate gene expression in the remnant kidneys of wild type, B1 and B2 receptor knockout animals. The microarray data indicate that gene families involved in vascular damage, inflammation, fibrosis, and proteinuria were upregulated, whereas gene families involved in cell growth, metabolism, lipid, and protein biosynthesis were downregulated in the remnant kidneys. Interestingly, the microarray analyses coupled to histological evaluations are suggestive of a possible protective role of kinins operating through the B2 receptor subtype in this model of renal disease. The results highlight the potential of microarray technology for unraveling complex mechanisms contributing to chronic renal failure.

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The prevalence and incidence of end-stage renal disease (ESRD) has dramatically increased over the last decade.[1] Regardless of the underlying etiology, chronic renal insufficiency is accompanied by a permanent loss of functional nephrons leading to a progressive loss of renal function.[1] The remnant kidney model (subtotal or 5/6 nephrectomy (SNX)) is an experimental animal model of chronic renal disease progression in which the development of segmental glomerulosclerosis and tubulointerstitial fibrosis are both clearly established after 20 weeks following SNX.[2]

It is now well admitted that angiotensin-converting enzyme inhibitors (ACEi) are the most efficient drugs to delay ESRD development.[3] ACE inhibition decreases angiotensin II (vasoconstrictor peptide) and increases kinin (vasodilator peptides) concentrations.[4] The beneficial effects of ACE inhibitors have been well determined; kinin implications are less clear, although in several studies a role for kinins was proposed.[5,6] Kinins execute their physiological function through the activation of two types of kinin receptors, the B_1 (B_1R) which is overexpressed in pathological states and the B_2 (B_2R) which is constitutively expressed.[7] A number of reports have shown involvement of kinin receptors, as components of the renal kallikrein–kinin system, in disease states that lead to renal failure (reviewed in Leeb-Lundberg *et al.*[8]). We have recently demonstrated that a number of genes relevant to renal physiology/pathology were differentially expressed in B_1R-knockout (B_1R-KO) and B_2R-knockout (B_2R-KO) mice, which is indicative for the important role of both kinin receptors in renal function.[9]

To date, kidney disease-oriented research has focused on mechanisms responsible for ESRD initiation and progression. However, because ESRD is a complex disease, interruption of a single pathway is unlikely to result in significant therapeutic benefit. Further understanding of the pathogenesis of renal failure and the development of new therapies will thus require global expression analysis of disease states using genomics and/or proteomics tools.

In this study, we examined changes of gene expression in 20-week remnant kidneys of SNX wild-type (SNX-WT) mice, SNX-B_1R-KO mice, and SNX-B_2R-KO mice to comprehend

molecular mechanisms of advanced renal disease better and possible implications of kinin receptors in renal failure. Numerous genes that encode proteins previously implicated in ESRD displayed drastic altered expression in 20-week remnant WT kidneys. However, and importantly, we observed strong variations in a number of genes and gene families (cytochrome P450 (CYPs), major urinary proteins (MUPs), serpins, glutathione S-transferase (GSTs), and enzymes of the urea cycle) and a novel pathway (linked with hepatocyte nuclear factor 4-alpha (Hnf4a) signaling) not previously linked with renal disease progression. The altered expression of some of these genes is also associated with possible protective and compensatory mechanisms. Additionally, our results are indicative for implications of kinin receptors, and especially the kinin B_2R subtype, in chronic renal disease.

RESULTS

Blood pressure measurements and biochemical analyses

As expected with mice on a C57BL6 genetic background, we found that our WT- and KO-SNX mice are rather resistant to glomerulosclerosis, proteinuria, and hypertension.[2,10] Indeed all animals survived 20 weeks of SNX. Consistent with previously described data,[11,12] no significant change in blood pressure was observed along the study period in the SNX-WT and SNX-KO animals (Figure 1a). However, the biochemical analyses performed on urine and blood samples collected before killing of all SNX mice were confirmative for ESRD progression (Figure 1b). Serum creatinine was significantly increased and urinary creatinine was significantly decreased in SNX animals compared with control animals. Proteinuria was not significantly different between WT and SNX animals, but a tendency was observed. No significant difference was observed in the biochemical parameters between SNX-WT and SNX-KO animals (Figure 1b).

Gene expression profiling in 20-week WT remnant kidneys

Initially, we identified global changes of renal gene expression in WT remnant kidneys (20-week post-SNX). The microarray experiments were performed in triplicates, as the renal gene expression was compared at random between three separate pairs of SNX-WT versus WT-sham-operated (WT-C) animals (SNX-WT/WT-C). A subset of 1131 differentially expressed genes was selected from all triplicate microarray data by filtering on expression level ($\geqslant$2-fold). Using these selection criteria, we found 543 genes to be upregulated and 588 genes to be downregulated in the 20-week remnant kidneys, compared to that of the control kidneys. Table 1A shows list of selected functional groups of genes that were upregulated ($\geqslant$2-fold) in the 20-week remnant kidneys. As seen from Table 1A, there is substantial number of upregulated genes with previously shown implication in mechanisms of renal pathology, including altered lipid metabolism, inflammation, fibrosis, vascular homeostasis, and/or reactive oxygen species formation. Some of them displayed very strong upregulation ($>$5-fold) in remnant kidneys (indicated in bold). These included albumin (Alb), fatty acid-binding protein 1 (Fabp1), retinol-binding protein 4 (Rbp4), carbamoylphosphate synthetase 1 (Cps1), apolipoproteins Apoa1, Apoa2, Apoh, and Apoc4, kininogen (Kng1), vitronectin (Vtn), fetuin-A (Ahsg), esterase 1 (Es1), paraoxonase 1 (Pon1), fibrinogen (Fg), orosomucoid 1 (Orm1), hemopexin (Hpxn), and different members of the cytochrome *P*450 gene family (Cyp2c39, Cyp2c40, Cyp3a44, Cyp2a12, Cyp2c29, Cyp3a11, and Cyp2c40). Interestingly, four members of the major urinary protein family (Mup1, Mup3, Mup4, and Mup5) displayed similar high range ($>$5-fold) of gene expression. Table S1 shows the complete list of upregulated genes ($\geqslant$2-fold) in the 20-week remnant WT kidneys.

Five hundred and eighty-eight genes were subject to at least twofold downregulation in the remnant WT kidneys (a list of selected downregulated genes is presented in Table 1B). Among the genes with known functions, major classifications comprised metabolism (35%), transport (17%), cell growth and maintenance (11%), transcription (11%), signal transduction (10%), protein turnover (8%), immune response (4%), apoptosis (2%), proteolysis and peptidolysis (2%), and more than half (323 genes) had unknown function. Table S2 displays the complete list of the 588 downregulated genes ($\leqslant$2-fold) in the 20-week remnant WT kidneys.

A network analysis based on the 1131 gene list with twofold altered expression identified 26 highly significant networks with score $\geqslant$11 (see Table S3). The four top-scoring networks are shown in Table 2 and were associated with the functions of cell-to-cell signaling and interaction, cellular growth and proliferation, lipid metabolism, molecular transport, and immune response. A common network obtained upon merging the four top-scoring networks (Figure 2) recognized several important nodes linked with numerous interaction partners, including the v-fos FBJ murine osteosarcoma viral oncogene homolog (Fos), thrombin (Thr), fibronectin 1 (Fn1), C-reactive protein (Crp), plasminogen (Plg), insulin-like growth factor 1 (Igf1), and Hnf4a. As the majority of these genes and related pathways were previously associated with renal pathology, the Hnf4a signaling represents a novel pathway (see also Figure 3), possibly linked with the mechanisms of advanced renal disease.

Kinin receptors ablation results in consistent patterns of altered gene expression in 20-week remnant kidneys

In parallel, we studied global gene expression changes in 20-week remnant kidneys of SNX-B_1R-KO and SNX-B_2R-KO mice. Gene expression analyses were performed at random in five separate pairs (biological replicates) of SNX-B_1R-KO mice versus SNX-WT mice (SNX-B_1R-KO/SNX-WT) and six biological replicates of SNX-B_2R-KO versus SNX-WT animals (SNX-B_2R-KO/SNX-WT). A subset of differentially expressed genes was selected displaying at least twofold difference in four of the five B_1R-KO microarray experiments and in four of the six B_2R-KO microarray experiments. Using these

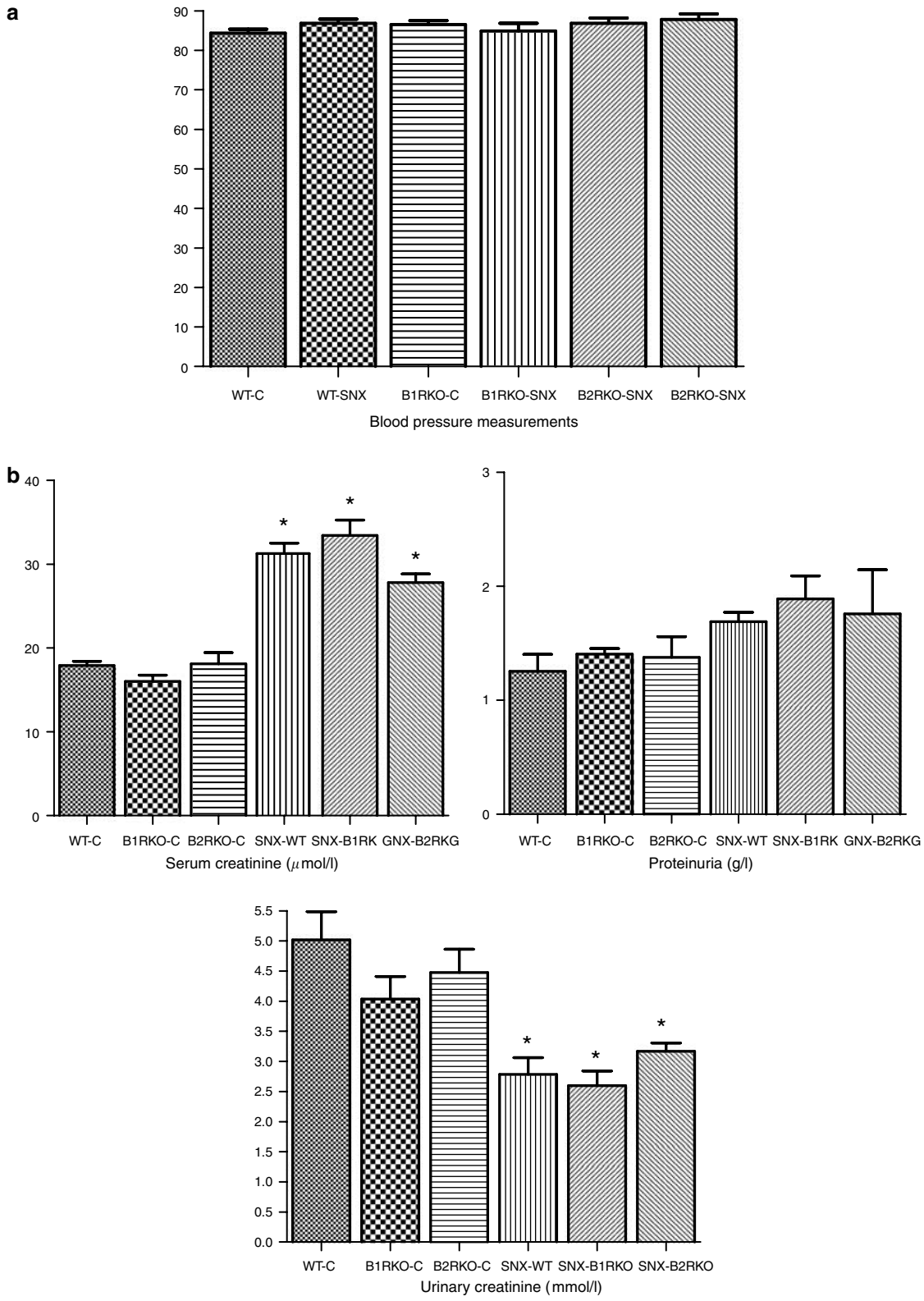

Figure 1 | BP measurements and biochemical parameters of ESRD progression in SNX-WT, SNX-B_1R-KO, and SNX-B_2R-KO mice 20 weeks after SNX. (**a**) BP measurements; (**b**) biochemical parameters. Serum creatinine increases (*$P<0.0001$) and urinary creatinine decreases (*$P<0.001$) 20 weeks after SNX in the different animals groups, versus respective controls; $n=4$–6 mice/group. A tendency of increased proteinuria was observed in SNX animals but it did not reach statistical significance.

Table 1 | Selected functionally related groups of differentially expressed genes (⩾2-fold)[a] in 20-week remnant WT kidneys

(A) Upregulated genes	
Cytochrome P450 family (vascular homeostasis and/or ROS formation)	*Cyp1a2*, ***Cyp2a12***, ***Cyp2b9***, *Cyp2b10*, ***Cyp2b13***, ***Cyp2c29***, ***Cyp2c37***, ***Cyp2c38***, ***Cyp2c39***, ***Cyp2c40***, ***Cyp2c54***, *Cyp2c70*, *Cyp2f2*, ***Cyp3a11***, ***Cyp3a16***, ***Cyp3a25***, ***Cyp3a44***, ***Cyp4a14***
Lipid metabolism and transport	***Apoa1***, ***Apoa2***, ***Apoa5***, ***Apoc1***, ***Apoc3***, ***Apoc4***, *Apoe*, ***Apof***, ***Apoh***, ***Fabp1***, ***Sult2a2***, ***Sth2a1***, ***Slc27a5***, ***Pon1***, ***Sth2***, *Acdc*
Serpins; proteolysis and peptidolysis	***Serpina1b***, ***Serpina1d***, ***Serpina1e***, ***Serpina3c***, ***Serpina3k***, ***Serpina3n***, ***Serpina6***, ***Serpina10***, ***Serpinc1***, *Serping1*, ***F2***, ***F10***
Solute carriers and other transporters	***Slc7a12***, ***Slc10a1***, ***Slc38a4***, ***Slco1b2***, ***Car3***, *Slc16a4*, *Slc26a8*, *Slc39a4*, *Ambp*
Inflammation; complement activation	***Es1***, ***C4bp***, ***Orm1***, ***Hpxn***, ***Es31***, ***C3***, ***Hamp***, ***Orm2***, ***Hp***, ***Cfh***, ***Saa4***, ***Cpb2***, ***Cfi***, ***Cfhl1***, ***Adn***, ***Mbl2***, *Retn*, *Plg*, *Hpgd*, *C1r*, *C9*, *C1s*, *Masp*, *Crp*
Immune response	***H2-Bl***, ***Ig-γ1***, ***Was***, ***Igh-VJ558***, ***Igl-6G14-F6***, ***H2-Q10***, ***Cd1d1***, ***Igj***, ***Igl-11***, ***C6-E3***, ***Ig-κ V-V***, ***Wasf3***, ***Igg1***, ***Igh-L2pecB***, ***Mug2***
Fibrosis	***Fgg***, ***Fgb***, ***Fga***, ***Fgl1***, ***Ppp1r9a***, *Fgfr1*, *Fn1*
Apoptosis	***Card14***, ***Csnk2a1***, ***Il21r***, *Clu*
ECM proteins	***Csf3r***, ***Vtn***, ***Lum***, *Ceacam2*
Cell growth and proliferation	***Igf2***, ***Rgn***, ***Igf1b***, *Igf1*, *Hel308*
Oxidative stress (pro- and antioxidants)	***Maob***, ***Uox***, ***Hao3***, *Cp*, *Hao1*, ***Trf***, *Trfr2*, ***Ucp1***, ***Gsta3***, *Gsta2*, *Gsta1*, ***Akr1c20***, ***Akr1c6***, ***Akr1c12***, ***Cbr1***
Enzymes of the urea cycle	***Cps1***, ***Arg1***, ***Arg2***, ***Otc***
Major urinary proteins	***Mup1***, ***Mup3***, ***Mup4***, ***Mup5***
Other genes relevant to renal pathology	***Alb1***, ***Ahsg***, ***Rbp4***, ***Kng1***, *Agxt*
(B) Downregulated genes	
Cell growth and metabolism	***Fgf5***, *Fgf2*, ***Odc***, *Odc1*, ***Tph1***, ***Dhrs8***, *Dhrs1*, ***Ldhd***, *Ldh1*, ***Atp11a***, *Mod1*, *B4galt5*, *Psap*, ***Sah***, ***Slc27a2***, ***Ugt8***, *Pcyt1a*, *Crot*, *Cyb5*, *Tgfbr2*, ***Acox1***, *Acox3*, ***Mapk6***
Transport	*Cacna2d2*, *Uty*, *Pitpnm1*, *Kcnk5*, *Slco1a1*, *Slco1a5*, *Slc7a13*, *Slco1a1*, *Tnpo3*
Protein biosynthesis	***Eif2s3y***, *Eif5a*, ***Polr3e***, *Gfm*, *AK018591*, *Mrpl12*
Cell adhesion	***Pcdhb9***, *Pcdh8*, *Itga2b*, *Lama1*, *Dscaml1*, ***Gjc1***, *Mmp5*
Cytochrome P450 family	***Cyp2j13***, ***Cyp4b1***, ***Cyp7b1***
Apoptosis	*Bcl2l1*, *Cideb*, *Dnase1*, *Pdcd8*
Genes relevant to renal pathology	*Hbα-a*, *Hb-β*, *Hb-β-b1*, *Pkd2*, *Nppb*, *Acel*

ECM, extracellular matrix; ROS, reactive oxygen species.
[a]Genes in bold are differentially expressed at least ⩾5-fold in all microarray experiments.

Table 2 | The four top-scoring genetic networks in the 20-week remnant WT mouse kidneys

Network	Genes in ingenuity networks[a]	Score[b]	Functions
1	↑***ANG***, ↑***APOH***, ***CDCP1***, ↑***CEACAM1***, ↑***CISH***, ↑***CP***, ↑***CPB2***, ↓***CSDA***, ↑***CSF3R***, ↑***DCN***, ↑***DF***, ↑***F10***, ↑***FABP4***, ↓***FOXE1***, ↑***IGF1***, ↑***IGF2***, ↓***IGFBP3***, ↓***INS***, ↑***LYZ***, ↓***MMP2***, ↑***PLG***, ↑***PRLR***, ↓***PTHR1***, ↑***RARRES2***, ↑***SERPINA3***, ↑***SERPINA10***, ↑***SERPINC1***, ↓***SERPINF2***, ↓***SLC20A1***, ↑***SOCS2***, ↑***TF***, ↑***TFPI***, ↑***TFR2***, ↑***VTN***, ↓***ZFP35***	46	Cell-to-cell signaling and interaction, cellular growth, and proliferation
2	↑***APOA1***, ↑***APOA2***, ↑***APOC1***, ↑***APOC3***, ↑***APOE***, ↓***BLMH***, ↑***C9***, ↑***C4A***, ↑***C8G***, ↑***CLU***, ↑***CUGBP2***, ↓***ENO1***, ↓***FARS2***, ↑***FGA***, ↑***FGB***, ↑***FOS***, ↑***GCNT1***, ↓***HNF4A***, ↑***HPD***, ↑***LCAT***, ↓***LDHA***, ↓***NP***, ↓***OAZ1***, ↓***OCM***, ↓***ODC1***, ↑***PON1***, ↑***PRKAR2B***, ↑***PYGL***, ↓***RNH***, ↓***RPS6KA3***, ↓***SLC27A2***, ↓***SMARCD1***, ↓***SPN***, ↑***TDO2***, ↓***TEGT***	46	Lipid metabolism, molecular transport, small molecule biochemistry
3	↑***ABCC3***, ↓***ACE***, ↑***ALB***, ↑***C3***, ↑***C1R***, ↓***CD9***, ↓***CD63***, ↑***CFH***, ↓***CYBA***, ↑***CYP2B2***, ↑***DGKQ***, ↑***F2***, ↓***FGF2***, ↑***GLUL***, ↑***GPNMB***, ↑***IF***, ↑***ITGA6***, ↓***ITGA2B***, ↑***KNG1***, ↑***MAFB***, ↑***MASP1***, ↑***MASP2***, ↑***MBL2***, ↓***MEP1B***, ↓***NAGK***, ↓***NOX3***, ↓***PBP***, ↓***PIK4CA***, ↓***PITPNM1***, ↑***PRKCB1***, ↑***SCD***, ↓***SERPINA5***, ↑***SERPING1***, ↓***SMPD2***, ↓***TNS***	46	Cardiovascular disease, tissue morphology, lipid metabolism
4	↓***ACOX1***, ↑***ADIPOQ***, ↑***APOA5***, ↓***ATP6V0C***, ↑***B2M***, ↑***CANX***, ↑***CD1D***, ↓***CPT1A***, ↑***CRP***, ↑***CSNK2A1***, ↑***CXCL9***, ↑***DCT***, ↑***FASN***, ↑***FCER1G***, ↑***FN1***, ↑***HLA-A***, ↑***HLA-B***, ↑***HLA-E***, ↑***IGHG1***, ↑***IGHM***, ↑***IL2***, ↓***IL2RA***, ↑***KLRC2***, ↑***LOC56628***, ↓***LPL***, ↑***MUG1***, ↓***MYH1***, ↓***NPPB***, ↓***PDCD8***, ↑***PTPN6***, ↑***SERPINA1***, ↓***SNRPN***, ↓***U2AF2***, ↑***UCP1***, ↓***ZBTB7***	46	Cell-to-cell signaling and interaction, immune response

[a]Upregulated (↑) and downregulated (↓) genes are indicated with arrows.
[b]A score >3 was considered significant.

selection criteria, we found 73 genes to be upregulated and 118 genes to be downregulated in B_1R-KO remnant kidneys, compared with that of WT remnant kidneys, whereas 71 genes were upregulated and 52 genes were downregulated in B_2R-KO remnant kidneys, when compared with WT remnant kidneys (Tables S4 and S5).

Interestingly, a substantial number of genes (43 upregulated and 30 downregulated) were commonly differentially expressed in the SNX-B_1R-KO/SNX-WT experiments and in the SNX-WT/WT-C experiments (Figure 4a). In contrast, the number of the differentially expressed genes that are common in the SNX-B_2R-KO/SNX-WT experiments and in the SNX-WT/WT-C experiments was considerably lower (18 upregulated and 0 downregulated genes – see Figure 4b). The number of overlapping genes in both experimental conditions including kinin receptors KO

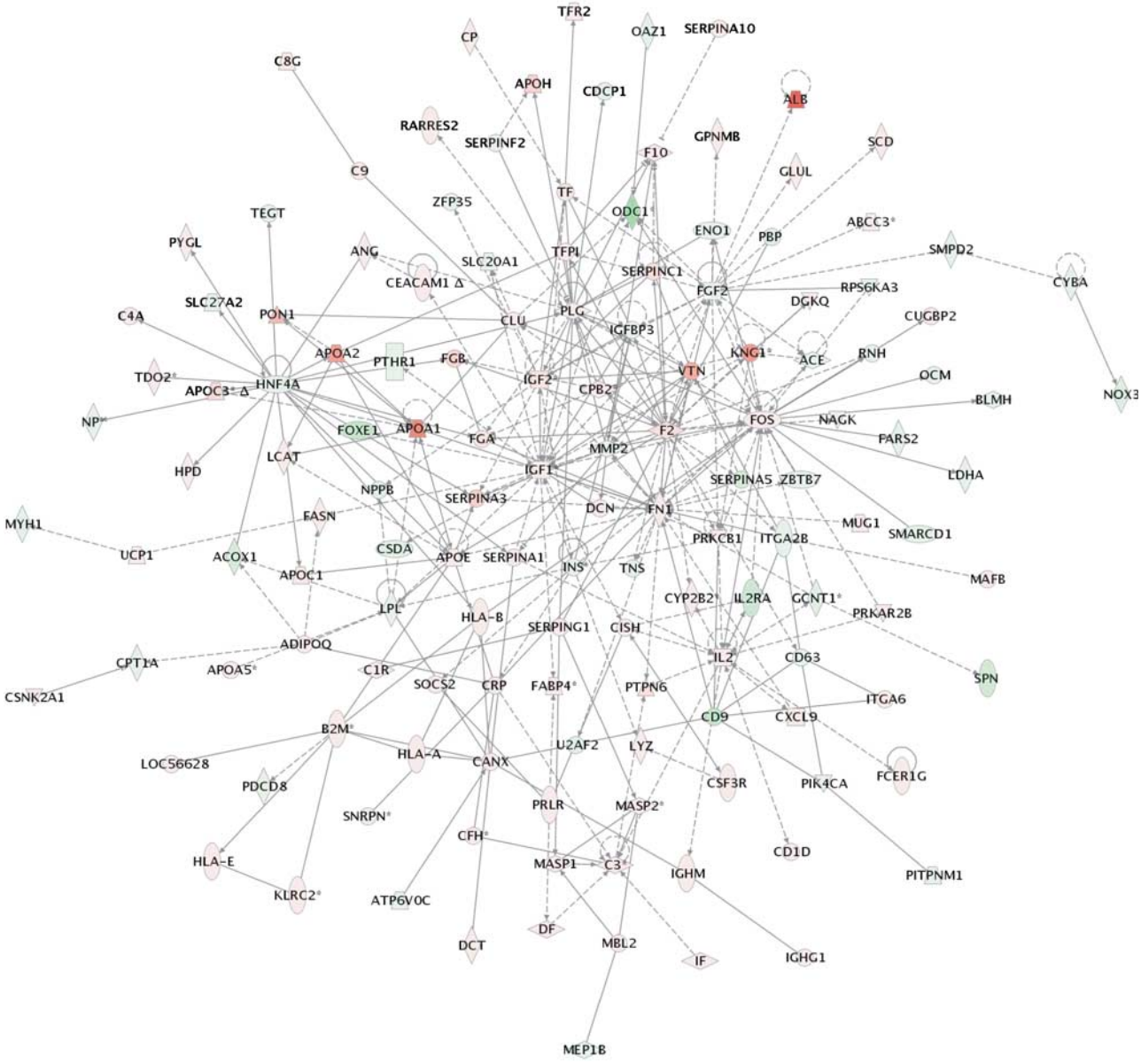

Figure 2 | Network analysis of dynamic gene expression in the 20-week remnant kidneys based on the twofold gene expression list. The top four networks were merged and are displayed graphically as nodes (genes/gene products) and edges (the biological relationships between the nodes). Intensity of the node color indicates the degree of upregulated (red) or downregulation (green). Nodes are displayed using various shapes that represent the functional class of the gene product (square, cytokine; vertical oval, transmembrane receptor; rectangle, nuclear receptor; diamond, enzyme; rhomboid, transporter; hexagon, translation factor; horizontal oval, transcription factor; and circle, other). Edges are displayed with various labels that describe the nature of the relationship between the nodes: — binding only; → acts on. The length of an edge reflects the evidence supporting that node-to-node relationship, in that edges supported by more articles from the literature are shorter. Dotted edges represent indirect interaction.

mice was also negligible (Figure 4c). However, some functionally important renal pathology genes, including genes implicated in renal fibrosis (Fga, Fgg, Saa2, Edn1, and Ren1) were uniquely overexpressed only in the B_2R-KO remnant kidneys. This also holds for a subset of genes, functionally implicated in control of cell proliferation (Nrg3, Sycp3, Plec1, Ptn, Kif11, and Kif13a), found to be exclusively downregulated in remnant kidneys of the B_2R-KO mice (Table S5).

Table 3 displays the classifications by function of the differentially expressed genes in both experimental conditions involving kinin receptors-KO mice (SNX-B_1R-KO/SNX-WT and SNX-B_2R-KO/SNX-WT). These data are indicative for some similarities, as well for some differences in the functional classes that are specific for each mouse KO strain. Thus among the upregulated genes, major classifications in the B_1R-KO remnant kidneys include transport, metabolism (especially lipid transport and metabolism),

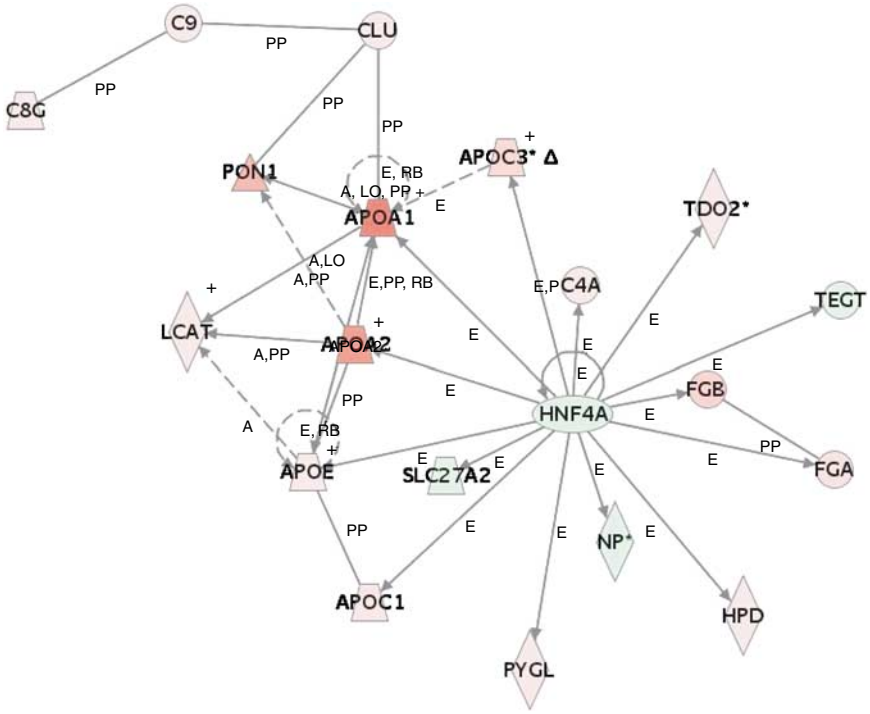

Figure 3 | The Hnf4a signaling pathway. Figure legends are as described in Figure 2. Edge labels: A, activation; B, binding; E, expression; PP, protein–protein binding; RB, regulation of binding; LO, localization.

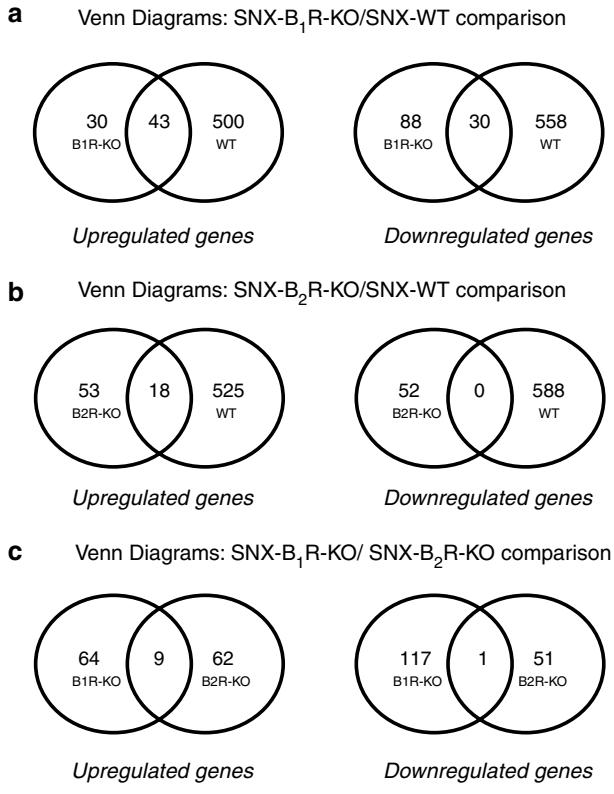

Figure 4 | Comparisons of the common and distinct gene expression across the different experimental conditions using the Venn diagram analysis. (**a**) Comparison for upregulated genes. (**b**) Comparison for downregulated genes. (**c**) The different experimental conditions subject to comparison are indicated (see details in text).

Table 3 | Functional distribution of the differentially expressed genes (twofold) in remnant kidneys of SNX kinin receptors KO mice compared with SNX-WT mice

SNX-B_1R-KO: functional groups	%	SNX-B_2R-KO: functional groups	%
(A) Upregulated genes (⩾2-fold)			
Transport	21.9	Metabolism	15.3
Metabolism	17.2	Signal transduction	12.5
Proteolysis and peptidolysis	14.1	Transport	12.5
Lipid metabolism and transport	10.9	Acute phase response	9.7
Immune response	9.4	Transcription	8.3
Inflammation	7.8	Inflammation	5.6
Other	6.2	Immune response	5.5
Unknown function	12.5	Vasoconstriction	2.8
		Unknown function	27.8
(B) Downregulated genes (⩽2-fold)			
Metabolism	12.4	Signal transduction	17.3
Transcription	8.3	Transcription	13.5
Signal transduction	6.6	Cell proliferation	11.5
Cell growth and proliferation	5.8	Transport	11.5
Transport	5.8	Metabolism	7.7
Immune response	4.9	Immune response	5.8
Apoptosis	3.3	Unknown function	32.7
Unknown function	52.9		

KO, knockout; SNX, subtotal or 5/6 nephrectomy; WT, wild type.

proteolysis and peptidolysis, whereas in B_2R-KO remnant kidneys, the top functional classes are associated with metabolism, signal transduction, transport, and acute-phase response (Table 3A). As for the downregulated genes, functional groups in the B_1R-KO remnant kidneys mostly

comprise metabolism, transcription, and signal transduction, whereas in the B_2R-KO remnant kidneys these mainly include signal transduction, transcription, cell proliferation, and transport (Table 3B). The differences between the two SNX-KO strains were further confirmed by clustering and pathway analysis. Supervised clustering based on a selected list of 65 genes revealed formation of two major cluster groups that perfectly distinguish between SNX-B_1R-KO and SNX-B_2R-KO kidney samples (Figure 5). The 65 gene list is presented in Table S6. Twenty genes from the 65 genes list were upregulated in the B_2R-KO remnant kidneys. Major classifications of these genes include metabolism and regulation of transcription. Genes, upregulated in B_1R-KO remnant kidneys (45 genes) are mainly involved in signal transduction, metabolism, transport, and apoptosis.

Network analysis identified three significant gene networks in both up and down-differentially expressed genes in the B_1R-KO remnant kidneys, whereas in the B_2R-KO remnant kidneys three networks for the upregulated genes and two networks for the downregulated genes displayed a significant score (Table 4). The discrepancies were mostly evident in the networks obtained with downregulated genes, as the SNX-B_1R-KO networks were associated with gene expression and cellular growth and proliferation (Table 4B), whereas the SNX-B_2R-KO networks were mainly linked to immune response, lipid metabolism, and transport (Table 4D).

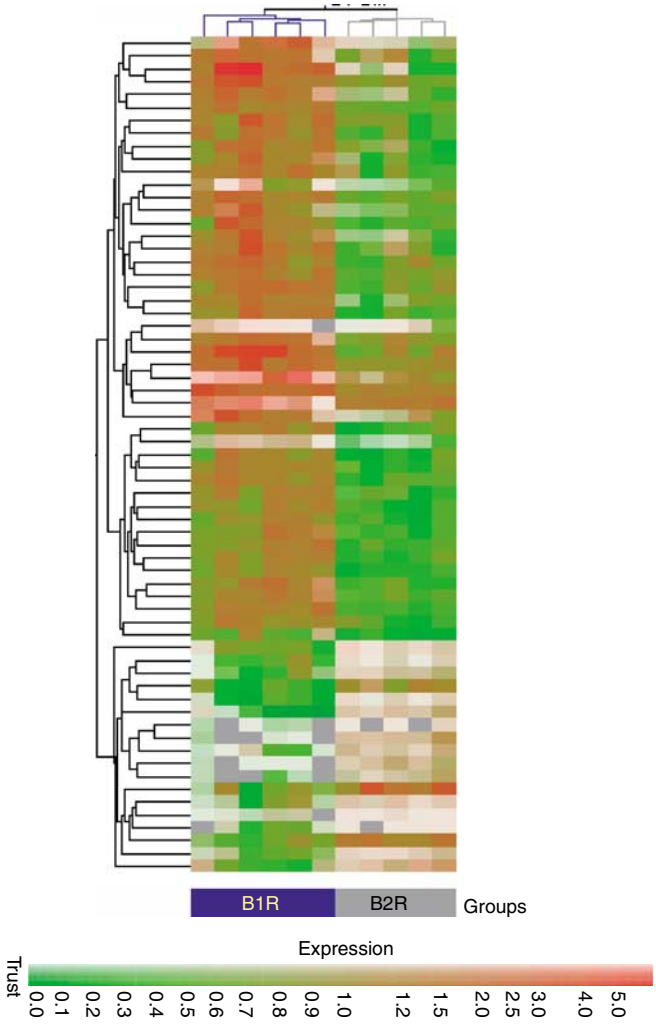

Figure 5 | Hierarchical clustering of 20-week remnant kidneys that discriminates between SNX-B_1R-KO and SNX-B_2R-KO kidney samples. A subset of candidate genes were initially obtained by filtering on signal intensity (twofold), retaining 306 genes. One-way analysis of variance parametric test (Welch *t*-test; variances not assumed equal; $P \leqslant 0.05$) further selected 65 genes. Clustering analysis based on the 65 gene list was performed using the standard Condition Tree algorithm provided in GeneSpring. The mean appears grey, whereas red signifies upregulation, and green signifies downregulation (see legend bar). B_1R SNX kidney samples are indicated in grey; B_2R SNX kidney samples are indicated in blue.

Validation of microarray findings with semi-quantitative RT-PCR

To validate microarray results, we arbitrarily selected 15 differentially expressed genes in 20-weeks remnant WT kidneys and quantified their expression by semi-quantitative real time-polymerase chain reaction (sqRT-PCR) in the available WT remnant and WT control kidney samples. Additionally, we validated a number of genes by RT-PCR, found to be differentially expressed in 20-week remnant kidneys of SNX-B_1R-KO or SNX-B_2R-KO mice compared with SNX-WT control animals. All sqRT-PCR analyses were performed in samples previously used for the microarray experiments. Table 5 summarizes the gene expression measurements of all validated genes. We found that both methods (microarray analysis and sqRT-PCR) detected similar patterns for the upregulated and downregulated genes selected for validation.

Histological analysis of remnant kidney tissues reveals higher levels of renal fibrosis in the B_2R-KO SNX mice

In remnant kidneys of the WT and kinin receptor-KO animals, examination (Masson's trichrome staining) of kidney tissue revealed classical alterations such as glomerular hypercellularity and mild glomerular sclerosis (Figure 6). We have also noticed some tubular dilation and scarce areas with tubulointerstitial fibrosis. α-Smooth muscle actin staining was only observed in the intrarenal arteries and macrophage infiltration was mainly located around the gomeruli (not shown).

Whereas there was no difference in the gomerular diameter between the WT, B_1R- and B_2R-KO kidney mice under physiological conditions (respectively, 70.55 ± 5.2, 68.09 ± 7.78, and $66.13 \pm 6.27\,\mu$m), we found that the glomerular diameter was significantly higher in the SNX-B_2R-KO kidney mice compared with those observed in the SNX-WT and B_1R-KO remnant kidney (Figure 6a–c). Moreover, the level of tubulointerstitial fibrosis was significantly higher in the B_2R-KO SNX mice ($4.8 \pm 0.7\%$) compared with that in the SNX-WT mice ($2.5 \pm 0.4\%$) (Figure 6d–f). Although not significant, a tendency to a lower macrophage infiltrate was observed in the B_1R-KO remnant kidneys (not shown).

Table 4 | Genetic networks in remnant kidneys of the kinin receptors-KO mice

Network	Genes in ingenuity networks[a]	Score[b]	Focus genes[c]	Functions
(A) Networks in remnant kidneys of SNX-B_1R-KO mice (upregulated genes)				
1	*AGXT, APOA1, APOC1, APOC3, COX8B, CYP3A2, F2, F10*,* ***FGB****, ITGA6, MUP4, PLG, PON1, SERPINC1, SERPIND1, TFR2*	29	16	Lipid metabolism, molecular transport
2	*AHSG,* ***ARG1*****, C5, CIDEA, DF*, ELOVL6, HP,* ***HPX****, MBL2, MYO5A,* ***ORM1****, SAA4, SERPINAC3 (Kalbp)*	22	13	Development, lipid metabolism
3	*AMBP,* ***ARG1*****, BAAT,* ***CYP3A7****, DF*, F10*, HDC, HLA-B, IGJ, MGC27165,* ***ORM2****, SERPINA3K, UCP1*	22	13	Cellular growth and proliferation
(B) Networks in remnant kidneys of SNX-B_1R-KO mice (downregulated genes)				
1	*BCL2L2, BMF, BRCA2, CD36*, HDAC2, ITGB1, MEOX2, NFKBIZ, PLF2, PTPN4, SDHA, SH3GLB1, TM4SF8, TSC22D1*	24	14	Gene expression, cellular movement
2	*ABHD5, CDH17, CYP4B1, G0S2, MPRA, POLR3E, RAP1B, SFRS1, SLAMF6, SLC20A1, SLC30A1, SLCO1A1, SNX6, UGT2B7*	24	14	Cellular growth and proliferation
3	*AKR1C2, BRF1, CD36*, CENPB, CES1, DARS, EIF5A, ENPEP, HSPA8, LACTB, NBR1, TP53BP2, TSC22D3*	21	13	Gene expression
(C) Networks in remnant kidneys of SNX-B_2R-KO mice (upregulated genes)				
1	*ANTXR1, APCS,* ***ARG1*****, CD163, CPB2, DHCR7, FGG*, HP, ITIH3, LCAT, LRG1, MAT1A,* ***ORM1*****, SAA2, SERPINA3*	27	15	Immune response, cellular growth and proliferation
2	*ALAD, CD3D, CPS1, CYP2F1, EDN1, FASN, GSTM2, HAND1, HAO1, HCK,* ***HPX****,* ***ORM1****, SOX18*	22	13	Lipid metabolism, molecular transport
3	***ARG1*****, CDGAP,* ***CYP3A7****, FGA,* ***FGB****, FGG*, FKBP5, GSTM1,* ***ORM2****, PC4, PSMD11, RPS6KA1*	20	12	Cell signaling
(D) Networks in remnant kidneys of SNX-B_2R-KO mice (downregulated genes)				
1	*ABCD3, CAMP, CCL27, CTSC, ERCC2, GHSR, IGH1A, PHIP, PLEC1, PRKACA, PRKCI, SP100*	23	12	Immune response, cellular growth and proliferation
2	*CAST (ERC2), KIF11, NRG3, NT5E, PTN, RASSF4, SYCP3, TFRC, ZBTB33*	16	9	Lipid metabolism, molecular transport

B_1R, kinin receptor, which is overexpressed in pathological states; B_2R, kinin receptor, which is constitutively expressed; KO, knockout; SNX, subtotal or 5/6 nephrectomy.
[a]Genes marked with asterisk are present in more than one network. Bold genes are present in networks identified by both experimental conditions (including B_1R-KO and B_2R-KO mice).
[b]The table displays only networks with significant scores (score >3 is considered as significant).
[c]Only genes identified by microarray analysis (focus genes) that are differentially expressed ($\geqslant$2-fold) are listed.

DISCUSSION

Molecular mechanisms of advanced renal disease

Functional classification of differentially expressed genes in 20-week remnant WT kidneys revealed marked overexpression of genes involved in inflammation/immune response, fibrosis, cell adhesion, apoptosis, and altered metabolism and transport (Table 1A). A consistent finding of our study was the dramatic upregulation of numerous members of the CYP gene family (Table 1A and Table S1). The induction of different CYP genes could lead to altered metabolism, inflammation, and oxidative stress in renal tissue.[13] However, our microarray data show predominant upregulation of CYP members from the 2b and the 2c gene subfamilies that are known to be involved in vasodilation and as anti-inflammatory and angiogenic mediators,[14,15] which could lead to possible compensatory (protective) effects during advanced renal disease.

The observed strong upregulation of several oxidases (Maob, Uox, Hao3, Cp, Hao1) probably contributes to oxidative stress and renal damage in the 20-week remnant kidneys. In contrast, we have also monitored significant overexpression of different genes with demonstrated anti-inflammatory and antioxidant effects in renal pathology. These genes comprise transferrin (Trf) and its transferrin receptor 2 (Trfr2),[16] uncoupling protein 1 (Ucp1),[17] as well as different members (Gsta1, Gsta2, Gsta3) of the GST gene family[18] and the aldo-keto reductase (AKR) gene family (Akr1c20, Akr1c6, Akr1c12, Cbr1).[19] Both GST and UCP genes were also found to be strongly upregulated in rat kidneys following ethylene glycol-induced urolithiasis[20] and upon renal ischemia–reperfusion (I/R) injury in mice and rats,[21] as Gsta1 was also shown to be overexpressed in kidneys of sickle-cell transgenic mice.[22]

Another important gene group that showed marked induction in 20-week remnant kidneys included transcripts of the apolipoprotein family encoding for molecules involved in lipid metabolism and transport. Disturbances in lipid metabolism are often observed in patients with chronic renal failure,[23] as the negative impact of proteinuria on ESRD progression could be mediated in part through increased filtration of lipoproteins.[24]

Transcripts belonging to the serine proteinase inhibitors (serpins) gene family were also consistently overexpressed in 20-week remnant kidneys. Almost no data are available for implications of serpins in renal pathology. However, given their recently proposed role in regulating renal inflammation

Table 5 | sqRT-PCR validation of microarray data

GenBank No.	Common name	Fold expression (SNX-WT/WT-C)	
		Microarray	sqRT-PCR[a]
(A) Comparison of the qualitative results obtained by microarray analysis and conventional method (sqRT-PCR) in kidney tissues from SNX-WT mice versus WT control mice			
NM_009654	Albumin 1 (Alb1)	+267.50	+55.29
NM_031188	Major urinary protein 1 (Mup1)	+94.96	+10.30
NM_011548	Serpin clade A, 3K (Serpina3k)	+94.27	+43.60
NM_017399	Fatty acid-binding protein 1 (Fabp1)	+64.35	+21.46
AK008765	Retinol-binding protein 4 (Rbp4)	+63.18	+9.73
NM_009692	Apolipoprotein A-1 (Apoa1)	+59.52	+23.12
NM_080852	Solute carrier family 7, member 12 (Slc7a12)	+52.90	+10.15
NM_023125	Kininogen 1 (Kng1)	+52.84	+4.87
NM_177380	Cytochrome P450 3a44 (Cyp3a44)	+34.86	+37.59
NM_011134	Paraoxonase 1 (Pon1)	+36.51	+7.83
NM_133862	Fibrinogen, gamma (Fgg)	+24.68	+5.75
NM_007576	Complement 4 binding protein (C4bp)	+22.67	+17.89
NM_023114	Apolipoprotein C-III (Apoc3)	+19.53	+8.04
NM_019946	Microsomal glutathione *S*-transferase 1 (Mgst1)	+8.94	+3.07
NM_013797	Solute carrier, member 1a1 (Slco1a1)	−55.25	−26.01
		Fold expression (SNX-B_1R-KO/SNX-WT)	
GenBank No.	Common name	Microarray	sqRT-PCR[a]
(B) Comparison of the qualitative results obtained by microarray analysis and conventional method (RT-PCR) in remnant kidneys from either SNX-B_1R-KO or SNX-B_2R-KO mice versus SNX-WT mice			
NM_134066	Aldo-keto reductase 1, member c18 (Akr1c18)	+10.47	+2.04
NM_023114	Apolipoprotein C-III (Apoc3)	+5.17	+1.36
BY426609	Immunoglobulin J558, heavy chain (IgJ558)	+4.52	+1.60
AK011118	Fibrinogen, B beta (Fbb)	+4.13	+6.05
NM_007443	Alpha 1 microglobulin (bikunin)	+4.09	+2.88
		Fold expression (SNX-B_2R-KO/SNX-WT)	
GenBank No.	Common name	Microarray	sqRT-PCR
AK031900	RIKEN cDNA library, clone: 6330442E02	+8.39	+2.34
NM_017371	Hemopexin (Hpxn)	+7.03	+3.89
NM_009252	Serpin clade A, 3N (Serpina3n)	+4.11	+3.21
AK007175	RIKEN cDNA library, clone: 1700112E06	+3.46	+2.61
BY426609	Immunoglobulin J558, heavy chain (IgJ558)	+3.24	+2.57
AK011118	Fibrinogen B-beta (Fgb)	+3.11	+3.80
NM_011016	Orosomucoid 2 (Orm2)	+2.91	+4.17
NM_010196	Fibrinogen alpha (Fga)	+2.66	+2.01
NM_133862	Fibrinogen gamma (Fgg)	+2.62	+1.94
NM_010104	Endothelin 1 (Edn1)	+2.04	+1.75
NM_031192	Renin 1 (Ren1)	+2.03	+1.84
NM_172841	Solute carrier, member 5A1 (Slco5a1)	−15.58	−12.30
NM_008973	Pleiotrophin (Ptn)	−2.95	−1.72

B_1R, kinin receptor, which is overexpressed in pathological states B_2R, kinin receptor, which is constitutively expressed; C, control; KO, knockout; RT-PCR, real time-polymerase chain reaction; SNX, sqRT-PCR, semi-quantitative real time-polymerase chain reaction; WT, wild-type.
[a]The sqRT-PCR data represent medium values of triplicate RT-PCR experiments, as the specific gene expression was compared at random between three separate pairs of experimental animals.

and tissue repair during kidney regeneration,[25] one can consider their overexpression in the remnant kidneys as a possible compensatory mechanism during disease progression. Interestingly, a number of serpin genes were also found to be strongly upregulated in mouse kidneys following renal I/R, and a role for serpins in conferring protection from I/R injury was proposed.[21]

Genes encoding for enzymes of the urea cycle, including both arginase enzymes (Arg1 and Arg2), were also consistently induced in remnant kidneys (Table 1A). It was previously shown that Arg2 is present in the proximal tubules and is implicated in tubular repair.[26] Comparable compensatory changes in enzymes of the urea cycle were also monitored in hypertrophied mouse kidneys after streptozotocin-induced diabetes,[27] renal I/R,[22] unilateral nephrectomy,[27] and in kidneys of sickle-cell transgenic mouse.[22]

Transcripts encoding for different MUPs were highly overexpressed in 20-week remnant kidneys. MUPs are involved in pheromone transport, detoxification, inflammatory responses, and animal behavior.[28] MUP expression was reported to be upregulated by androgen stimulation in the mouse kidney.[29] Although the entire spectrum of biological

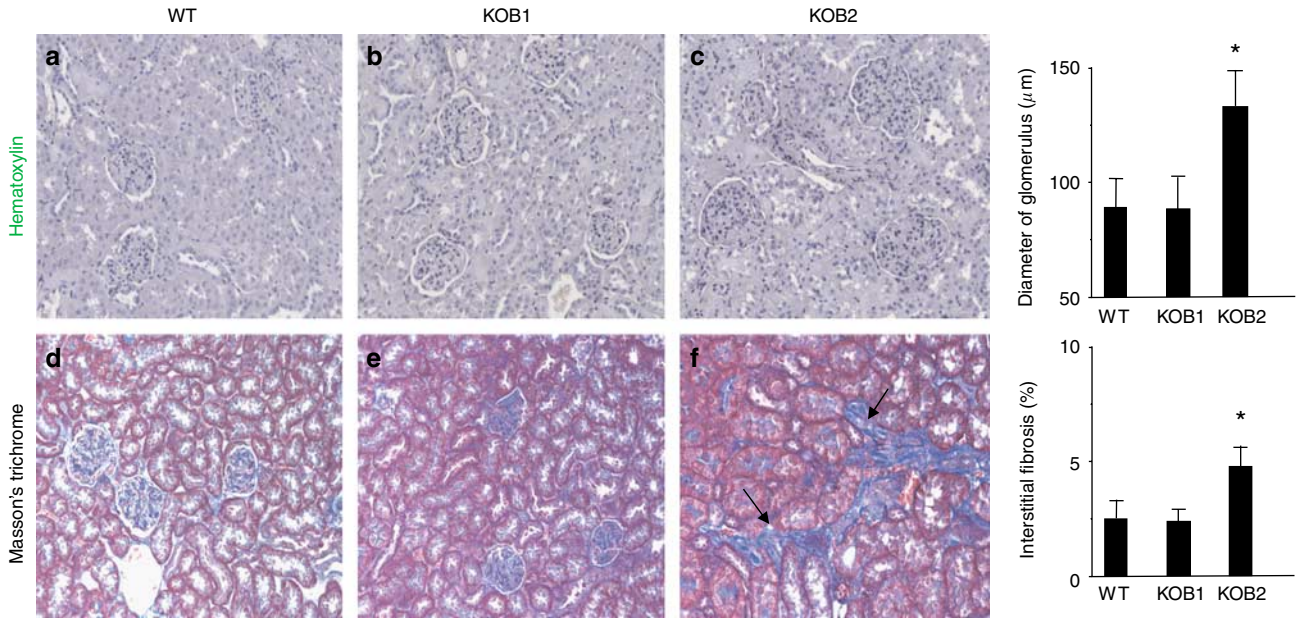

Figure 6 | Histological analysis of remnant kidney tissues. Histological appearance of glomeruli in (**a**) SNX-WT mice, (**b**) SNX-B_1R-KO mice, and (**c**) SNX-B_2R-KO mice. Representative photographs of Masson trichrome-stained renal sections of (**d**) 20-week remnant kidney tissues of SNX-WT mice, (**e**) SNX-B_1R-KO mice, and (**f**) SNX-B_2R-KO mice. Arrows indicated accumulation of interstitial collagen (blue). Bars represent the quantitative analysis where data are expressed as the mean $\pm$ s.d. *$P < 0.05$ versus SNX-WT. Original magnification $\times$ 200. Histological analyses were performed on five mice/group.

functions of MUPs are still unknown, the upregulated expression of androgen-regulated genes suggests that MUPs may mediate the androgen-dependent tissue injury in nephrectomized kidneys and may be additionally involved in the disruption of protein secretion and transport in ESRD. Interestingly, similar overexpression of MUP genes was demonstrated in 2-week remnant mouse kidneys,[30] and in a mouse model of autosomal recessive polycystic kidney disease.[31]

We want to especially point out several genes relevant to kidney pathology, which displayed strong upregulation in 20-week remnant kidneys. These include Alb1, Ahsg, Crp, Rbp4 and Kng1. Among these, Alb1, Ahsg, Rbp4 and Crp represent proven protein markers for clinical outcome in ESRD patients.[32]

As expected, remnant kidneys displayed strong downregulation of genes implicated in cell growth and metabolism, including genes involved in lipid (fatty acid) and protein biosynthesis (Table 1B and Table S2). Indeed, increased protein catabolism and decreased lipid and protein synthesis are common phenomena in renal failure.[33] Lower expression rates were also found for several cell adhesion genes (Pcdhb9, Pcdh8, Itga2b, Lama1), which probably contribute to ESRD progression, as loss of epithelial cell adhesion represents the initial step to epithelial-to-mesenchymal transition, a phenotypic conversion that is fundamentally linked to the pathogenesis of renal interstitial fibrosis.[34]

Several genes relevant to renal pathology were downregulated in the 20-week remnant kidneys. These include several hemoglobins (Hbα-a, Hb-β, Hb-β-b1), the natriuretic peptide precursor type B gene (Nppb), the polycystic kidney disease 2 (Pkd2) and ACE1. The downregulation of the hemoglobin genes probably contributes to anemia, which is a common complication of ESRD.[35] Although the roles of Nppb and Pkd2 in chronic renal failure are still not well defined, a suppression of ACE1 gene expression could obviously mediate protective effects against ESRD progression in remnant kidneys.

Network analysis identified several signaling pathways (Fos, Thr, Fn1, Fgf2, Crp, Plg, Igf1, and Hnf4A signaling) that displayed maximal transcriptional activity in 20-week remnant kidneys (Figure 2). Indeed, FOS induction has been previously associated with activation of mechanisms that induce and amplify the damage during progression of renal disease.[36] As seen in Figure 2, the Thr, Fn1, Crp, and Plg signaling pathways are highly interactive as these molecules have been associated with abnormalities of coagulation (vascular thrombosis), increased fibrosis, and inflammation in ESRD patients.[37] The IGF signaling is suggested to influence the pathogenesis of renal diseases,[38] and a link was shown between inflammation and Igf1 activity, which is decreased in inflamed individuals.[39] Thus, our data are suggestive for protective effects of IGFs (Igf1, Igf2, IgfB) overexpression in advanced renal failure. Similar upregulation of different members of the IGF gene family was also observed in other animal models of renal pathology, including renal I/R[21] and spontaneous congenital unilateral obstructive uropathy.[40]

Additionally, we have identified a novel pathway associated with the Hnf4A signaling that could be potentially implicated in mechanisms of chronic renal disease. Hnf4A belongs to the steroid/thyroid hormone receptor superfamily

of transcription factors and is involved in the regulation of serum lipid and glucose levels.[41] Mutations in this gene are responsible for maturity onset diabetes of the young, a monogenic dominant inherited form of diabetes mellitus characterized by defective insulin secretion of the pancreatic beta-cells;[42] however, there are no literature data available that directly link Hnf4a expression and activity to renal physiology and/or pathology, The Hnf4a signaling pathway (Figure 3) includes genes mainly involved in fatty acid and glycerophospholipid metabolism and transport (Apoc1, Apoe, Apo2, Apo1, Apoc3, Lcat, Slc27a2, and Pyg1), as well as different members of the complement and coagulation cascade (C4a, Tdo2, Fga, Fgb, C8g, C9, and Clu). Recent data indicate that Hnf4a coordinates the developmental expression of an extensive array of cell junction and adhesion proteins in the liver.[43] Thus, the detailed mechanisms for implication of Hnf4a signaling in renal pathology remain to be elucidated.

Different animal models of renal pathology have been used to study disease mechanisms applying the microarray technology.[20–22,40,44–46] However to our knowledge, the present work represents the first effort to define global changes in renal gene expression during nephropathy progression in SNX animal models using high-density microarrays. As our microarray experiments were performed in whole kidney tissues, future cellular localization experiments will be needed in remnant kidney tissues for better selection and characterization of potential ESRD biomarkers.

Role of kinin receptors in ESRD

A large body of evidence supports a role of the endogenous kallikrein–kinin system on various aspects of renal function and pathology.[8] We have observed different differential gene expression patterns in B_1R-KO and B_2R-KO 20-week remnant kidneys that are supportive for distinct mechanisms of implications for each kinin receptor subtype in chronic renal disease. According to our data, kinin B_1R ablation potentiates some gene expression alterations observed in the 20-week WT remnant model although less marked than invalidation of the B_2R. This correlated with the absence of effect on the level of fibrosis (Figure 6). However, the role of the B_1R might be more important in the early stages of chronic renal disease where the inflammatory component is more exacerbated.[47]

The effect of the B_2R gene knockout in 20-week remnant kidneys seems to be more profound, as B_2R-KO 20-week remnant kidneys display strong overexpression for a number of mediators of inflammation, immune, and acute phase response (Fga, Fgb, Fgg, Hp, Hpxn, IgJ588, Orm1, Orm2, SerpinA3N, Cnlfsf2b). Additionally, some genes with potential importance in renal fibrosis, including haptoglobin (Hp),[48] serum amyloid A2 (Saa2),[49] Fga, Fgb and Fgg,[50] Edn1,[51] and Ren1[52] were overexpressed in remnant kidneys of SNXB2R-KO mice. Overall this suggests that the B_2R is an endogenous protector in this chronic renal disease model. This is also confirmed by the significantly increased glomerular diameter and level of renal fibrosis in the SNX-B_2R-KO mice, compared with SNX-WT and SNX-B_1R-KO mice (Figure 6). Our data support previous findings for the endogenous protective effect of the B_2R against renal fibrosis, inflammation, and glomerular hypertrophy.[53–55]

In conclusion, we used the remnant kidney model to define global changes in renal gene expression during nephropathy progression in 20-week SNX animals, to understand better the molecular mechanisms of advanced renal disease. We discussed several gene groups and corresponding signaling pathways that were differentially expressed in 20-week remnant kidneys and that are functionally related to vascular damage, inflammation, oxidative stress, altered metabolism and transport, fibrosis, and proteinuria, events seen in the evolution of ESRD. A potentially new ESRD pathway was identified, linked to Hnf4a signaling. We also observed altered expression of genes linked with protective and compensatory mechanisms to disease progression. Our data also suggest for a protective role of the kinin B_2R subtype in chronic renal disease and its progression toward ESRD. However, this needs to be further confirmed by performing chronic blockade of kinin receptors in SNX-WT mice with specific antagonists.

Results described in this study underscore the potential of the microarray technology for unraveling the complex mechanisms of advanced renal failure. In perspective, we intend to perform similar gene expression analyses using our remnant WT and kinin receptors KO models in earlier stages following SNX, to better understand the complex molecular mechanisms involved in the kinetics of progression to ESRD and the involvement of kallikrein–kinin system in renal pathology.

MATERIALS AND METHODS

Animals

B_1R-KO and B_2R-KO mice were generated by gene targeting and homologous recombination.[56,57] All experiments were performed with 12–16-week-old male mice from breedings that have been previously backcrossed for 10 generations against a C57Bl/6J strain to homogenize the genetic background of our B_1R-KO and B_2R-KO mice. Initial microsatellite analysis showed that our B_2R-KO mice are genetically comparable with C57Bl/6j mice.[58] Similar results were obtained for the B_1R-KO mice. Accordingly, C57BL/6J male mice (The Jackson Laboratories, Bar Harbor, ME, USA) were used as WT controls. The mice were housed in the animal quarters with a 12-h light/dark cycle and maintained on a standard chow diet (Purina Rodent Chow 5002) and distilled water *ad libitum*. This study was conducted in accordance with the guidelines for the Care and Use of Animals approved by the Animal Protection Committee of Laval University.

Subtotal (5/6) nephrectomy

Three groups of mice (B_1R-KO, B_2R-KO and WT; $n = 6$–8) were submitted to subtotal nephrectomy. The procedure was performed in anesthetized mice (pentobarbital 50 mg/kg intraperitoneally). The left kidney was exposed via a flank incision and both poles were excised (approximately two-thirds of the kidney), leaving a small amount of renal tissue around the left ureter and hilar vessels. Blood loss was minimized by the application of gelatin sponges. After a

7-day recovery period, again under anesthesia, the right kidney was removed, leaving 20–25% of the total renal mass. Renal function was allowed to stabilize for 1 week before further treatments. Sham-operated B_1R-KO, B_2R-KO, and WT animals ($n = 4$–5) that have only underwent laparotomy were used as controls. The animals were killed 20 weeks after the last surgical intervention. A part of the remnant or the control kidney was snap-frozen and stored at -80°C for RNA extraction and microarray analyses, whereas the remaining part was processed for histological analyses. Several biochemical analyses (serum creatinine, urine creatinine, and proteinuria measurements) were performed on urine and blood samples collected before killing of the experimental animals ($n = 4$/group). In unanesthetized mice measurements of binding potential were performed before killing by the tail-cuff plethysmography method, as described previously.[58] Unpaired t-test was used to analyze these data. $P < 0.05$ were considered statistically significant.

Gene expression profiling and data analysis

These experiments included 3–6 pairs of mice per study group. All microarray experiments were performed in multiple replicates. Gene expression analysis was carried out as described previously.[9,59] Labeled cRNAs were applied to the Agilent 44K Mouse Whole Genome Oligonucleotide Microarray (containing 41 534 genes). Cyanine-labeled cRNA (0.75 μg) from one remnant WT kidney (SNX-WT) sample was mixed with the same amount of reverse-color cyanine-labeled cDNA from one WT control (WT-C) kidney sample. Alternatively, cyanine-labeled cRNA (0.75 μg) from one B_1R-KO or B_2R-KO remnant kidney (SNX-B_1R-KO or SNX-B_2R-KO) sample was mixed with the same amount of reverse-color cyanine-labeled cDNA from one WT remnant kidney (SNX-WT) sample. Array hybridization, washing, scanning, data extraction, and analyses were performed as described previously.[9,59] Network analysis of the microarray data was completed using the Ingenuity Pathway Analysis (IPA) software (see http://www.Ingenuity.com).

Semi-quantitative duplex RT-PCR

Validation of microarray data was performed for selected differentially expressed genes by sqRT-PCR as described previously.[9] The glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene was used as internal standard. Primers were designed for these loci with the sequences freely available from the Entrez Nucleotide database and the Primer3 algorithm for primer design (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

Immunohistomorphometric analyses

The development of histological lesions in the renal tissue of the SNX animals and protein expression of different markers was examined using a combination of immunohistochemistry and histomorphometric analyses, as shown previously.[54] The diameter of glomeruli, spanning from the vascular pole to the opposite Bowman's capsule, was measured by using the Explora Nova Mosaïc software (Explora Nova, La Rochelle, France). Statistical analyses were performed using the Graphpad Prism software. Unpaired t-test was performed for comparison between the different groups. $P < 0.05$ were considered statistically significant.

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SUPPLEMENTARY MATERIAL

Table S1. Upregulated genes ($\geqslant$2-fold) in the 20-week remnant WT kidneys as compared with that of the WT control kidneys.
Table S2. Downregulated genes ($\geqslant$2-fold) in the 20-week remnant WT kidneys as compared with that of the WT control kidneys.
Table S3. Genetic networks in the 20-week remnant WT mouse kidneys.
Table S4. Differentially expressed genes (twofold) in the 20-week remnant B1R-KO kidneys as compared with that of the 20-week remnant WT kidneys.
Table S5. Differentially expressed genes (twofold) in the 20-week remnant B2R-KO kidneys as compared with that of the 20-week remnant WT kidneys.
Table S6. Genes, upregulated or downregulated in the B2R-KO SNX experiment in comparison with the B1R-KO SNX experiment (twofold; $P = 0.05$).

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