ONLINE SUPPLEMENT

ELIMINATION OF SEVERE ALBUMINURIA IN AGING HYPERTENSIVE RATS BY EXCHANGE OF TWO CHROMOSOMES IN DOUBLE-CONSOMIC RATS

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Supplemental Methods

Animals and experimental design
For our consomic studies we first generated the single-consomic strains MWF-6^{SHR} and MWF-8^{SHR} by analysing a panel of 240 microsatellite markers, respectively. For breeding of the double-consomic strain MWF-6^{SHR}8^{SHR} we used the same set of 240 microsatellite markers. The interval between the polymorphic markers was on average about 10 centMorgan. All chromosomes except the Y-chromosome were covered. Genotypes were generated by PCR from 50 ng of genomic DNA in a final reaction volume of 10 µl, containing 100 nM each primer, 200 µM dNTPs, 1.5 mM MgCl₂, 10x PCR buffer (Rapidozym, Germany), and 0.4 U/µl of Taq DNA polymerase. The forward primer was labelled with [γ³²P]ATP by T4 polynucleotide kinase. PCR products were processed and subsequently analyzed by autoradiography after polyacrylamide gel electrophoresis. We tested the purity of our double-consomic strain in 22 animals (8 males and 14 females). All tested animals showed only homozygosity for SHR alleles only on chromosome 6 and 8, respectively, and on the other chromosomes exclusively homozygosity for MWF alleles. We selected 3 males and 5 females to establish a colony of the double-consomic strain at our facility. All investigated animals of the current studies were taken from this colony.

Laboratory methods

Urinary albumin excretion (UAE)
Animals were placed in metabolic cages for 2 days. The first day was used for adaptation and urine was collected for the last 24h for UAE analysis measured with a rat-specific ELISA technique.

Glomerular histology and immunohistochemical analysis
In kidney of young animals at 6 weeks of age stainings were performed for desmin and podoplanin as previously described. Briefly, paraffin sections were dewaxed and endogenous peroxidase was blocked. Sections were incubated with primary antibodies, followed by incubation with peroxidase-labeled secondary antibody. Sections were counterstained with hematoxylin. Staining for podoplanin was analyzed by counting the percentage of glomeruli that showed loss of podoplanin in podocytes in one or more segments. For desmin the percentage of glomeruli that showed extra-mesangial expression of desmin were counted. 30 glomeruli per section were scored. Ten randomly chosen regions of the outer glomerular cortex were photographed at 200x magnification with a Zeiss Axioplan microscope equipped with a Sony DXC-950P 3CCD color camera (Sony, Tokyo, Japan). The surface area of all glomeruli in the photographs was measured using Image-J 1.34 software (National Institutes of Health, http://rsb.info.nih.gov/ij). From these measurements, the mean glomerular surface was calculated as described previously. Structural glomerular changes in old MWF, SHR and double-conomic animals were analyzed at 24 weeks of age by determination of glomerular sclerosis index (GSI).

Realtime PCR analysis for Kim-1
Quantitative gene expression analysis of Kim-1 was performed by PCR analysis using the following primers: Kim-1\textsubscript{forward} ATTGTGGCCGAGTGGAGAT and Kim-1\textsubscript{reverse} TGTGGTTGGTGGGTCTTTTGAT. At 24 weeks of age rats (n=7-8, each strain) were killed and the left kidney was excised and snap frozen in liquid nitrogen for subsequent expression analysis. RNA was isolated by the TRIzol® reagent (Invitrogen, Karlsruhe, Germany), according to the manufacturer’s instructions, and was resuspended in DEPC (diethyl pyrocarbonate)-treated water. First-strand cDNA synthesis was carried out on 2 µg of total RNA in a 20 µl reaction using the First Strand cDNA Synthesis Kit (Fermentas Life Sciences,
St. Leon-Rot, Germany), following the manufacturer’s recommendations. To quantify mRNA expression of \textit{Kim-1} we employed a real-time quantitative reverse transcriptase (TaqMan) PCR method using the standard curve method. To normalize our expression data, PBGD (porphobilinogen deaminase) was used as a housekeeping gene (GeneBank® accession no. X06827) \textsuperscript{6}.

\textit{Total nephron number determination}
Total glomerular number per kidney was analyzed in an independent set of young male consomic MWF-6\textsuperscript{SHR}8\textsuperscript{SHR} (n=7) at 4 weeks of age by using the stereoscopic physical fractionator method \textsuperscript{7}. The data were compared to previously reported and in replication experiments confirmed and compared to previously reported nephron numbers of kidneys from SHR, MWF, single-consomic MWF-6\textsuperscript{SHR} and MWF-8\textsuperscript{SHR} animals \textsuperscript{1,2}.

\textit{Blood pressure measurements}
Direct blood pressure measurements by indwelling arterial catheters were performed in MWF and consomic animals at 12 and 24 weeks of age as reported \textsuperscript{8}. Catheters were connected to a pressure transducer system (ADIInstruments, Spechbach, Germany) and 3 repetitive blood pressure recordings were obtained in awake animals on 2 consecutive days, respectively; data were averaged to obtain individual blood pressure values for each rat.
Supplemental Reference List


Figure S1. Desmin and podoplanin expression in spontaneously hypertensive rats (SHR) and double-consomic MWF-6\textsuperscript{SHR8SHR} rats at 6 weeks of age. Panel A, representative photographs of desmin protein staining in glomeruli. Both SHR and MWF-6\textsuperscript{SHR8SHR} exhibited a similar small amount of desmin expression in their glomeruli. Panel B, representative photographs of podoplanin protein staining in glomeruli. Both SHR and MWF-6\textsuperscript{SHR8SHR} exhibited a similar amount of podoplanin expressed in a similar pattern.