

# Gene loci protect the Dahl rat against the development of tubulointerstitial inflammation and microangiopathy in the kidney despite severe salt-sensitive hypertension

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## Introduction

Salt-sensitive hypertension plays a significant role as a factor contributing to the manifestation and progression of cardiovascular and chronic renal diseases. It is a well recognized clinical phenomenon that subgroups of patients with essential hypertension exhibit salt sensitivity and more severe progression of hypertensive target-organ damage over time. Familial aggregation and the higher prevalence of salt-sensitive hypertension in specific ethnic populations point to the potential importance of genetic factors. This is also supported by several genetic rat models that display salt-sensitive hypertension and related target-organ damage as an inherited trait, thus representing an attractive substitute for the investigation of the polygenetic basis of the human disease.

To study the effect of high-salt diet on the genetics of urinary albumin excretion (UAE) and the salt-induced renal tissue damage we used the Dahl salt-sensitive rat (SS) as a model of salt-sensitive spontaneous hypertension and compared it with a spontaneously hypertensive rat (SHR) strain with salt-resistant spontaneous hypertension.

## Methods

All animals used in the present study were males and were obtained from our colonies at the Freie Universität Berlin, Benjamin Franklin Campus.

### Phenotyping

Parental- and F2-animals were treated with a high salt diet (4%) from week 6 to 14. Phenotyping at 15 weeks of age included analysis of systolic blood pressure (SBP), urinary albumin excretion (UAE), urinary protein excretion (UPE), and histological scoring of renal injury parameters. We performed genome-wide linkage analysis to identify quantitative trait loci (QTL) contributing to salt-induced renal injury in an F2-population derived from SS and SHR (n=230).

### Genome screen and QTL mapping

210 polymorphic microsatellite markers were spaced at an average genetic distance of 10 cM. For the first genome screen 23 animals of the lowest and the highest phenotype for UAE, UPE, and SBP, respectively (in total 46 animals) were selected for analysis. Genotyping was completed in all 230 animals for regions with a p-value < 0.01 and for flanking markers.

### Statistical Analysis

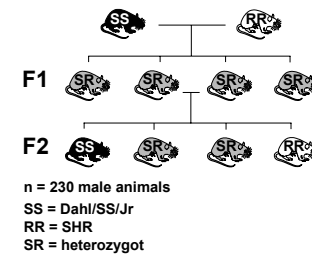
Statistical analysis was performed using MAP-MAKER/QTL and ANOVA.

Statistical criteria (Lander / Kruglyak):

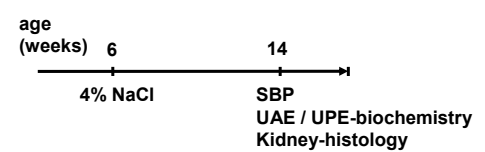
lod ≥ 4.3 ⇒ significant linkage

2.8 ≤ lod < 4.3 ⇒ suggestive linkage

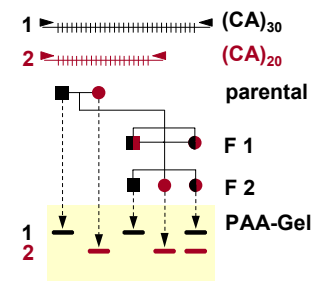
### F2-Intercross



### Experimental design

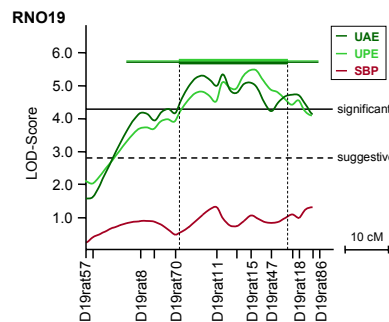
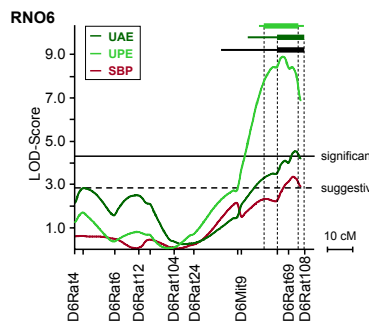
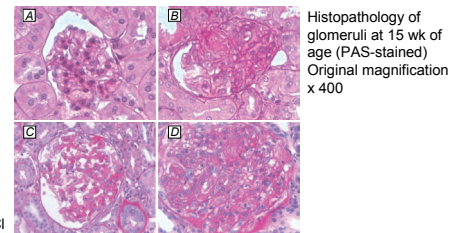
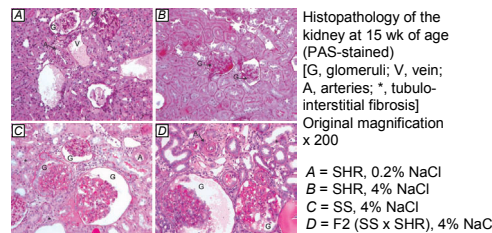
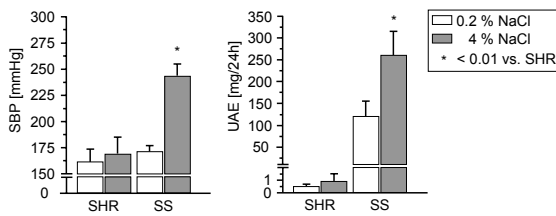


### Genome analysis



## Results

In response to high-salt diet SS developed a striking increase in SBP and urinary albumin excretion (UAE) compared to SHR (244 +/- 12 vs. 169 +/- 16 mmHg and 260 +/- 55 vs. 1.4 +/- 1.2 mg/24h, p<0.001, respectively). SS developed severe glomerulosclerosis (p<0.05) but only mild forms of microangiopathy, tubulointerstitial fibrosis and inflammation compared to SHR. We detected two QTL with significant linkage to UAE and UPE on rat chromosomes (RNO) 6 and 19. Interestingly, some F2-animals demonstrated severe microangiopathy and tubulointerstitial inflammation, that exceeded the mild degree observed in the parental SS strain. Genome-wide QTL analysis revealed 2 loci with suggestive linkage to these phenotypes on RNO3 and RNO5, while no linkage to glomerular damage was found. The maximum effect was found for linkage to tubulointerstitial inflammation on RNO3 (p=0.000085). Further analyses at these loci indicated, that the severity of damage was related to the SHR-allele. Thus, the SS-allele protected against microangiopathy (MICRANG) and tubulointerstitial inflammation (INTINF) at these loci. Moreover, the SHR-allele at D3Mgh9 on RNO3 also demonstrated suggestive linkage to increased SBP (p= 0.0045) and UPE (p= 0.0011).



Parameter	locus	phenotype (means ± SD) according to genotype SHR/SHR	SS/SHR	SS/SS	p-value
UAE [mg/24h]	D6Rat108	43.12 ± 73.60	11.63 ± 31.17	16.20 ± 34.13	0.00011
	D19Rat11	7.50 ± 19.54	22.64 ± 55.47	28.94 ± 42.72	<0.0001
UPE [mg/24h]	D6Rat69	43.52 ± 44.09	20.97 ± 19.72	23.02 ± 24.35	<0.0001
	D19Rat15	16.67 ± 6.03	31.39 ± 38.54	34.98 ± 29.07	<0.0001
SBP [mmHg]	D3Mgh9	43.46 ± 49.65	25.38 ± 27.61	19.89 ± 7.79	0.0011
	D6Rat69	203.8 ± 25.2	190.5 ± 22.3	191.8 ± 21.2	0.0038
MICRANG [grades]	D3Mgh9	203.7 ± 25.3	192.3 ± 22.2	189.4 ± 22.6	0.0045
	D5Mit10	0.40 ± 0.72	0.12 ± 0.33	0.14 ± 0.40	0.028
INTINF [grades]	D5Mit10	0.41 ± 0.69	0.10 ± 0.33	0.12 ± 0.33	0.0014
	D3Mgh9	0.44 ± 0.99	0.78 ± 0.42	0.00 ± 0.00	<0.0001
	D5Mit10	0.37 ± 0.96	0.05 ± 0.35	0.09 ± 0.34	0.0069

## Conclusion

We identified gene loci that confer protection against the development of UAE, UPE, microangiopathy and tubulointerstitial inflammation in salt-sensitive hypertension. Partially these loci are blood-pressure independent. In contrast to all previous studies which indicated collectively that UAE or UPE is largely determined by recessive genetic effects, the SS-allele on RNO19 demonstrated a dominant effect on UAE and UPE. Moreover, our data support a genetic link between microvascular and tubulointerstitial injury in salt-sensitive hypertension.