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Albuminuria is associated with too few glomeruli and too much testosterone

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Normally, the glomerular filtration barrier almost completely excludes circulating albumin from entering the urine. Genetic variation and both pre- and postnatal environmental factors may affect albuminuria in humans. Here we determine whether glomerular gene expression in mouse strains with naturally occurring variations in albuminuria would allow identification of proteins deregulated in relatively 'leaky' glomeruli. Albuminuria increased in female B6 to male B6 to female FVB/N to male FVB/N mice, whereas the number of glomeruli/kidney was the exact opposite. Testosterone administration led to increased albuminuria in female B6 but not female FVB/N mice. A common set of 39 genes, many expressed in podocytes, were significantly differentially expressed in each of the four comparisons: male versus female B6 mice, male versus female FVB/N mice, male FVB/N versus male B6 mice, and female FVB/N versus female B6 mice. The transcripts encoded proteins involved in oxidation/reduction reactions, ion transport, and enzymes involved in detoxification. These proteins may represent novel biomarkers and even therapeutic targets for early kidney and cardiovascular disease.

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Normally, macromolecules such as albumin are almost completely excluded from entering the filtrate by the glomerular filtration barrier consisting of endothelia, podocytes, and glomerular basement membrane.[1] Major barrier disruptions, as occur in individuals with mutations of slit diaphragm genes, cause massive protein leakage.[2,3] More moderate albumin excretion above the normal range, or 'microalbuminuria' (30–300 mg per 24 h), may also be clinically important, as may variations within the so-called normal range. In individuals with diabetes mellitus, microalbuminuria generally precedes, and may predict progression to nephropathy.[4,5] Indeed, filtered proteins, or bound molecules, may be tubulotoxic.[6,7] Microalbuminuria is an independent risk factor for cardiovascular mortality and morbidity not only in individuals with diabetes mellitus or systemic hypertension but also in the general population.[8] This association may be explained by increased albuminuria being just one manifestation of a generalized microvascular disturbance.[8]

In normotensive US adolescents, albumin excretion rate is higher in blacks than in whites.[9] In US adults, the prevalence of microalbuminuria is greater in non-Hispanic blacks and Mexican Americans as compared with non-Hispanic whites.[10] The importance of genetic background in determining albuminuria is supported by observations of inbred 'normal' mice.[11] Tsaih *et al.*[11] examined mice at advanced ages of 12–24 months, reporting up to a 100-fold difference of albuminuria between strains. Within certain strains (for example, A/J, C57BL/10J, and FVB/NJ), males excreted more albumin than females, whereas the opposite held in other strains (for example, BUB/BnJ and SJL/J). In healthy adults in the Netherlands, men had a higher average urinary albumin excretion rate than women (10 vs. 8 mg per 24 h), and the prevalence of microalbuminuria was twofold higher in males, even after controlling for smoking, hypercholesterolemia, and obesity.[12] In nondiabetic white UK adults, men had a higher albumin excretion rate than women; microalbuminuria in men was associated with short stature, whereas hypertension positively correlated with albumin excretion rate in women.[13]

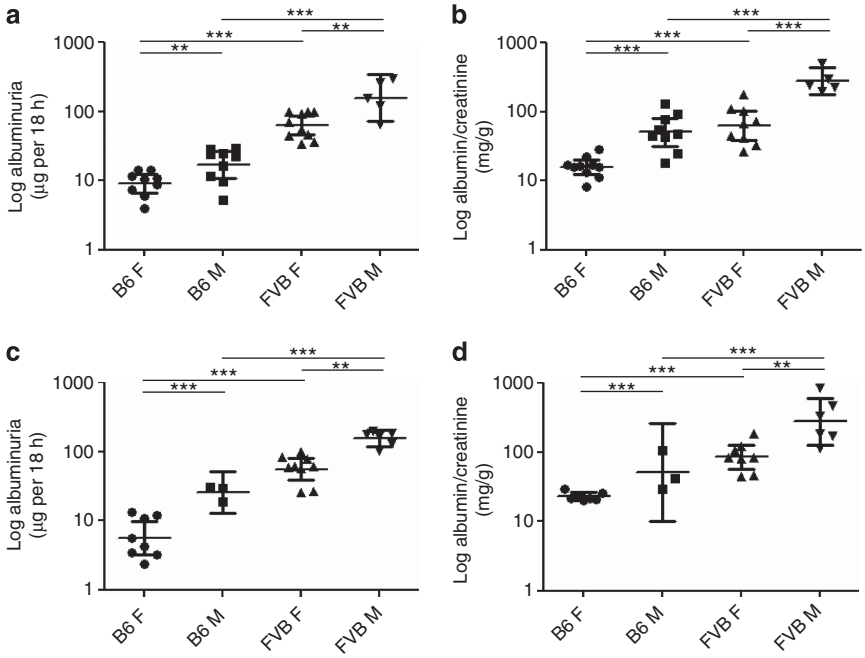

Figure 1 | Albuminuria in B6 and FVB/N mice. Overnight (**a**, **c**) albumin excretion and (**b**, **d**) albumin-to-creatinine ratios were evaluated in (**a**, **b**) 18-week-old and (**c**, **d**) 13-week-old adult mice. Data were log transformed before analysis and are presented as geometric means and confidence interval. There was a significant increase in urinary albumin in male (M) and female (F) FVB/N mice compared with sex-matched B6 animals. Within each strain, males had elevated albuminuria versus females (**$P<0.01$, ***$P<0.001$ between groups).

The antenatal environment to which an individual is exposed may influence urinary albuminuria. Adults gestated during the Dutch famine in World War II had an increased risk of microalbuminuria.[14] Possibly, maternal undernutrition led to birth of individuals whose kidneys contained fewer nephrons than normal and subsequent compensatory changes would have resulted in loss of filtration barrier functionality.[15,16] Indeed, rodent embryos exposed to maternal low-protein diet form kidneys with fewer glomeruli than normal.[17] Interestingly, within the general human population there exists considerable variation in the numbers of glomeruli per kidney,[18] with normotensives having about $1–2\times10^6$ glomeruli per kidney and adults with essential hypertension having approximately $0.5–1\times10^6$ glomeruli per kidney.

The above evidence is consistent with the contentions that genetic background and sex modify albumin excretion rate. We speculated such variations in albuminuria would relate to alterations in glomerular biology and/or numbers. We hypothesized that studying mice with naturally occurring variations in albuminuria would allow us to identify genes deregulated in 'leaky' glomeruli.

RESULTS

Urinary albumin excretion in B6 and FVB/N mice

The urinary albumin excretion rate in adult (18 weeks old) female and male FVB/NHanHsd (FVB/N) mice was, on average, eightfold more than age- and sex-matched C57BL/6JOlaHsd (B6) mice (Figure 1a). Within each strain, males had increased albumin excretion rates than females (twofold elevations in both B6 and FVB/N strains; Figure 1a). The same patterns were apparent when albumin/creatinine concentration was measured (Figure 1b). Albumin excretion rates (Figure 1c) and albumin/creatinine (Figure 1d) were quantified in separate sets of mice aged 13 weeks, with the same patterns noted between the strains and sexes. There were no significant differences in albuminuria between mice of the same sex and strain at 13 versus 18 weeks.

Glomerular gene expression *in vivo*

Eighteen-week-old mice were perfused with magnetic beads that accumulate in glomerular capillaries (Figure 2a). Magnetically isolated glomeruli usually consisted of the tuft alone, although others also contained a capsule (Figure 2b). mRNA integrity was preserved in isolated glomeruli (Figure 2c). For each group, we undertook three sets of RNA microarrays, each from a separate mouse. We identified a common set of 39 genes significantly differentially expressed in each of the comparisons: female FVB/N versus female B6 mice; male FVB/N versus male B6 mice; male versus female B6 mice; and male versus female FVB/N mice. Expression levels of 34 transcripts positively correlated with albuminuria (Table 1), whereas levels of five others negatively correlated with albuminuria (Table 2). Several upregulated transcripts coded for proteins involved in oxidation/reduction reactions (*Aass*, *Aldh1l1*, *Cyp4a12a*, *Hsd3b2*, and *Ldhd*) or ion transport (*Slc22a2*, *Slc22a6*, *Slc5a8*, and *Slco1a1*). Other upregulated transcripts included: *Acy3* and *Tst*, coding for enzymes involved in detoxification; *Treh*, an enzyme hydrolyzing trehalose; *Aqp1*, coding for aquaporin-1; and *Hpn*, coding for hepsin, a serine protease. Downregulated transcripts