Uromodulin gene variants and their association with renal function and blood pressure in cats: a pilot study

[a#]**Rosanne E. Jepson BVSc MVetMed PhD DipACVIM DipECVIM MRCVS**

[c]**Helen R. Warren BSc, PhD**

[a]**Harriet M. Syme BSc BVetMed PhD DipACVIM DipECVIM MRCVS**

[b]**Jonathan Elliott MA Vet MB PhD DipECVPT MRCVS**

[c]**Patricia B. Munroe BSc, MSc, PhD**

[#]*Corresponding author:*

rjepson@rvc.ac.uk

Tel: +44 (0) 1707 666366

Office: +44 (0) 1707 667033

[a]Royal Veterinary College, Hawkshead Lane, Nr Hatfield, Herts, AL9 7TA

[b]Royal Veterinary College, Royal College Street, London, NW1 0TU

[c]Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ

This study was supported by a PetSavers Clinical Research Project Grant.

Objectives: In human medicine, genome wide association studies have identified genetic variants in uromodulin (*UMOD*), which have been associated with blood pressure (BP) and renal function. Given the homology of *UMOD* between mammalian species, the goal of this study was to evaluate the association of currently annotated single nucleotide polymorphisms (SNPs) at the feline *UMOD* locus with both renal function and BP.

Methods: Cats aged 14 years with systolic blood pressure (SBP) and renal function measures, and DNA samples were retrospectively selected for analysis. SNPs in the feline *UMOD* gene were identified, and association between *UMOD* SNPs and renal function (assessed by plasma creatinine concentration), and systolic blood pressure (SBP) as continuous variables were explored. Longitudinal data was used to determine associations between genotype and the dichotomous diagnoses of chronic kidney disease (CKD) and systemic hypertension.

Results: Eight intronic SNPs, one 1372 base-pairs up-stream from UMOD and two exonic SNPs were evaluated in 227 cats with renal and BP data. An analysis of 188 cats (where BP modifying therapy was not used) found four SNPs ($p<0.01$) to be significantly associated with SBP (g.9879T>C, g.9858T>C, g.9764A>C, g.8539A>C) although all were in linkage disequilibrium (LD). No significant associations were identified between SNPs and renal function or CKD.

Clinical Significance: The results of this pilot study suggest that genetic variation in *UMOD* might influence BP in cats, similar to findings in humans and provides potential insights into the pathophysiology of hypertension in this species. The

pathophysiology of this association is incompletely understood but is hypothesized to relate to sodium and water homeostasis involving the apical Na^+ K^+ $2Cl^-$ cotransporter in the thick ascending limb of the loop of Henle.

Keywords: Cats, kidney, uromodulin, hypertension, chronic kidney disease

Introduction:

Uromodulin, otherwise known as Tamm-Horsfall protein, is a glycosylphosphatidylinositol-anchored protein that is expressed on the luminal surface of renal tubular cells of the thick ascending limb (TAL) of the loop of Henle (Vyletal et al. 2010). From this location, yet to be identified proteases release uromodulin into the urine, where it represents one of the most abundant urinary proteins in all mammalian species, and forms high molecular weight polymers (Vyletal et al. 2010). Despite uromodulin being identified in the 1950s its physiological function is still incompletely characterised. Nevertheless, uromodulin has been proposed to play an important role in the formation and trafficking of apical membrane-targeted cargo vesicles. The complex gel-like filamentous structure that it forms on the apical surface of the TAL is believed to provide a barrier to water permeability in that region and simultaneously regulate ion transportation. It has also been hypothesised that uromodulin is a receptor for binding of certain ligands which may link uromodulin to cell surface events and that localisation to cilia may indicate that uromodulin plays a role in mechanosensitisation to urinary flow and therefore intracellular signaling pathways. Within urine, it has been suggested that uromodulin maintains its gel-like properties retarding the passive passage of positively-charged electrolytes

such as sodium and potassium through the TAL whilst facilitating active absorptive mechanisms. In the distal tubule, it has been suggested that uromodulin may bind pathogenic bacterial strains helping to prevent urinary tract infections and in a similar manner may also act as an inhibitor of urinary stone formation (Vyletal et al. 2010, Rampoldi et al. 2011).

In feline medicine, similar to other mammalian species, uromodulin has been localised to the TAL of loop of Henle (Brandt et al. 2012). Urinary uromodulin has predominantly been explored in relation to struvite and calcium oxalate calculus formation (Rhodes et al. 1993, Buffington et al. 1994, Matsumoto & Funaba 2008, Lulich et al. 2012). More recently, differential expressions of urinary proteins including uromodulin were identified in the urine of cats with chronic kidney disease (CKD) by two-dimensional gel electrophoresis (Ferlizza et al. 2015).

In humans, urinary uromodulin concentrations decline with a variety of renal diseases (Thornley et al. 1985, Torffvit et al. 1998, Kottgen et al. 2010, Lhotta 2010, Prajczer et al. 2010). Mutations in the UMOD gene encoding for uromodulin have been identified and associated with a series of conditions collectively referred to as uromodulin-associated kidney disease (UAKD)(Rampoldi et al. 2011, Eckardt et al. 2015). UAKD represent autosomal dominant disorders for which over 50 mutations have been identified and that are characterised by tubulointerstitial fibrosis, hyperuricaemia, development of renal cysts at the corticomedullary junction and loss of urine concentrating ability (Bleyer et al. 2011, Eckardt et al. 2015). More recently, interest in genetic variation within the UMOD gene has extended beyond monogenic conditions. Genetic variations

within UMOD have been identified, from genome-wide association studies (GWAS) and meta-analyses, to be associated with estimated glomerular filtration rate (eGFR), CKD, incident CKD, decline in renal function and end-stage renal disease (Kottgen et al. 2009, 2010, Gudbjartsson et al. 2010, Boger et al. 2011, Reznichenko et al. 2012, Gorski et al. 2015). Genetic variants in UMOD have also been associated with hypertension (HT) using both GWAS and candidate gene approaches (Padmanabhan et al. 2014, Cabrera et al. 2015). An extreme case–control GWAS study of European individuals identified that the minor allele for SNP rs13333226 was protective against HT (Padmanabhan et al. 2010). When the association of rs13333226 with continuous blood pressure measurement was evaluated, the minor allele was found to be significantly associated with a 0·5 mmHg lower systolic blood pressure (SBP) and 0·3 mmHg lower diastolic blood pressure, results of which are concordant with the odds of HT (Padmanabhan et al. 2010). This discovery study was subsequently validated in a large-scale case–control study in which this protective effect was replicated (Padmanabhan et al. 2010). Studies by Han et al. and Iwai et al. evaluated single nucleotide polymorphisms (SNPs) in UMOD as a candidate gene in Chinese and Japanese populations, respectively (Iwai et al. 2006, Han et al. 2012).

Both CKD and systemic HT are common conditions in the ageing feline population, which may be considered as complex disease traits likely influenced by both genetic and environmental factors (Lulich et al. 1992, Syme et al. 2002, 2006, Jepson 2011, Marino et al. 2014). To date there have been no studies that have evaluated potential genetic associations in these conditions. Indeed the concept of investigating genetic associations in complex disease traits in cats is novel. The

aim of this study was to build on the known homology and conservation of the UMOD gene across mammalian species, and using publically-available genetic polymorphism data for the feline UMOD gene to evaluate associations between genotype and the continuous traits of renal function and SBP, and the dichotomous traits of CKD and systemic HT. Further information about the design and interpretation of genetic association studies can be found in the following review articles: Cordell & Clayton 2005, Hattersley & McCarthy 2005, Palmer & Cardon 2005, Bush & Moore 2012.

Materials and Methods:

Case selection:

Cats included in this study were selected retrospectively from a computerised database containing clinical data for cats that had participated in a longitudinal geriatric cat monitoring programme. All cats had been evaluated at one of two first opinion clinics in (Beaumont Sainsbury Animal Hospital, Camden, London and People's Dispensary for Sick Animals, Bow, London) and at enrollment to the longitudinal programme a full history had been obtained, physical examination performed and SBP assessed as previously described using the Doppler technique (Syme et al. 2002).

The collection and storage of blood samples was performed with owner consent and the protocols adhered to within this study had been approved by the Ethics and Welfare Committee at the Royal Veterinary College, London, UK. Blood samples were obtained by jugular venipuncture and collected into lithium heparin and EDTA. Samples were held on ice (4°C) for a maximum of 6 hours before

centrifugation and separation. Plasma biochemical analysis (Idexx laboratories), packed cell volume and total protein evaluation were routinely performed for all cats on enrollment to the longitudinal monitoring programme. Total serum thyroxine concentration was measured in all cats in which the history (e.g. polyphagia, weight loss), physical examination findings (e.g. tachycardia, arrhythmia, poor body condition, palpable goiter), or serum biochemical findings (increased alanine transferase or alkaline phosphatase activities) raised concern for hyperthyroidism. In all cases in which the urinary bladder was palpable, a urine sample was collected by cystocentesis. For every cat enrolled in the longitudinal monitoring programme residual sample (EDTA, serum, heparinised plasma) and EDTA cell pellets mixed with a 1:1 ratio of EDTA-phosphate buffered saline were stored at −80°C, the latter to be used for genomic DNA extraction.

Cats that were considered healthy on the basis of these data were offered re-examination on a 6-monthly basis. Cats were diagnosed with azotaemic CKD if plasma creatinine was greater than laboratory reference interval (177 μmol/L) on two occasions a minimum of 4 weeks apart in association with inappropriate urine concentrating ability (urine specific gravity<1·035). Cats were diagnosed with systemic HT if SBP was greater than 170 mmHg on one occasion in association with hypertensive ocular target organ damage or if SBP greater than 170 mmHg on at least two occasions.

Cats diagnosed with hyperthyroidism, HT and/or CKD either at enrollment or at subsequent visits in the longitudinal monitoring programme were offered standard management and re-examined every 8 weeks. At each re-examination

visit clinical information was reviewed and physical examination including repeat assessment of SBP and bodyweight performed. At every other visit (i.e. every 16 weeks), blood and urine samples were obtained. For cats diagnosed with hyperthyroidism this included assessment of total thyroxine and renal parameters providing euthyroidism was maintained. For cats with a diagnosis of CKD or systemic HT this included assessment of renal parameters. When urine could be obtained a full urinalysis including specific gravity, dipstick and sediment examination was performed. Urine culture was performed for patients with compatible clinical signs (stranguria, dysuria, pollakiuria, haematuria) or where there was indication on urine sediment examination (pyuria, bactiuria, haematuria) for a urinary tract infection.

Cats diagnosed with CKD were provided with a commercially available renal diet free of charge and, where indicated in accordance with the IRIS guidelines, additional intestinal phosphate binder and potassium supplementation. Systemic HT was routinely treated with amlodipine besylate (0·625 to 2·5 mg/cat once a day) to a target SBP less than 160 mmHg. Hyperthyroidism was initially managed medically with the option for uni-/bi-lateral surgical thyroidectomy. Data from every cat enrolled in the longitudinal monitoring programme from every visit were collated within a searchable computerised database. Longitudinal monitoring was provided for cats for the duration of their life or until the client elected to withdraw from the study. Cats were excluded from the longitudinal monitoring programme only if significant concurrent disease precluded provision of care through the clinic, e.g. diabetes mellitus.

Data from approximately 2900 cats greater than nine years old available on the computerised database were initially screened. Cats for inclusion in the current genetic association study were selected from the computerised database by identification of the first visit after they turned 14 years old, where full biochemical and SBP data were available, as well as a stored cell pellet for genomic DNA extraction. Cats were excluded if they were identified as newly-diagnosed or uncontrolled hyperthyroid at the visit of interest. Cats diagnosed with hyperthyroidism but adequate control of hyperthyroidism (total T4 10 to 45 nmol/L) documented at the time of biochemical assessment were included in the study. Ultimately, all cats identified from the computerised database fulfilling the inclusion and exclusion requirements and from which genomic DNA was available were used in this study. Sample size was therefore driven by availability. In addition, when evaluating the association of genotype with SBP at enrollment, cats were excluded if they were receiving antihypertensive medication, typically amlodipine besylate, or medications that might modify BP, e.g. angiotensin receptor blocker, angiotensin converting enzyme inhibitor, beta-blocker.

Data available permitted cross-sectional evaluation of the outcome variables relating to renal function (quantitative creatinine and dichotomous CKD diagnosis) and quantitative SBP at the first visit when cats were aged 14 years and also longitudinal evaluation of the development of systemic HT throughout follow-up.

<u>Single nucleotide polymorphism identification:</u>

The currently reported coding sequence of UMOD was explored (Ensembl http://www.ensembl.org/Felis_catus/Info/Index; ENSFCAG00000004381. GenBank Assembly ID GCA_000181335.1, Felis_catus_6.2 ChrE3: 27156434-27168778). Previously published annotated single nucleotide polymorphisms (SNPs) within either intron and exon regions of the *UMOD* gene were identified using a previously available genome assembly (http://genome-euro.ucsc.edu NHGRI/GTP V17e/felcat4/GenBank assembly accession: GCA_000003115.1) and a recent release of annotated feline SNPs (http://public.dobzhanskycenter.ru/Hub/hub.txt) which were mapped to the Felis_catus_6.2 genome assembly (Mullikin, Hansen et al. 2010, Tamazian, Simonov et al. 2014). Location of SNPs within the *UMOD* gene sequence was confirmed by blast search against the feline, canine and human nucleotide collection (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Polyphen (http://genetics.bwh.harvard.edu/pph2) was used to predict the impact of the amino acid substitution on the structure and function of *UMOD* for non-synonymous SNPs within *UMOD* exons.

Genotyping:

Genomic DNA (gDNA) was extracted from buffy coat enriched packed cells using a commercially available kit (Sigma GenElute Blood Genomic DNA kit, Sigma-Aldrich Company Ltd.) according to the manufacturer's instructions and subsequent spectrophotometric (Nanodrop 1000 Spectrophotometer, Thermo Scientific) quantification. gDNA was diluted with nucleic acid-free H2O (Water, Molecular Biology Reagent, Sigma-Aldrich Company Ltd) to a final concentration of 5 ng/µL for genotyping.

For all evaluated SNPs, primers were designed based on the SNP locus sequence for a PCR-based competitive allele-specific PCR (KASP™) genotyping assay (KASP™, LGC Genomics) using Primerpicker [Primepicker (previously KBiosciences) LGC Genomics, KBS-1016-022 (25 mL) KASP™ Master Mix, LGC Genomics]. This produced two allele-specific oligonucleotides with unique 5′ tails and one common reverse oligonucleotide. For each genotyping assay, the allele-specific and common primers were diluted in nucleic acid-free H2O to a final concentration of 100 μM and combined as a SNP-specific assay mix (12 μL allele-specific primer 1+12 μL allele-specific primer 2+30 μL common primer+46 μL dH2O). Buffer mix was prepared using KASP™ assay mix (KBS-1016-022 (25 mL) KASP™ Master Mix, LGC Genomics) [containing universal fluorescent resonance energy transfer (FRET) cassettes with the dyes FAM and VIC, high ROX™ passive reference dye, Taq polymerase and free nucleotides] and MgCl2 (50 mM) to give a final concentration of 1·8 mM.

For genotyping assays, 7·5 ng of gDNA was applied per well using a 384 well format (AB Biosystems) and air-dried at 65°C for a minimum of 2 hours or overnight. A master mix was prepared by the addition of 16 μL SNP-specific assay mix and 1008 μL buffer mix with 2·5 μL added to each well before covering with optical adhesive PCR film. The following PCR cycling sequence (Tetrad PTC 225 Peltier Thermocycler (previously MJ Research), BIO-RAD, Hemel Hempstead) was used for all assays: 94°C for 15 minutes, 10 cycles of 94°C for 20 seconds with touch down over 65 to 70°C for 60 seconds (reducing by 0·8°C per cycle), 26 cycles of 94°C for 20 seconds and 57°C for 60 seconds. Allelic discrimination was

274 performed (ABI PRISM® 7900HT, Applied Biosystems, Thermo Fisher Scientific)
275 with manual cluster detection (SDS 2.3, Applied Biosystems, Thermo Fisher
276 Scientific). Genotyping assays were initially tested across 24 cats and assays that
277 performed well were taken forwards for genotyping of all cats using a randomised
278 format and including nucleic acid-free H2O (n=8) as a negative control.
279
280 Statistical analysis:
281 Summaries of the clinical data are presented in Table 1, showing the median and
282 interquartile range (IQR) for each variable. A non-parametric Mann–Whitney U
283 test was used to compare these clinical variables as well as the duration of follow-
284 up between normotensive and hypertensive cats and between cats diagnosed
285 with azotaemic CKD aged 14 years and those, which were non-azotaemic aged 13
286 years (see Tables 2 and 3). For all clinical statistical analyses $P<0{\cdot}05$ was
287 considered significant.
288
289 Associations between quantitative and binary response variables and SNPs were
290 performed using PLINK with either linear or logistic regression models
291 respectively (Purcell et al. 2007). Quantitative variables were assessed for
292 normality by visual inspection of histograms and also the Kolmogorov–Smirnov
293 test. Due to skewness, creatinine concentration was logarithmically transformed
294 before analysis. Genotype frequencies, minor allele frequency (MAF), Hardy–
295 Weinberg equilibrium (HWE; using the exact Hardy–Weinberg test) and linkage
296 disequilibrium (LD) were evaluated. MAF greater than 10% was chosen to include
297 only common variants and therefore to improve power to detect a significant
298 association given the relatively small sample size. For quality control, summary

299 statistics were checked for the genotype call rates per SNP and per cat, and a SNP
300 call rate threshold of greater than 90% was used. Pairwise LD values were
301 calculated in PLINK using data from our cohort of cats due to lack of available
302 known feline LD reference data (unlike in human GWAS). An r2 value of >0·5 was
303 used based on suggested threshold from PLINK of 0·5 "being necessary to declare
304 that one SNP tags another" (Purcell et al. 2007). An additive model was used for
305 all SNP associations, with results corresponding to a per allele unit effect. For
306 genotyping analyses Bonferroni correction was applied to adjust for multiple
307 testing. Based on pairwise LD the SNPs reduced to a set of five pairwise
308 independent SNPs (Table S2) and therefore for genotyping analyses statistical
309 significance was defined as P<0·01 (using Bonferroni correction: P=0·05/5).
310
311 Associations were evaluated for the quantitative variables log-creatinine and SBP
312 at the time of biochemical assessment aged 14 years and for the binary outcome
313 of diagnosis of azotaemic CKD aged 14 years. Longitudinal clinical data available
314 from computerised records for all cats were assessed in order to determine
315 whether, during their entire period of monitoring, cats had ever been diagnosed
316 with systemic HT. A single case–control association was then subsequently
317 explored with the binary outcome of ever becoming hypertensive versus
318 remaining normotensive during the available period of follow-up. For both the
319 quantitative trait SBP and the binary outcome hypertensive/normotensive
320 analyses, log-creatinine concentration was included as a covariate for adjustment
321 based on potential association between renal function and likelihood of
322 developing HT. For the quantitative variable log-creatinine and the binary
323 outcome of diagnosis of CKD aged 14 years no covariates were included.

Results:

Stored cell pellets were available for 227 cats. The median age of cats was 14·4 years (IQR 14·2 to 4·6 years). Of this population of cats 78·4% (n=178) were domestic shorthair, 8·8% domestic longhair (n=20) with the following breeds also represented; Burmese n=10 (4·4%), Persian n=6 (2·6%), Persian cross n=3 (1·3%), with two each of the following breeds; British blue, Russian blue cross, Siamese and one each of the following breeds; American shorthair, Maine coon, occicat, Russian blue.

Clinical and biochemical data for cats at recruitment to the study are provided in Table 1. Hyperthyroidism had previously been diagnosed in 19% (44/227) of cats and was documented to be well-controlled, both on the basis of clinical signs and total thyroxine measurement (n=44 median total thyroxine 22·9 nmol/L; IQR 17·1 to 31·7 nmol/L). Twenty-one of the cats that had been diagnosed with hyperthyroidism had previously undergone either uni- or bi-lateral thyroidectomy and therefore were not receiving any antithyroid medication at the point of enrollment. At the time of initial assessment 33·9% (77/227) of cats had been diagnosed with azotaemic CKD. Clinical data are compared between cats diagnosed with azotaemic CKD and non-azotaemic cats at entry to the study in Table 2. As may be anticipated cats diagnosed with azotaemic CKD had significantly higher plasma creatinine concentration, lower packed cell volume and urine specific gravity. Potassium concentration was unexpectedly significantly higher in cats with azotaemic CKD (P=0·034) and, despite selection

of cats from the 14th year of life, cats with azotaemic CKD at enrollment were younger (P=0·02) than non-azotaemic cats.

Systemic HT had previously been diagnosed in 17% (39/227) of cats at enrollment in the study of which all were receiving amlodipine besylate therapy and 46% (18/39) of these hypertensive cats had been diagnosed with azotaemic CKD. Clinical records for all 227 cats were reviewed to determine whether systemic HT developed during their period of follow-up. The median period of follow-up for all cats from the date of sampling in their 15th year until death, euthanasia or the study end point (end of December 2014) was 850 days (399, 1218 days). During this period, 81 cats were diagnosed with systemic HT and 146 remained normotensive and there was no significant difference in duration of follow-up between groups (Table 3). Twenty-one cats went on to develop hyperthyroidism of which 23·8% (5/21) underwent thyroidectomy and the remainder received medical management. Clinical data at entry to the study are compared between cats that developed systemic HT and those that remained normotensive throughout follow-up (Table 3). Potassium and urine specific gravity were significantly lower (P<0·05) in cats at enrollment if they were diagnosed during follow-up with systemic HT than if they remained normotensive.

<u>Single nucleotide polymorphism identification:</u>

Eight intronic SNPs and one SNP 1372bp upstream of the feline UMOD reference sequence were identified (Table 4) using published data by Mullikin and colleagues (2010) from a previously available genome assembly (NHGRI/GTB

V17e/felcat4) GenBank assembly GCA_000003115.1). KASP™ assays were designed for genotyping (Supplemental Table A)(Mullikin et al. 2010). Location within the predicted *UMOD* gene sequence (Supplemental data Figure 1) from the current genome assembly was confirmed by performing a nucleotide BLAST search against the feline nucleotide collection. Genotype frequencies are reported in Table 4.

A further three exonic UMOD SNPs were identified using recently published data by Tamazian et al. (Table 4). Two of these SNPs (g.1381T>A and g.1664A>G) are non-synonymous and located in exon 2; one is a synonymous SNP (g.4635T>C) located in exon 5 (Fig S1). Polyphen was used to predict the impact of the amino acid substitution on the structure and function for both non-synonymous SNPs (Adzhubei et al. 2010). The two SNPs were considered as benign (g.1381T>A, p.ser74thr: score 0·009, sensitivity 0·96, specificity 0·77 and g.1664A>G, p.asp168gly: score 0·002, sensitivity 0·99, specificity 0·3). KASP™ assays were designed for all three SNPs but were successful for only two (exonic g.1381T>A and intronic g.4635T>C). Ultimately the exonic non-synonymous SNP g.1381T>A which was considered benign using Polyphen modelling did not have a MAF greater than 10% and therefore was not evaluated within association studies. Genotyping frequency data are presented in Table 4.

One reported SNP (g.1664A>G) failed to genotype in any of the cats and was excluded from further analysis with uncertainty whether this represented primer failure or that this was not a true SNP. Overall genotype rate was 0·967561 and all SNPs demonstrated a genotype failure rate less than 10%. Forty-eight cats failed

to genotype in ≥1 SNP (28 cats failed in one SNP, 10 cats failed two SNP, four cats failed three SNPs, four cats failed four SNPs and one cat failed in five SNPs). However, given the relatively small sample size all cats were retained in the study. Evaluating genotype data from all cats, seven SNPs had a MAF greater than 10% (Table 4) and were used for further evaluation, restricting to analysis of common variants as appropriate for this sample size. HWE data are presented in Table 4. LD pairwise comparison identified that there were five independent SNPs (Table S2; g.4635T>C, g.1381T>A, g.6902C>T, g.3390G>A and 5′ upstream 1372 bp G>A).

Association between UMOD genotype and renal function

Seven SNPs with MAF greater than 10% (Table 4) were analysed for associations with renal function using the quantitative trait log-creatinine and the binary outcome of being diagnosed with azotaemic CKD in 15th year of life or being non-azotaemic. We observed no significant association between SNPs and log-creatinine (Table S3). Similarly there were no SNPs significantly associated with the diagnosis of CKD as a binary variable (all had P>0·01; Table S4).

Association between UMOD genotype, systolic blood pressure and systemic hypertension

After exclusion of cats that were receiving antihypertensive or BP-modifying medication at the time of enrollment, 188 cats were available for evaluation of association between genotype and SBP as a continuous variable (Table S6). Seven SNPs demonstrated MAF greater than 10% (Table S5) and were included in the analysis (Table S6). Four SNPs were significantly associated (P<0·01; Table 5)

with SBP as a quantitative variable, adjusted for plasma creatinine as a covariate (Table S6) but all four were in LD, suggesting one overall distinct association signal.

Clinical record data for all 227 cats were reviewed in order to categorise cats as normotensive or hypertensive during their period of follow-up at the clinics (Table 3). No SNPs with MAF greater than 10% were significantly associated with the hypertensive state (all had P>0·01; Table S7).

Discussion:

This study demonstrates that genetic variants in UMOD are significantly and positively associated with SBP but not with systemic HT as a specific outcome. This finding is comparable to the associations that have been made to date in human medicine (Iwai et al. 2006, Padmanabhan et al. 2010, Han et al. 2012). The SNP identified in human medicine (rs13333226), located within the promotor region of UMOD has been associated with a lower risk of HT (Padmanabhan et al. 2010). However, in our current study, SNPs that reached statistical significance were associated positively with SBP. The four SNPs that demonstrated association with SBP were not independent and shown to be in LD. There is relatively little known about LD in cats and the values generated for the current study were inferred from this population alone using an LD r2 value that was lower than typically applied to human studies (Alhaddad et al. 2013). The SNPs where significant association was identified were intronic. Therefore any effect from these SNPs will not be the result of structural change in the uromodulin amino acid sequence but could reflect, for example, alteration in splicing or post-

translational modifications (Shastry 2009). The overall effect of SNPs significantly associated with SBP in this study appears proportionally large compared to effects identified in human medicine. It can be hypothesised that potentially the cat may be different from the human in terms of the complexity of SBP as a trait giving rise to this greater effect. However, further work is required to validate the SNPs identified in independent cohorts of cats in order to establish this association.

The mechanism by which genetic variation in UMOD is associated with control of BP is incompletely understood. However, studies suggest that this may relate to alteration in permeability of the TAL of the loop of Henle to water and modulation of sodium handling by the apical Na+K+2Cl− cotransporter (NKCC2). Recent studies have used uromodulin knockout mice to further elucidate the role played by uromodulin. Uromodulin knockout (UMOD−/−) mice demonstrate no abnormalities in electrolyte balance but do show significantly reduced creatinine clearance and impaired urine-concentrating ability and decreased NKCC2 activity (Bachmann et al. 2005, Mutig et al. 2011). Transfection of TAL cells with uromodulin resulted in increased concentration of phosphorylated NKCC2 and increased intracellular chloride concentration indicating that uromodulin plays an important facilitating role in absorption of sodium and activity of the NKCC2 cotransporters within the TAL (Mutig et al. 2011, Trudu et al. 2013). In addition, knockout studies suggest that uromodulin may regulate expression of other channels including, amongst others, the renal outer medullary potassium channel (ROMK2) (Bachmann et al. 2005, Renigunta et al. 2011). This is a potentially interesting concept given that cats with systemic HT have previously been shown to have significantly lower plasma potassium concentrations than their

473 normotensive counterparts, a finding which was also seen in the present study
474 (Syme et al. 2002, Bijsmans et al. 2015).
475
476 A study by Graham et al. has demonstrated that UMOD−/− mice have significantly
477 lower SBP (116·6 ±0·3 mmHg) than wild-type mice (136·2 ±0·4 mmHg) and that
478 the knockout mice show no response in terms of alteration in BP to sodium
479 loading (Graham et al. 2014). The pressure-natriuresis curve was also shifted to
480 the left in UMOD−/− mice (Graham et al. 2014). A further study conversely
481 demonstrated that over-expression of uromodulin resulted in increased
482 uromodulin excretion and increased BP (Trudu et al. 2013).
483
484 TNFα has also been shown to downregulate NKCC2 expression in an autocrine
485 manner and it has been suggested that TNFα may be a link between the intra- and
486 extra-cellular roles of uromodulin and BP regulation (Battula et al. 2011);
487 UMOD−/− mice showed increased urinary TNFα concentrations compared to
488 wild-type mice (Graham et al. 2014). Cells from the TAL were isolated from wild-
489 type mice and stimulated with TNFα resulting in a reduction in NKCC2 expression,
490 and simultaneous increase in UMOD mRNA expression (Graham et al. 2014). This
491 work suggests that uromodulin modulates the effect of TNFα on NKCC2
492 expression and hence may affect BP regulation. However, further work is required
493 to exactly characterise this molecular mechanism. To date, although studies report
494 the measurement of uromodulin in cats using experimental collection of large
495 volumes of urine, it has not been possible to validate a human-based ELISA system
496 for urine uromodulin quantification (Lulich et al. 2012). Nevertheless, this would

be an interesting avenue for further study in order to explore the relationship between genetic variation and uromodulin expression in cats.

In contrast to data from human medicine, no association could be identified between genetic variants in UMOD and plasma creatinine as a marker of renal function or the outcome of a diagnosis of CKD. In human medicine, a significant association has been identified between UMOD variant rs12917707 and CKD defined as an estimated GFR (eGFRcreat) using creatinine (eGFRcreat) of <60 mL/kg/minute/1·73 m2 in both discovery and replication groups (Kottgen et al. 2009, Psaty et al. 2009). However, it is important to note that within the meta-analysis, even when combining six risk alleles, only 0·7% of the variance in eGFRcreat could be explained (Kottgen et al. 2009). The association between UMOD variants and renal function have been replicated in an independent population in which the UMOD tag-variant rs4293393 was significantly associated with both CKD and serum creatinine concentration (The International HapMap C 2005, Gudbjartsson et al. 2010). UMOD variants (rs12917707 and rs4293393) have also been significantly associated with the risk of incident CKD in humans and more recently they have been associated with development of end-stage renal disease in humans (Kottgen et al. 2009, 2010, Boger et al. 2011).

It can be hypothesised that, if the effect of genetic association between UMOD and renal function is smaller than that for BP, lack of association with renal function in the current study may reflect the small sample size. In the current study every available cat meeting the study criteria was included. Given this available sample size, we have performed power calculations retrospectively (Purcell et al. 2003)

to estimate the expected power achievable from an analysis of N=227 cats for detecting effects of associated SNPs with MAF≥10%. For BP traits, which are known to only have small effects for each SNP individually in humans, the sample of 227 cats may only have ~20% power. For renal trait associations, for which we anticipate higher power, as human studies indicate larger effect sizes, the sample of 227 cats may have ~40% power. These calculations include estimates and assumptions for heritability and LD structure known from human genetics. However, with no prior GWAS of BP and renal traits in cats, the accuracy of these estimates is unknown. Analysis of this pilot study data and the quality control diagnostics performed suggest that there may be stronger LD structure within cats, compared to humans. If this is the case, the power could actually be higher than has been estimated, which may explain the successful identification of associations among UMOD SNPs and SBP. Equally the small sample size means that there is insufficient evidence to conclude absence of a significant association between UMOD variants and HT or renal traits from this study.

Although a range of intronic and exonic SNPs were evaluated in this study only 5 out of 11 were ultimately identified to be independent, and therefore there were only a limited number of distinct signals that could be analysed for these data. Furthermore, in the current study, plasma creatinine was used as a marker of GFR whilst in comparable human studies estimated GFR based on either creatinine or cystatin C were commonly used. Creatinine is recognised to be a less precise marker of GFR particularly in the early stages of CKD than estimated GFR calculations. It is therefore possible that as yet undiscovered SNPs in the feline

546 UMOD gene may be associated with renal function or that an association may be
547 identified if more precise markers of renal function are employed.
548
549 A further limitation of the current study was inclusion of cats which either had a
550 prior diagnosis of hyperthyroidism and had undergone surgical thyroidectomy or
551 medical management, or which were identified to become hyperthyroid during
552 follow-up. Hyperthyroidism had previously been diagnosed in 19% of cats at
553 enrollment to this study. From the feline literature, approximately 10% of cats are
554 diagnosed with systemic HT at diagnosis of hyperthyroidism with approximately
555 20% demonstrating HT after treatment and return to euthyroidism (Morrow et al.
556 2009, Williams et al. 2010). In human patients and experimental studies,
557 hyperthyroidism results in a reduction in systemic vascular resistance that is
558 offset by an increase in cardiac output, thus the net effect of hyperthyroidism is
559 towards a small decline in blood pressure (Syme 2007). The underlying
560 pathophysiology of systemic HT documented in cats with hyperthyroidism
561 remains to be determined, but may relate to the decline in renal function identified
562 with return to euthyroidism (Williams et al. 2010, 2013). If this is the case then it
563 remains possible that genetic variants, such as those identified in uromodulin,
564 could be common to all cats and still play a predisposing role in the development
565 of systemic HT. Every attempt was made to ensure that cats were truly euthyroid
566 at the point of inclusion aged 14 years. It is therefore hoped that any effect of a
567 prior diagnosis of hyperthyroidism on both SBP and renal function as assessed by
568 plasma creatinine will have been minimised at this time. The association analysis
569 for SBP was repeated excluding cats that were receiving medical therapy for their
570 hyperthyroidism without documenting any change in association results (data not

presented) implying that inclusion of these cats did not adversely affect the results. However, despite careful longitudinal monitoring, it is possible that either failure to make an early diagnosis of hyperthyroidism or medical management of hyperthyroidism in those patients where a diagnosis was made, could have impacted on our ability to define cats as hypertensive during follow-up.

Further novel SNP discovery is warranted and continued exploration with novel renal markers, e.g. symmetric dimethylarginine. In particular, the SNP associations in human medicine both with renal function and BP have been located within the promoter region for the UMOD gene (Kottgen et al. 2009, Padmanabhan et al. 2010). Focusing on SNPs within the promoter region of the feline UMOD gene may be of greatest benefit. A further aspect of association that was not performed in the current study on cats but which has been evaluated in human medicine is the association between genetic variants and progression of renal disease (Gorski et al. 2015).

In conclusion, this exploratory pilot study suggests that there may be similarities between humans and cats in the underlying mechanisms of BP regulation and the role that genetic variants in UMOD play in modifying BP. Further work is required to replicate and validate these preliminary findings in a separate cohort of cats and to explore the relationship between uromodulin excretion and UMOD genetic variation in cats.

[a]Idexx laboratories, Wetherby, UK

[b]Sigma GenElute Blood Genomic DNA kit, Sigma-Aldrich Company Ltd, Dorset, UK

596 [c]Nanodrop 1000 Spectrophotometer, Thermo Scientific, Wilmington, DE, USA

597 [d]Water, Molecular Biology Reagent, Sigma-Aldrich Company Ltd, Dorset, UK

598 [e]KASP™, LGC Genomics, Teddington, Middlesex, UK

599 [f] Primepicker, (previously KBiosciences) LGC Genomics, Teddington, Middlesex,

600 UK

601 [g]KBS-1016-022 (25ml) KASP™ Master Mix, LGC Genomics, Teddington, Middlesex,

602 UK

603 [h] AB Biosystems, Paisley, UK

604 [i] Tetrad PTC 225 Peltier Thermocycler (previously MJ Research), BIO-RAD, Hemel

605 Hempstead, Hertfordshire, UK

606 [j]ABI PRISM® 7900HT, Applied Biosystems, Thermo Fisher Scientific, Paisley, UK

607 [k]SDS 2.3, Applied Biosystems, Thermo Fisher Scientific, Paisley, UK

608 [l]IBM SPSS 20, Portsmouth, UK

609

610 Table 1: Clinicopathological data for cats at enrollment to the study
611
612

Clinical parameter	Median (25^{th}, 75^{th})	N
Age (years)	14·4 (14·2, 14·6)	227
Urea (mmol/L)	12·0 (9·7, 15·9)	227
Creatinine (µmol/L)	154·3 (127·6, 192·7); range 63·8 to 550·8	227
Phosphorus (mmol/L)	1·28 (1·10, 1·48)	227
USG	1·030 (1·020, 1·042)	191
UP/C	0·15 (0·00, 2·99)	70
Total thyroxine (nmol/L)	22·3 (16·5, 28·2)	141
SBP (mmHg)	140 (127·2, 155·6)	227
Weight (kg)	4·06 (3·42, 4·65)	219
Diagnosed with azotaemic CKD at enrollment	-	52/227 22·9%
Previous diagnosis of systemic hypertension at enrollment	-	39/227 17%
Proportion of cats with systemic hypertension diagnosed with azotaemic CKD	-	18/39 46%

613
614

615 Table 2: Comparison of clinical parameters between cats diagnosed with
616 azotaemic CKD at enrollment versus non-azotaemic cats

Variable (units)	Non-azotaemic cats	n	Cats diagnosed with azotaemic CKD	n	Significance (P)
Age at visit during 15th year	14·4 (14·2, 14·6)	150	14·3 (14·1, 14·6)	77	0·02
Creatinine (µmol/L)	137·0 (116·7, 155·3)	150	215·0 (190·0, 251·0)	77	<0·0001
Phosphorus (mmol/L)	1·27 (1·09, 1·44)	150	1·31 (1·16, 1·61)	77	0·127
Potassium (mmol/L)	3·90 (3·70, 4·20)	150	4·07 (3·72, 4·30)	77	0·034
Weight (kg)	4·01 (3·42, 4·64)	146	4·10 (3·48, 4·70)	73	0·502
Systolic blood pressure (mmHg)	138 (126·0, 156·0)	150	143 (128·5, 156)	77	0·257
Packed cell volume (%)	37·0 (34·0, 40·0)	149	34·5 (30·0, 38·0)	76	0·005
Urine specific gravity	1·036 (1·028, 1·050)	120	1·020 (1·016, 1·024)	71	<0·0001
Urine protein to creatinine ratio	0·16 (0·12, 0·29)	38	0·14 (0·09, 0·26)	32	0·328
Duration of follow-up (days)	899·0 (484·5, 1274·5)	150	799·0 (246·0, 1106·0)	77	0·069

617
618
619
620

621 Table 3: Comparison of clinical parameters between cats documented to be
622 hypertensive and those, which remained normotensive during follow-up
623
624

Variable (units)	Cats remaining normotensive	n	Cats developing hypertension	n	Significance (P)
Age at visit during 15th year	14·4 (14·1, 14·6)	146	14·3 (14·1, 14·6)	81	0·106
Creatinine (µmol/L)	151·7 (125·1, 186·4)	146	159·2 (132·4, 201·0)	81	0·127
Phosphorus (mmol/L)	1·28 (1·12, 1·46)	146	1·31 (1·07, 1·51)	81	0·778
Potassium (mmol/L)	4·0 (3·7, 4·3)	146	3·9 (3·6, 4·1)	81	0·02
Weight (kg)	4·01 (3·46, 4·62)	146	4·17 (3·31, 4·68)	81	0·853
Packed cell volume (%)	36 (32, 39)	146	36 (33, 39)	79	0·845
Urine specific gravity	1·031 (1·021, 1·046)	119	1·025 (1·019, 1·034)	72	0·01
Urine protein to creatinine ratio	0·16 (0·11, 0·27)	40	0·16 (0·10, 0·34)	30	0·476
Duration of follow-up (days)	822 (371, 1151)	81	968 (486, 1401)	81	0·138
Diagnosis of hyperthyroidism at enrollment during 15th year	-	28	-	16	
Cats diagnosed with hyperthyroidism during follow-up	-	10	-	11	

References:

Adzhubei, I. A., S. Schmidt, L. Peshkin, V. E. Ramensky, A. Gerasimova, P. Bork, A. S. Kondrashov and S. R. Sunyaev (2010). "A method and server for predicting damaging missense mutations." Nature methods **7**(4): 248-249.

Alhaddad, H., R. Khan, R. A. Grahn, B. Gandolfi, J. C. Mullikin, S. A. Cole, T. J. Gruffydd-Jones, J. Häggström, H. Lohi, M. Longeri and L. A. Lyons (2013). "Extent of Linkage Disequilibrium in the Domestic Cat, <italic>Felis silvestris catus,</italic> and Its Breeds." PLoS ONE **8**(1): e53537.

Bachmann, S., K. Mutig, J. Bates, P. Welker, B. Geist, V. Gross, F. C. Luft, N. Alenina, M. Bader, B. J. Thiele, K. Prasadan, H. S. Raffi and S. Kumar (2005). "Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice." American Journal of Physiology - Renal Physiology **288**(3): F559-F567.

Battula, S., S. Hao, P. L. Pedraza, C. T. Stier and N. R. Ferreri (2011). "Tumor necrosis factor-α is an endogenous inhibitor of Na+-K+-2Cl− cotransporter (NKCC2) isoform A in the thick ascending limb." American Journal of Physiology - Renal Physiology **301**(1): F94-F100.

Bijsmans, E. S., Jepson, R. E., Chang, Y. M., et al. (2015) Changes in systolic blood pressure over time in healthy cats and cats with chronic kidney disease. Journal of Veterinary Internal Medicine.

Bleyer, A. J., M. Zivna and S. Kmoch (2011). "Uromodulin-associated kidney disease." Nephron Clin Pract **118**(1): c31-36.

Boger, C. A., M. Gorski, M. Li, M. M. Hoffmann, C. Huang, Q. Yang, A. Teumer, V. Krane, C. M. O'Seaghdha, Z. Kutalik, H. E. Wichmann, T. Haak, E. Boes, S. Coassin, J. Coresh, B. Kollerits, M. Haun, B. Paulweber, A. Kottgen, G. Li, M. G. Shlipak, N. Powe, S. J. Hwang, A. Dehghan, F. Rivadeneira, A. Uitterlinden, A. Hofman, J. S. Beckmann, B. K. Kramer, J. Witteman, M. Bochud, D. Siscovick, R. Rettig, F. Kronenberg, C. Wanner, R. I. Thadhani, I. M. Heid, C. S. Fox and W. H. Kao (2011). "Association of eGFR-Related Loci Identified by GWAS with Incident CKD and ESRD." PLoS Genet **7**(9): e1002292.

Brandt, L. E., A. A. Bohn, J. B. Charles and E. J. Ehrhart (2012). "Localization of Canine, Feline, and Mouse Renal Membrane Proteins." Veterinary Pathology Online **49**(4): 693-703.

Buffington, C. A., J. L. Blaisdell and T. Sako (1994). "Effects of Tamm-Horsfall glycoprotein and albumin on struvite crystal growth in urine of cats." Am J Vet Res **55**(7): 965-971.

Bush, W. S. & Moore, J. H. (2012) Chapter 11: genome-wide association studies. PLoS Computational Biology 8, e1002822

Cabrera, C. P., Ng, F. L., Warren, H. R., et al. (2015) Exploring hypertension genome-wide association studies findings and impact on pathophysiology, pathways, and pharmacogenetics. Wiley Interdisciplinary Reviews: Systems Biology and Medicine 7, 73-90

Cordell, H. J. & Clayton, D. G. (2005) Genetic association studies. The Lancet 366, 1121-1131

Eckardt, K.-U., S. L. Alper, C. Antignac, A. J. Bleyer, D. Chauveau, K. Dahan, C. Deltas, A. Hosking, S. Kmoch, L. Rampoldi, M. Wiesener, M. T. Wolf and O. Devuyst (2015). "Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management[mdash]A KDIGO consensus report." Kidney Int **88**(4): 676-683.

672 Ferlizza, E., A. Campos, A. Neagu, A. Cuoghi, E. Bellei, E. Monari, F. Dondi, A. M.
673 Almeida and G. Isani (2015). "The effect of chronic kidney disease on the urine
674 proteome in the domestic cat (Felis catus)." The Veterinary Journal **204**(1): 73-
675 81.
676 Gorski, M., A. Tin, M. Garnaas, G. M. McMahon, A. Y. Chu, B. O. Tayo, C. Pattaro, A.
677 Teumer, D. I. Chasman, J. Chalmers, P. Hamet, J. Tremblay, M. Woodward, T.
678 Aspelund, G. Eiriksdottir, V. Gudnason, T. B. Harris, L. J. Launer, A. V. Smith, B. D.
679 Mitchell, J. R. O'Connell, A. R. Shuldiner, J. Coresh, M. Li, P. Freudenberger, E. Hofer,
680 H. Schmidt, R. Schmidt, E. G. Holliday, P. Mitchell, J. J. Wang, I. H. de Boer, G. Li, D.
681 S. Siscovick, Z. Kutalik, T. Corre, P. Vollenweider, G. Waeber, J. Gupta, P. A.
682 Kanetsky, S.-J. Hwang, M. Olden, Q. Yang, M. de Andrade, E. J. Atkinson, S. L. R.
683 Kardia, S. T. Turner, J. M. Stafford, J. Ding, Y. Liu, C. Barlassina, D. Cusi, E. Salvi, J. A.
684 Staessen, P. M. Ridker, H. Grallert, C. Meisinger, M. Muller-Nurasyid, B. K. Kramer,
685 H. Kramer, S. E. Rosas, I. M. Nolte, B. W. Penninx, H. Snieder, M. Fabiola Del Greco,
686 A. Franke, U. Nothlings, W. Lieb, S. J. L. Bakker, R. T. Gansevoort, P. van der Harst,
687 A. Dehghan, O. H. Franco, A. Hofman, F. Rivadeneira, S. Sedaghat, A. G. Uitterlinden,
688 S. Coassin, M. Haun, B. Kollerits, F. Kronenberg, B. Paulweber, N. Aumann, K.
689 Endlich, M. Pietzner, U. Volker, R. Rettig, V. Chouraki, C. Helmer, J.-C. Lambert, M.
690 Metzger, B. Stengel, T. Lehtimaki, L.-P. Lyytikainen, O. Raitakari, A. Johnson, A.
691 Parsa, M. Bochud, I. M. Heid, W. Goessling, A. Kottgen, W. H. L. Kao, C. S. Fox and C.
692 A. Boger (2015). "Genome-wide association study of kidney function decline in
693 individuals of European descent." Kidney Int **87**(5): 1017-1029.
694 Graham, L. A., S. Padmanabhan, N. J. Fraser, S. Kumar, J. M. Bates, H. S. Raffi, P.
695 Welsh, W. Beattie, S. Hao, S. Leh, M. Hultstrom, N. R. Ferreri, A. F. Dominiczak, D.
696 Graham and M. W. McBride (2014). "Validation of Uromodulin as a Candidate Gene
697 for Human Essential Hypertension." Hypertension **63**(3): 551-558.
698 Gudbjartsson, D. F., H. Holm, O. S. Indridason, G. Thorleifsson, V. Edvardsson, P.
699 Sulem, F. de Vegt, F. C. d'Ancona, M. den Heijer, J. F. Wetzels, L. Franzson, T. Rafnar,
700 K. Kristjansson, U. S. Bjornsdottir, G. I. Eyjolfsson, L. A. Kiemeney, A. Kong, R.
701 Palsson, U. Thorsteinsdottir and K. Stefansson (2010). "Association of variants at
702 UMOD with chronic kidney disease and kidney stones-role of age and comorbid
703 diseases." PLoS Genet **6**(7): e1001039.
704 Han, J., Y. Chen, Y. Liu, Y. Liang, X. Wang, L. Liu, F. Wang, L. Zhang, H. Zhang and H.
705 Wang (2012). "Common variants of the UMOD promoter associated with blood
706 pressure in a community-based Chinese cohort." Hypertens Res.
707 Hattersley, A. T. & McCarthy, M. I. (2005) What makes a good genetic association
708 study? The Lancet 366, 1315-1323
709 Iwai, N., K. Kajimoto, Y. Kokubo and H. Tomoike (2006). "Extensive Genetic
710 Analysis of 10 Candidate Genes for Hypertension in Japanese." Hypertension
711 **48**(5): 901-907.
712 Jepson, R. E. (2011). "Feline systemic hypertension: Classification and
713 pathogenesis." J Feline Med Surg **13**(1): 25-34.
714 Kottgen, A., N. L. Glazer, A. Dehghan, S. J. Hwang, R. Katz, M. Li, Q. Yang, V.
715 Gudnason, L. J. Launer, T. B. Harris, A. V. Smith, D. E. Arking, B. C. Astor, E.
716 Boerwinkle, G. B. Ehret, I. Ruczinski, R. B. Scharpf, Y. D. Chen, I. H. de Boer, T.
717 Haritunians, T. Lumley, M. Sarnak, D. Siscovick, E. J. Benjamin, D. Levy, A.
718 Upadhyay, Y. S. Aulchenko, A. Hofman, F. Rivadeneira, A. G. Uitterlinden, C. M. van
719 Duijn, D. I. Chasman, G. Pare, P. M. Ridker, W. H. Kao, J. C. Witteman, J. Coresh, M.

G. Shlipak and C. S. Fox (2009). "Multiple loci associated with indices of renal function and chronic kidney disease." Nat Genet **41**(6): 712-717.
Kottgen, A., S. J. Hwang, M. G. Larson, J. E. Van Eyk, Q. Fu, E. J. Benjamin, A. Dehghan, N. L. Glazer, W. H. Kao, T. B. Harris, V. Gudnason, M. G. Shlipak, Q. Yang, J. Coresh, D. Levy and C. S. Fox (2010). "Uromodulin levels associate with a common UMOD variant and risk for incident CKD." J Am Soc Nephrol **21**(2): 337-344.
Lhotta, K. (2010). "Uromodulin and chronic kidney disease." Kidney Blood Press Res **33**(5): 393-398.
Lulich, J. P., C. A. Osborne, M. Carvalho and Y. Nakagawa (2012). "Effects of a urolith prevention diet on urine compositions of glycosaminoglycans, Tamm-Horsfall glycoprotein, and nephrocalcin in cats with calcium oxalate urolithiasis." American Journal of Veterinary Research **73**(3): 447-451.
Lulich, J. P., C. A. Osborne, T. D. O'Brien and D. J. Polzin (1992). "Feline renal failure: Questions, Answers, Questions." Compend Cont Ed Pract Vet **14**(2): 127-152.
Marino, C. L., B. D. X. Lascelles, S. L. Vaden, M. E. Gruen and S. L. Marks (2014). "Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies." Journal of Feline Medicine and Surgery **16**(6): 465-472.
Matsumoto, K. and M. Funaba (2008). "Factors affecting struvite (MgNH4PO4.6H2O) crystallization in feline urine." Biochim Biophys Acta **1780**(2): 233-239.
Mullikin, J., N. Hansen, L. Shen, H. Ebling, W. Donahue, W. Tao, D. Saranga, A. Brand, M. Rubenfield, A. Young, P. Cruz, N. C. S. Program, C. Driscoll, V. David, S. Al-Murrani, M. Locniskar, M. Abrahamsen, S. O'Brien, D. Smith and J. Brockman (2010). "Light whole genome sequence for SNP discovery across domestic cat breeds." BMC Genomics **11**(1): 406.
Mutig, K., T. Kahl, T. Saritas, M. Godes, P. Persson, J. Bates, H. Raffi, L. Rampoldi, S. Uchida, C. Hille, C. Dosche, S. Kumar, M. Castañeda-Bueno, G. Gamba and S. Bachmann (2011). "Activation of the Bumetanide-sensitive Na+,K+,2Cl– Cotransporter (NKCC2) Is Facilitated by Tamm-Horsfall Protein in a Chloride-sensitive Manner." Journal of Biological Chemistry **286**(34): 30200-30210.
Padmanabhan, S., L. Graham, N. R. Ferreri, D. Graham, M. McBride and A. F. Dominiczak (2014). "Uromodulin, an Emerging Novel Pathway for Blood Pressure Regulation and Hypertension." Hypertension **64**(5): 918-923.
Padmanabhan, S., O. Melander, T. Johnson, A. M. Di Blasio, W. K. Lee, D. Gentilini, C. E. Hastie, C. Menni, M. C. Monti, C. Delles, S. Laing, B. Corso, G. Navis, A. J. Kwakernaak, P. van der Harst, M. Bochud, M. Maillard, M. Burnier, T. Hedner, S. Kjeldsen, B. Wahlstrand, M. Sjögren, C. Fava, M. Montagnana, E. Danese, O. Torffvit, B. Hedblad, H. Snieder, J. M. C. Connell, M. Brown, N. J. Samani, M. Farrall, G. Cesana, G. Mancia, S. Signorini, G. Grassi, S. Eyheramendy, H. E. Wichmann, M. Laan, D. P. Strachan, P. Sever, D. C. Shields, A. Stanton, P. Vollenweider, A. Teumer, H. Völzke, R. Rettig, C. Newton-Cheh, P. Arora, F. Zhang, N. Soranzo, T. D. Spector, G. Lucas, S. Kathiresan, D. S. Siscovick, J. a. Luan, R. J. F. Loos, N. J. Wareham, B. W. Penninx, I. M. Nolte, M. McBride, W. H. Miller, S. A. Nicklin, A. H. Baker, D. Graham, R. A. McDonald, J. P. Pell, N. Sattar, P. Welsh, P. Munroe, M. J. Caulfield, A. Zanchetti, A. F. Dominiczak and B. C. Global (2010). "Genome-Wide Association Study of Blood Pressure Extremes Identifies Variant near *UMOD* Associated with Hypertension." PLoS Genet **6**(10): e1001177.

Palmer, L. J. & Cardon, L. R. (2005) Shaking the tree: mapping complex disease genes with linkage disequilibrium. The Lancet 366, 1223-1234

Prajczer, S., U. Heidenreich, W. Pfaller, P. Kotanko, K. Lhotta and P. Jennings (2010). "Evidence for a role of uromodulin in chronic kidney disease progression." Nephrol Dial Transplant **25**(6): 1896-1903.

Psaty, B. M., C. J. O'Donnell, V. Gudnason, K. L. Lunetta, A. R. Folsom, J. I. Rotter, A. G. Uitterlinden, T. B. Harris, J. C. M. Witteman, E. Boerwinkle and o. B. o. t. C. Consortium (2009). "Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of Prospective Meta-Analyses of Genome-Wide Association Studies From 5 Cohorts." Circulation: Cardiovascular Genetics **2**(1): 73-80.

Purcell, S., Cherny, S., & Sham, P. (2003) Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19, 149-150.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. W. de Bakker, M. J. Daly and P. C. Sham (2007). "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses." American journal of human genetics **81**(3): 559-575.

Rampoldi, L., F. Scolari, A. Amoroso, G. Ghiggeri and O. Devuyst (2011). "The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease." Kidney Int **80**(4): 338-347.

Renigunta, A., V. Renigunta, T. Saritas, N. Decher, K. Mutig and S. Waldegger (2011). "Tamm-Horsfall Glycoprotein Interacts with Renal Outer Medullary Potassium Channel ROMK2 and Regulates Its Function." Journal of Biological Chemistry **286**(3): 2224-2235.

Reznichenko, A., C. Boger, H. Snieder, J. van den Born, M. de Borst, J. Damman, M. van Dijk, H. van Goor, B. Hepkema, J.-L. Hillebrands, H. Leuvenink, J. Niesing, S. Bakker, M. Seelen, G. Navis and O. b. o. t. R. G. group (2012). "UMOD as a susceptibility gene for end-stage renal disease." BMC Medical Genetics **13**(1): 78.

Rhodes, D. C., E. J. Hinsman and J. A. Rhodes (1993). "Cation-induced aggregation of cat Tamm-Horsfall glycoprotein and its possible role in feline urolithiasis." Zentralbl Veterinarmed A **40**(4): 283-291.

Shastry, B. S. (2009) SNPs: impact on gene function and phenotype. In: Single nucleotide polymorphisms: methods and protocols. Ed A. A. Komar . Humana Press, Totowa, NJ, USA. pp 3-22.

Syme, H. M., P. J. Barber, P. J. Markwell and J. Elliott (2002). "Prevalence of systolic hypertension in cats with chronic renal failure at initial evaluation." J Am Vet Med Assoc **220**(12): 1799-1804.

Syme, H. M., P. J. Markwell, D. Pfeiffer and J. Elliott (2006). "Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria." J Vet Intern Med **20**(3): 528-535.

Tamazian, G., S. Simonov, P. Dobrynin, A. Makunin, A. Logachev, A. Komissarov, A. Shevchenko, V. Brukhin, N. Cherkasov, A. Svitin, K.-P. Koepfli, J. Pontius, C. A. Driscoll, K. Blackistone, C. Barr, D. Goldman, A. Antunes, J. Quilez, B. Lorente-Galdos, C. Alkan, T. Marques-Bonet, M. Menotti-Raymond, V. A. David, K. Narfström and S. J. O'Brien (2014). "Annotated features of domestic cat – Felis catus genome." GigaScience **3**(1): 1-3.

The International HapMap, C. (2005). "A haplotype map of the human genome." Nature **437**(7063): 1299-1320.

817 Thornley, C., A. Dawnay and W. R. Cattell (1985). "Human Tamm-Horsfall
818 glycoprotein: urinary and plasma levels in normal subjects and patients with renal
819 disease determined by a fully validated radioimmunoassay." Clin Sci (Lond)
820 **68**(5): 529-535.
821 Torffvit, O., P. E. Jorgensen, A. L. Kamper, N. H. Holstein-Rathlou, P. P. Leyssac, S. S.
822 Poulsen and S. Strandgaard (1998). "Urinary excretion of Tamm-Horsfall protein
823 and epidermal growth factor in chronic nephropathy." Nephron **79**(2): 167-172.
824 Trudu, M., S. Janas, C. Lanzani, H. Debaix, C. Schaeffer, M. Ikehata, L. Citterio, S.
825 Demaretz, F. Trevisani, G. Ristagno, B. Glaudemans, K. Laghmani, G. Dell'Antonio,
826 t. the Swiss Kidney Project on Genes in Hypertension, J. Loffing, M. P. Rastaldi, P.
827 Manunta, O. Devuyst and L. Rampoldi (2013). "Common noncoding UMOD gene
828 variants induce salt-sensitive hypertension and kidney damage by increasing
829 uromodulin expression." Nat Med **advance online publication**.
830 Vyletal, P., A. J. Bleyer and S. Kmoch (2010). "Uromodulin biology and
831 pathophysiology--an update." Kidney Blood Press Res **33**(6): 456-475.
832 Williams, T. L., Peak, K. J., Brodbelt, D., et al. (2010) Survival and the development
833 of azotemia after treatment of hyperthyroid cats. Journal of Veterinary Internal
834 Medicine 24, 863-869.
835 Williams, T. L., Elliott, J., & Syme, H. M. (2013) Renin-angiotensin-aldosterone
836 system activity in hyperthyroid cats with and without concurrent hypertension.
837 Journal of Veterinary Internal Medicine 27, 522-529.
838
839

840