Screening for increased plasma urea levels in a large-scale ENU mouse mutagenesis project reveals kidney disease models

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Aigner B, Rathkolb B, Herbach N, Kemter E, Schessl C, Klaften M, Klempt M, Hrabé de Angelis M, Wanke R, Wolf E. Screening for increased plasma urea levels in a large-scale ENU mouse mutagenesis project reveals kidney disease models. Am J Physiol Renal Physiol 292: F1560–F1567, 2007. First published January 30, 2007; doi:10.1152/ajprenal.00213.2006.—Kidney diseases can be generated by genetic engineering techniques which result in defined alterations of the mouse genome. However, the resulting phenotypes of the mutant mice cannot be predicted and do not always mirror the respective human disease. A complementary strategy is to generate new alleles by random mutagenesis and to screen for clinically relevant phenotypes. The underlying mutations are then identified by forward genetics strategies and may lead to the identification of genes/alleles which may have counterparts relevant for human diseases (2, 24, 28).

Ethynitrosourea (ENU) has been used in various mouse mutagenesis programs to produce random mutations. Specific pathological states have been identified by appropriate routine procedures allowing the screening of large numbers of mice for a broad spectrum of parameters (9, 19). In the Munich ENU mouse mutagenesis project (http://www.gsf.de/ieg/groups/genome/enu.html), a screening profile of clinical chemistry blood parameters was established for the analysis of offspring of chemically mutagenized mice to detect phenotypic variants with defects of various organ systems or changes in metabolic pathways. Breeding of the affected mice and screening of the offspring confirmed the transmission of the altered phenotype to subsequent generations, thereby revealing a mutation as cause for the aberrant phenotype (21, 22).

Urea is the waste product of protein catabolism. In mammals, it is excreted in the urine thereby representing the most abundant urinary solute. Compared with blood, urea is highly concentrated in the urine of humans and rodents and plays an important role in the urine concentration mechanism. The ability to concentrate urine depends on the accumulation of urea in the renal medulla by specific transporter proteins (26). Urea excretion per unit body weight in mice is several times higher than in humans which relies on an effective excretory system (33). Increased blood urea levels may be caused by prerenal, renal, and postrenal alterations. Regarding renal diseases, blood urea concentrations increase if kidney function is severely reduced (14).

Nephropathies include various multifactorial disorders caused by genetic and/or environmental factors. Several monogenic disorders are known to result in progressive renal insufficiency. Among these are glomerular basement membrane defects as well as tubular and interstitial alterations. Once chronic renal insufficiency is established, it tends to progress to end-stage kidney failure, which is characterized by progressive glomerulosclerosis irrespective of the initiating nephropathy (25). Few appropriate animal models are available to date for the examination of genes involved in chronic kidney diseases (1, 4, 13, 30, 32). Quantitative trait loci (QTL) in rodents were found to be concordant with kidney disease loci in humans, suggesting conserved disease genes in these species (12).

Today published ENU project results did not include reports of generating mutant lines by measuring increased plasma urea levels. Here, we present the successful establishment and comparative phenotypic characterization of mutant lines exhibiting increased plasma urea levels which were derived by the clinical chemical screen within the Munich ENU mouse mutagenesis project.
Materials and Methods

Mutagenesis and breeding of mice. The Munich ENU mouse mutagenesis project was carried out on the inbred C3HeB/FeJ (C3H) genetic background as described (16). Briefly, 10-wk-old male mice were injected intraperitoneally with ENU. The screen for dominant mutations was performed on G1 animals which were derived by mating mutagenized G0 males to wild-type C3H females. Inheritance of phenotypes in the inbred C3H genetic background was tested on G4 intercross or G4 × G3 backcross offspring. Mouse husbandry was done under a continuously controlled specific pathogen-free (SPF) hygiene standard according to the FELASA protocols (http://www.felasa.org) (18). The standard rodent diet 1314 (Altromin, Lage, Germany; http://www.altromin.de/downloads/1000_ratten_maeuse.pdf) and water were provided ad libitum. All animal experiments were carried out with permission from the responsible animal welfare authority (Regierung von Oberbayern).

Clinical chemistry analysis. Blood samples from 3-mo-old G1 and G3 mice fasted overnight were obtained by puncture of the retroorbital sinus under ether anesthesia. Plasma from Li-heparin-treated blood was analyzed using an Olympus AU400 autoanalyzer (Olympus, Hamburg, Germany) and the adapted reagents (Olympus). Calibration and quality control were performed according to the manufacturer’s protocols. Physiological parameter values were determined in male and female inbred C3H controls. Plasma urea concentrations were examined using the reagent OSR6134 (Olympus) with the linear measurement range of 5–300 mg/dl.

Moreover, the clinical chemistry screen contained additional plasma parameters including substrates, electrolytes, and enzymes. Furthermore, hematologic parameters including erythrocyte indexes and white blood cell counts were measured in EDTA-treated blood using an ABC blood analyzer (Sci!l, Viernheim, Germany) (11).

Urine parameters were measured in 24-h urine samples collected in metabolic cages (Tecniplast, Buguggiate, Italy; UREHR1: n = 3 male and 1 female mutants, UREHR1: n = 3 males and 1 female, UREHR3: n = 2 males and 2 females) and spot urine samples were collected for subsequent DNA isolation.

Morphological kidney analysis of the mutant lines. Animals were killed by ether inhalation. Kidneys of mice of the lines UREHR1-R4 were fixed with 4% paraformaldehyde in PBS (pH 7.2) via orthograde vascular perfusion as described previously (7). Following perfusion fixation, the kidneys were removed, weighed to the nearest milligram, postfixed by immersion in 4% paraformaldehyde for 24 h, and cut perpendicular to the longitudinal axis into 1- to 2-mm-thick slices. The kidney samples were processed in a Shandon Citadel 1000 (Thermo Electron, Waltham, MA) and subsequently embedded in plastic as described (6). Plastic sections (1.5 μm) were stained with hematoxylin and eosin (H&E) as well as periodic-acid Schiff (PAS). In addition, kidney slices as well as representative samples of lung, heart, liver, spleen, salivary gland, stomach, and intestine were routinely processed and embedded in paraffin according to standard procedures. Paraffin sections were stained with H&E, as well as Giemsa, Masson-Trichrom, PAS, and Congo red.

Kidneys derived from the line UREHD1 were fixed by immersion in 10% buffered formalin for 24 h, cut perpendicular to the longitudinal axis into 2-mm-thick slices, routinely processed, and embedded in paraffin.

Immunohistochemical analysis was carried out on paraffin sections mounted on sialized slides (Superfrost Plus; Menzel Gläser, Braunschweig, Germany), using the indirect immunoperoxidase technique. Sections were deparaffinized, blocked with 1% H2O2 for 15 min, and pretreated with Proteinase K for 10 min. Immunohistochemistry was performed with a polyclonal rabbit anti-IgA, IgM, and C1q antibody (DAKO, Hamburg, Germany), respectively. Immunoreactivity was visualized using 3,3-diaminobenzidine tetrahydrochloride dihydrate.

For electronmicroscopic analysis, kidneys of 3-mo-old UREH1 mice were fixed with 3% glutaraldehyde in PBS (pH 7.2) via orthograde vascular perfusion, and 1-mm3 samples of the renal cortex were postfixed by immersion in the same fixative for 1 day and processed as described previously (7).

Linkage analysis of the mutation. In the lines UREHR3 and UREHR4, homozygous phenotypic mutant C3H males were mated to C57BL/6 (for line UREHR4) or BALB/c (for line UREHR3) female mice. The resulting G1 hybrid mice were bred. G2 offspring were phenotypically classified in mice exhibiting normal or increased plasma urea levels, respectively. After death, tissue samples were collected for subsequent DNA isolation.

Statistical analysis. The statistical analysis of the data was carried out using the software program Microsoft Excel 2000. Values are presented as means ± SD unless stated otherwise. Statistical significance (defined as P < 0.05) was evaluated using Student’s t-test.

RESULTS

ENU-induced phenotypic variants showing increased plasma urea. The search for mutant mice showing increased plasma urea levels in the Munich ENU mouse mutagenesis project was carried out on the inbred C3H genetic background 3 mo postpartum after overnight fasting of the animals. Mean total urea levels in plasma from male and female C3H controls corresponded to 48 and 41 mg/dl, respectively (11). In females, the 95% range of the values contained higher values than the mean. Thus pathological plasma urea levels were defined in our test for both male and female mice showing values above the cut-off level of 70 mg/dl in two measurements within a 3-wk interval (Table 1).

Table 1. Physiological range of plasma urea (mg/dl) in 3-mo-old C3H wild-type mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Mean±2 SD</th>
<th>Median</th>
<th>95% Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>192</td>
<td>29–79</td>
<td>48</td>
<td>11</td>
<td>26–70</td>
<td>47</td>
<td>31–71</td>
</tr>
<tr>
<td>Female</td>
<td>235</td>
<td>20–76</td>
<td>41</td>
<td>11</td>
<td>19–63</td>
<td>39</td>
<td>23–71</td>
</tr>
</tbody>
</table>

Cut-off level for males and females: 70 mg/dl.

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The search for phenotypic variants observed increased plasma urea levels in the two measurements in 17 of more than 15,000 G1 offspring of ENU-treated mice screened for dominant mutations. For revealing recessive mutations, G3 offspring from more than 500 different pedigrees of ENU-treated G0 mice were examined. Twenty-seven G3 offspring exhibited increased plasma urea levels in two measurements. Referring to the number of G1 mice and G3 pedigrees examined, a much higher number of G1 phenotypic variants was expected in proportion to the number of G3 phenotypic variants detected. In total, 30 of 44 observed phenotypic variants (68%) were male mice (Table 2). This may be partly due to the use of the same cut-off level for both males and females despite the lower physiological mean plasma urea level in female mice.

**Transmission of the increased plasma urea phenotype to subsequent generations.** The inheritance of the increased plasma urea levels of 23 mice originally exhibiting this phenotype was analyzed on G2 offspring derived from the mating of the G1 phenotypic variants to wild-type mice in the screen for dominant mutations and on G4 intercross or G4 × G3 backcross offspring after breeding the G3 phenotypic variants to wild-type mice in the screen for recessive mutations. In the case no offspring were obtained, the respective phenotypic variants were mated to another wild-type animal. Eleven of the mated phenotypic variants (48%) produced no offspring due to severe illness and/or sterility in repeated breeding approaches (Table 2).

Twelve of the 23 mated mice showing increased plasma urea levels (52%) produced offspring. A mutation as cause for the increased plasma urea levels was assumed in the phenotypic variants when plasma urea values above the cut-off level (70 mg/dl) were detected in the offspring in two measurements within a 3-wk interval. Five of the mice which produced offspring (42%) transmitted the increased plasma urea levels to subsequent generations (Table 2). Establishment of another mutant line from a female variant derived from the recessive screen failed due to the low penetrance of the mutation. Therefore, this variant was classified as negative (Table 2). In our project, breeding of almost five phenotypic variants was necessary to establish one mutant line exhibiting an increased plasma urea level.

The mutant line derived from the screen for dominant mutations and the four mutant lines derived from the screen for recessive mutations were designated UREHD1 and UREHR1-R4, respectively. Mutant offspring showing pathological plasma urea levels were produced by mating heterozygous mutants to wild-type animals (line UREHD1) and by breeding heterozygous mutant mice (lines UREHR1-R4). The phenotypic mutants of the five lines showed mean plasma urea levels between 83 and 106 mg/dl, respectively (Table 3).

The phenotypic penetrance of the increased plasma urea levels in the five lines was analyzed by defining 100% phenotypic penetrance in the case of the appearance of 50 and 25% offspring exhibiting the phenotypic deviation after mating phenotypic mutants to wild-type mice and breeding heterozygous mutant mice in the lines with dominant and recessive mutations, respectively. A suitable phenotypic penetrance of the pathological urea levels above 50% was observed in all five lines which facilitates the effective subsequent phenotypic and molecular genetic analyses (Table 3). Similar results were found for both sexes within all lines. Reanalysis of the animals after a few weeks increased the phenotypic penetrance of pathological urea levels. The 5 lines were bred for 7 (lines UREHR2 and UREHR4) and more than 10 (lines UREHD1, UREHR1, and UREHR3) generations starting from the ENU mutagenized G0 founder animal without losing the abnormal phenotype. In all five lines, the numbers of animals exhibiting plasma urea levels above the cut-off level were significantly ($\chi^2$-test, $P < 0.001$) higher than the expected 2.5% of the C3H mouse population (Table 3).

**Additional clinical chemical alterations in the mutant lines.** For the analysis of additional plasma parameters, mean values and standard deviations of the means of phenotypic mutants within the litters were compared with those of sex-matched littermate controls. Plasma creatinine levels were increased in the phenotypic mutants of all five lines (Fig. 1). The Jaffé method used is described to overestimate the creatinine level in mice. In the comparative analysis of phenotypic mutants and controls, further techniques might improve the detected differences of the plasma and urinary creatinine levels (5, 15). The plasma electrolytes sodium, potassium, and chloride showed line- and/or sex-specific differences. A significant alteration in both sexes ($P < 0.05$) was only obtained for the decrease of chloride in the phenotypic mutants of line UREHR3 ($P < 0.001$). Reduced plasma glucose levels were observed in the phenotypic mutants of all lines examined (lines UREHD1, UREHR1, UREHR2, and UREHR3). In addition, the mutant mice of line UREHR1 showed strong hypercholesterolemia ($P < 0.001$) which was linked to the plasma urea retention phenotype of this line.

The clinical examination showed obvious additional deviations in the phenotypic mutants of the lines UREHD1, UREHR1, and UREHR3. In line UREHD1, the mutants exhibited increased food intake but decreased body weight gain and decreased body fat. The mutant mice of line UREHR1 had a decreased lifetime and commonly died at 5 mo of age.

### Table 2. Screen and analysis of the inheritance for increased plasma urea in ENU mutant mice

<table>
<thead>
<tr>
<th>Screen</th>
<th>n Pedigrees</th>
<th>Variants*: n (m/f)</th>
<th>Variants mated</th>
<th>No offspring</th>
<th>Negative</th>
<th>Mutants‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant (G1)</td>
<td>&gt;15,000</td>
<td>nd</td>
<td>17 (13/4)</td>
<td>8 (7/1)</td>
<td>2 (1/1)</td>
<td>5 (5/0)</td>
</tr>
<tr>
<td>Recessive (G3)</td>
<td>&gt;500</td>
<td>27 (17/10)</td>
<td>15 (10/5)</td>
<td>9 (5/4)</td>
<td>2 (1/1)</td>
<td>4 (4/0)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (30/14)</td>
<td>23 (17/6)</td>
<td>11 (6/5)</td>
<td>7 (6/1)</td>
<td>5 (5/0)</td>
<td></td>
</tr>
</tbody>
</table>

*G1/G3 offspring of ENU-treated mice showing urea levels of 2 measurements within a 3-wk interval above the cut-off level of 70 mg/dl. Males/females.
†A mutation as cause for the appearance of the abnormal phenotype was diagnosed in phenotypic variants with offspring showing plasma urea levels of 2 measurements within a 3-wk interval above the cut-off level of 70 mg/dl.
‡Variants inheriting increased plasma urea to the subsequent generations. nd, Not determined.
probably due to renal failure. The penetrance of the plasma urea retention phenotype increased in older animals, whereas complete penetrance of hypercholesterolemia was already present in 12-wk-old mice (16). In the phenotypic mutants of line UREHR3, polydipsia and polyuria were observed. Examination of the 24-h urine collected in metabolism cages was carried out in the lines UREHD1, UREHR1, and UREHR3. Additional spot urine samples were analyzed in these lines and in line UREHR2. The phenotypic mutants showing increased plasma urea levels were compared with sex-matched littermate controls.

All phenotypic mutants showed increased urine volumes (Fig. 2A) and water consumption in the metabolism cage. As a tendency, urinary creatinine (Fig. 2B) and urea/creatinine (Fig. 2C) were mostly decreased in the 24-h urine in the phenotypic mutants of both sexes. Analysis of spot urine samples of additional animals confirmed these findings. The glomerular filtration rate of creatinine showed the same changes in both sexes only for line UREHR1, where decreased values were found for the phenotypic mutants. Thus renal clearance has to be analyzed in additional animals. The urinary sodium, potassium, and chloride levels were also analyzed in relation to the creatinine level. As a tendency, urinary sodium/creatinine and chloride/creatinine ratios of the phenotypic mutants were decreased in the 24-h urine and spot urine samples of all lines, whereas urinary potassium/creatinine ratio was increased in the 24-h urine and spot urine samples of the lines UREHR1 and UREHR3.
The phenotypic mutants of the lines UREHD1 and UREHR1 were examined for albuminuria using SDS-PAGE. Severe albuminuria was found in the phenotypic mutants of line UREHR1, whereas this was not detected in line UREHD1 (23).

Nephropathological findings in the mutant lines. Morphological analysis was carried out on kidneys from phenotypic mutants exhibiting increased plasma urea levels as well as sex-matched litters with normal plasma urea levels. Kidneys from the phenotypic mutant mice of the lines UREHD1 (n = 4; 8 mo postpartum) and UREHR4 (n = 2; 3 mo postpartum) were both macroscopically and light microscopically indistinguishable from those of littermate controls (data not shown).

Compared with sex-matched littermate controls showing no pathological kidney changes (Fig. 3A), 3-mo-old phenotypic mutant mice of line UREHR1 (n = 7) consistently showed renal lesions including both glomerular and tubulointerstitial changes (Fig. 3, B and C). Light microscopic examination revealed diffuse glomerular lesions with considerable variation among glomeruli. Prominent findings of less damaged glomeruli included glomerular hypertrophy and segmental to global mesangiolysis with widened and reticulated mesangial areas. The capillary walls were thickened, sometimes to many times of their original width, due to the accumulation of a pale staining material beneath the endothelium, sometimes leading to obliteration of capillary lumina. Thickened capillary walls frequently showed double-contour formation of the glomerular basement membrane. Further glomerular lesions included extravasation into the mesangial compartment, glomerular obsolescence, hyalinosis, as well as sclerosis with segmental to circumferential synechiae between the glomerular tuft and Bowman’s capsule. Occasionally, capillaries contained microthrombi. No amyloid deposits were detectable using Congo red stain. Tubulointerstitial lesions included tubular atrophy, hyaline droplets in proximal tubular epithelia, and proteinaceous casts in distal tubules. Furthermore, larger intrarenal arterial vessels showed cellular intimal thickening and media hypertrophy.

Immunohistochemical analysis revealed a positive staining of IgA, IgM, and C1q in glomeruli of phenotypic mutant UREHR1 mice (data not shown).

Predominant electronmicroscopic findings of glomeruli of phenotypic mutant UREHR1 mice (Fig. 3D) were capillary

Fig. 3. Pathomorphological findings in kidneys of mice of the line UREHR1. Overview of the renal cortex of a 3-mo-old healthy littermate control (A) and a phenotypic mutant (B) mouse. Glomerular hypertrophy, thickening of capillary walls, glomerulosclerotic changes with circumferential adhesion between the glomerular tuft and Bowman’s capsule, and glomerular obsolescence as well as tubular atrophy and numerous proteinaceous casts are evident in the mutant mouse kidney (B). A, B: H&E, magnification ×10 objective. C: glomerular profile of a phenotypic mutant mouse, showing mesangiolytic changes and thickening of capillary walls due to accumulation of pale staining material beneath the endothelium (H&E, magnification ×40 objective). D: transmission electron microscopy of a phenotypic mutant mouse. Glomerular capillary walls are widened by accumulation of lucent material. Mesangial cell interposition into widened subendothelial space and endothelial cells swelling is shown. Final magnification ×3,800.
The established lines showed an incomplete penetrance of the mutant phenotype of various degrees. Appearance of the pathological plasma urea levels due to the interaction of multiple unlinked mutations leads to lower numbers of affected offspring. However, all five lines were bred for multiple generations starting from the ENU-mutagenized G0 founder animal without losing the abnormal phenotype. In addition, reanalysis of the lines after a few weeks increased the phenotypic penetrance of the pathological urea levels, thereby indicating that the penetrance was generally underestimated in the analysis after 12 wk of age.

**DISCUSSION**

In this paper, we describe the successful establishment of five mouse lines harboring dominant and recessive mutations by the high-throughput screening of a large number of randomly mutagenized mice for increased plasma urea levels. Elevated plasma urea levels were mostly confirmed in the reanalysis of the affected animals. The breeding approaches for the establishment of mutant lines detected more than half of the fertile phenotypic variants which did not transmit the increased plasma urea levels to the offspring. A high ratio in the failure of the transmission of the defect to the offspring was also found for other phenotypic deviations in our clinical chemistry screen (16). One reason may be the occurrence of not genetically fixed increased plasma urea levels due to the inherent impossibility of carrying out complete standardization of husbandry and experiment.

The genome-wide linkage analysis of 85 and 136 informative single nucleotide polymorphism (SNP) marker assays were analyzed in 47 and 45 phenotypic mutant mice for the lines UREHR3 and UREHR4, respectively. In line UREHR3, the examination revealed a strong linkage $[\chi^2=125.5; -\log_{10}(P) = 27.25]$ of the mutation to a defined single chromosomal site on chromosome 2 represented by the marker rs4223511 at 132 Mb (mouse genome build 36.1). In line UREHR4, a strong linkage $[\chi^2=43.8; -\log_{10}(P) = 9.51]$ of the mutation to a defined single chromosomal site on chromosome 7 represented by the marker rs3654133 at 115 Mb was detected.

**Table 4. Body weight, kidney weight, and relative kidney weight of 4-mo-old female mice of the line UREHR2**

<table>
<thead>
<tr>
<th>Phenotype*</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Kidney Weight, mg</th>
<th>Relative Kidney Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant</td>
<td>4</td>
<td>21.7 ± 2.2</td>
<td>251 ± 52</td>
<td>1.15 ± 0.07</td>
</tr>
<tr>
<td>Wild-type</td>
<td>4</td>
<td>28.5 ± 6.2†</td>
<td>429 ± 36†</td>
<td>1.54 ± 0.20†</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Offspring of heterozygous mutant mice were classified according to their plasma urea levels. †P < 0.05.
Deviations in the number of offspring per litter and/or in the sex ratio of the offspring indicating early loss of mutants as alternative reason for the decreased frequency of mice showing an increased plasma urea level were not examined. The phenotypic analysis of the mutation may be improved by analyzing the animals more frequently and/or by challenge experiments (31). Strain-specific susceptibility to renal damage (1) and/or the animals more frequently and/or by challenge experiments (http://www.jax.org/phenome; http://www.eumorphia.org) (20) have been found. Therefore, generation and subsequent analysis of hybrids and/or congenic strains may give rise to variations in the appearance of increased plasma urea levels.

In addition to the clinical chemistry screen in the Munich ENU mouse mutagenesis project, a systematic clinical chemistry analysis has been described to date in another ENU mouse mutagenesis program (19). The appearance of lines exhibiting increased plasma urea values has not yet been reported on a different genetic background (BALB/c x C3H) (8). Other ENU-based screens for renal dysfunction (3, 12) used primary albuminuria examination with urinary dipstick and ELISA techniques and subsequent calculation of the ratio of urinary albumin to urinary creatinine (http://mouse-genome.bcm.tmc.edu). Alternatively, renal dysfunction was analyzed by searching for abnormalities in blood electrolytes and creatinine combined with urinalysis (http://www.cmhd.ca) (3).

We recently carried out the large-scale analysis of ENU-induced mouse mutants for albuminuria in the Munich ENU project by using qualitative SDS-PAGE. Examination of more than 2,000 G1 animals for dominant mutations and nearly 50 G3 pedigrees for recessive mutations resulted in two lines showing a low phenotypic penetrance of albuminuria. This might be caused by the appearance and segregation of multiple causative mutations within the lines. However, the analysis demonstrated the principle feasibility to identify nephropathy phenotypes in ENU-mutagenized mice by screening for albuminuria (23).

The morphological analysis of the kidneys revealed different results for the established lines. The kidney pathology of line UREHR1 obviously explains the observed plasma urea retention. The morphological features of UREHR1 mutants resemble the findings that have been suggested to be classified as thrombotic microangiopathy (17). The reduction of the kidney weight and kidney-to-body weight ratio in line UREHR2 may contribute to the altered phenotype but per se does not appear to be sufficient to cause the plasma urea retention. Therefore, further investigations concerning ultrastructural and pathological changes of the kidneys are required to clarify the etiology of the clinical chemistry plasma alteration in line UREHR2. The structural changes of the kidneys of line UREHR3 may be primarily responsible for the increased plasma urea levels and for polydipsia and polyuria, or they may be a secondary change due to polydipsia and polyuria. In the lines UREH1D1 and UREHR4, no structural kidney changes were apparent; therefore, pathophysiological alterations have to be taken into account as a cause for increased plasma urea levels. Further phenotyping of the renal defects will include kidney function analysis (31 and refs. therein) as well as expression profiling of isolated tissues and/or cultured cells from the mutant lines (27, 29).

The number and the chromosomal examination of the causative mutations in ENU projects are examined by linkage analysis. The results in the lines UREHR3 and UREHR4 revealing a single chromosomal site as cause for the altered phenotype showed the feasibility of the project with plasma urea as parameter. Further linkage analysis on the defined chromosomal site and candidate gene analysis is currently performed to identify the causative mutation (10, 28).

Due to the triggering of random mutations by ENU, discovery of genes not yet known to be involved in the plasma urea excretion may occur in our mouse mutants. The mutants may also carry hypomorphic, hypomorph, or null alleles of known genes which may be useful in the further understanding of the role of these genes in kidney diseases. After having carried out the chromosomal linkage analysis, the subsequent identification of the causative mutations including the analysis of their functional relevance by reverse genetics methods as well as the examination of the pathological consequences of the mutations may establish suitable models for the subsequent research on human nephropathies.

REFERENCES


