The First \textit{Scube}3 Mutant Mouse Line with Pleiotropic Phenotypic Alterations

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ABSTRACT

The vertebrate \textit{Scube} (Signal peptide, CUB and EGF-like domain-containing protein) family consists of three independent members, \textit{Scube}1–3, which encode secreted cell surface-associated membrane glycoproteins. Limited information about the general function of this gene family is available, and their roles during adulthood. Here, we present the first \textit{Scube}3 mutant mouse line (\textit{Scube}3\textsuperscript{N294K/N294K}), which clearly shows phenotypic alterations by carrying a missense mutation in exon 8, and thus contributes to our understanding of \textit{SCUBE3} functions. We performed a detailed phenotypic characterization in the German Mouse Clinic (GMC). \textit{Scube}3\textsuperscript{N294K/N294K} mutants showed morphological abnormalities of the skeleton, alterations of parameters relevant for bone metabolism, changes in renal function, and hearing impairments. These findings correlate with characteristics of the rare metabolic bone disorder Paget disease of bone (PDB), associated with the chromosomal region of human \textit{SCUBE3}. In addition, alterations in energy metabolism, behavior, and neurological functions were detected in \textit{Scube}3\textsuperscript{N294K/N294K} mice. The \textit{Scube}3\textsuperscript{N294K/N294K} mutant mouse line may serve as a new model for further studying the effect of impaired \textit{SCUBE3} gene function.

KEYWORDS

\textit{SCUBE3} systemic phenotype pleitropy Paget disease of bone (PDB) mouse model

The vertebrate \textit{Scube} (Signal peptide, CUB and EGF-like domain-containing protein) family consists of three independent members, \textit{Scube}1–3. These encode secreted cell surface-associated glycoproteins that share a domain, organization of at least five recognizable motifs and the ability to both homo- and heterodimerize (Xavier et al. 2013). Human \textit{SCUBE3} was originally identified following transcriptional profiling of vascular endothelial cells and demonstrated significant enrichment in primary osteoblasts and long bones (Wu et al. 2004). \textit{SCUBE3} is a signal protein that is expressed during embryonic development in several tissues (Xavier et al. 2013). In mice, \textit{Scube}3 is
expressed in ectodermal, endodermal, and mesodermal derivatives, as are other members of the SCube gene family (Haworth et al. 2007). Expression of these genes has been shown to be dynamic, and both reciprocal and complementary to each other (Xavier et al. 2013; Haworth et al. 2007).

Although our understanding of the function of SCUBE3 in embryonic development as well as during adulthood is still marginal, one major role appears to be in bone development and homeostasis, with another one in neurological functions. Interestingly, human SCUBE3 maps to chromosome 6p21.3, a region that has been linked to Paget disease of bone 1 (PDB1) (Rotin et al. 1977; Tilgham et al. 1982), which is characterized by focal areas of increased bone turnover (Ralston et al. 2008). SCUBE3 function is also associated with other tissues for example, SCube3 overexpression in transgenic mice induced cardiac hypertrophy (Yang et al. 2007), and zebrafish SCube3 was recently identified as a key regulator of fast muscle development by modulating fibroblast growth factor signaling (Tu et al. 2014). Further associations of SCube3 have been reported with hedgehog signal transduction (Johnson et al. 2012), angiogenesis (Yang et al. 2013), and the immune system (Luo et al. 2012). In addition, deregulation of SCUBE3 has been found in different tumor tissues such as lung cancer (Wu et al. 2011; Zhao et al. 2013) or renal carcinomas (Morris et al. 2011).

Although SCUBE3 seems to be involved in many different organ systems and diseases, there is no suitable mouse model so far for the study of functional alterations. Recent publications on mice lacking SCube3 did not show any obvious phenotype (Xavier et al. 2010; Xavier et al. 2013). In this study, we present the first SCube3 mutant mouse line with phenotypic alterations: SCube3N294K/N294K. The mutant mouse line carries a recessive point mutation in Scube3 and was derived from the Munich N-ethyl-N-nitrosourea (ENU) mouse mutagenesis project (MEP, Hrabé de Angelis et al. 2000; Sabrautzki et al. 2012). A systemic phenotypic characterization (Hrabé de Angelis et al. 2015) of this new mutant mouse line annotates SCube3 gene function in mice to bone metabolism and morphology, renal function, and hearing, as well as neurological and behavioral functions and energy metabolism.

**MATERIALS AND METHODS**

**Generation of Scube3N294K/N294K mutants**

ENU mutagenesis and breeding were performed as described on a pure C3HeB/F6J (CH3) background (Hrabé de Angelis et al. 2000; Sabrautzki et al. 2012; Aigner et al. 2011). Briefly, C3H mice were originally purchased from the Jackson Laboratory (Bar Harbor, ME) and ENU (Serva Electrophoresis, Heidelberg, Germany) was applied in three weekly intervals by intraperitoneal injections of 90 mg/kg body weight to 10–12-week-old male mice (G0). G0 mice were mated with wild-type C3H females to produce F1 offspring. F1 males not showing any obvious phenotypic alterations were mated with wild-type C3H females to obtain the G2 generation. We either choose 6–8 female G2 mice for matings with their F1 father or performed intercross matings of G2 mice to produce at least 20 mice (G3 families). Phenotyping for dysmorphic alterations was performed according to a standardized protocol (Fuchs et al. 2000). A mutation was confirmed by showing a Mendelian distribution of expected homozygous mutant mice. The Scube3N294K/N294K mouse line was maintained on the C3H genetic background for more than 10 generations.

**Chromosomal mapping**

Homozygous carriers of the G3 generation were mated to C57BL/6J (B6) wild-type mice and the progeny (F1 generation) were intercrossed. DNA was prepared from tail tips of affected offspring (F2 generation). For chromosomal mapping, a microsatellite panel for polymorphic markers between C3H and B6 was used (Hrabé de Angelis et al. 2000).

**Whole exome sequencing**

For enrichment of exonic sequences, we used the SureSelectXT Mouse All Exon 50 Mb kit (Agilent) followed by Illumina HiSeq2000 sequencing as 100 bp paired-end runs with an average 108 × coverage (≥ 93% of the target being covered > 20x). To search for the causative variants, we compared the sequences of one Scube3aN294K/N294K mouse to one mouse from the C3HeB/F6J background strain.

**Phenotypic analysis**

For the phenotypic characterization of young adult (starting at the age of 7 wk) Scube3aN294K/N294K mutants, a cohort of 15 male and 15 female
Scube3<sup>N294K/N294K</sup> mutants, as well as 15 male and 15 female wild-type littermate controls (Scube3<sup>WT</sup>), was analyzed in the primary phenotyping screen of the GMC (Hrabé de Angelis et al. 2015; Gailus-Durner et al. 2005; Fuchs et al. 2012, 2011). The tests within the phenotyping pipeline and the corresponding age of the animals, as well as references for the protocols, are shown in Supplemental Material, Table S1. For further detailed investigation of observed phenotypes, secondary tests were carried out. Details for the applied protocols are listed below.

**Progression study**

Since Scube3<sup>N294K/N294K</sup> mice showed signs of an impaired bone metabolism, we tested groups of Scube3<sup>N294K/N294K</sup> and Scube3<sup>WT</sup> mice at 12, 24, 36, and 52 wk of age for the clinical chemical plasma parameters inorganic calcium (Ca), total inorganic phosphate (Pi), total alkaline phosphatase (ALP), cholesterol (CHO), triglycerides (TGL), glucose (GLUC), total protein (TP), urea (U), uric acid (UA), and albumin (ALB) by using an AU480 clinical chemistry analyzer (Beckman-Coulter, Krefeld, Germany) and adapted reagent kits provided by Beckman-Coulter. Additionally, pQCT (peripheral QuantitativeComputed Tomography, Stratec, Pforzheim, Germany) analyses of the femoral metaphysis and diaphysis were performed at the age of 9 and 12 months. We measured CTX-1 in plasma of 52 wk old mice using RatLaps (carboxy-terminal collagen crosslinks, CTX-1) EIA ELISA from IDS (Frankfurt am Main, Germany) according to the manufacturer’s protocol.

**Analysis of renal function in metabolic cages**

A subgroup of 48 animals (12 mutant and control mice each of both sexes) at the age of 34 wk was subjected to a renal function test using metabolic cages for single mice (Tecniplast, Buguggiate, Italy) to collect 48 hr urine samples and monitor water and urine production. During the test, mice had free access to water and pulverized food. Tests were conducted as described previously, starting with a single blood sample collection followed by urine collection over 48 hr (Fuchs et al. 2011). Urine and plasma samples were analyzed for a set of 12 clinical chemistry parameters including concentrations of sodium, potassium, and chloride (Na, K, and Cl), Ca, Pi, creatinine (CREA), U, UA, and GLUC, as well as TP and ALB, as described above.

**Transcriptome analysis**

Transcriptome analyses from kidney samples of four Scube3<sup>N294K/N294K</sup> and four Scube3<sup>WT</sup> male mice at the age of 29 wk were performed following total RNA extraction (RNAeasy Midi kit, QIAGEN). Illumina Mouse Ref8 v2.0 Expression BeadChips were employed as previously described (Horsch et al. 2008; Kugler et al. 2013). Illumina Genomestudio 2011.1 was used for data normalization (cubic spline) and statistical analysis for the identification of differential gene expression was performed with SAM (Significant Analysis of Microarrays, fold change > 1.6, FDR < 6%) (Saeed et al. 2006; Tusher et al. 2001). Overrepresented functional annotations were obtained through the use of QIAGEN’s Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity). Expression data are available at the GEO database under GSE56402.

**Inner ear preparation**

Inner ears were dissected from the temporal bones of killed animals and fixed in 4% formalin (Carl Roth GmbH, Karlsruhe, Germany). Following dehydration in ethanol, inner ears were immersed in methyl salicylate (Sigma Aldrich, Taufkirchen, Germany) and incubated overnight. Analysis of cleared inner ears was documented by photographic images.

![Figure 1: Skeletal abnormalities in Scube3<sup>N294K/N294K</sup> mice. (A) Femur length of female animals plotted by body length (red, Scube3<sup>N294K/N294K</sup> and blue, Scube3<sup>WT</sup>). (B) Bone mineral content plotted by body weight (green, Scube3<sup>N294K/N294K</sup> males; yellow, Scube3<sup>N294K/N294K</sup> females; blue, Scube3<sup>WT</sup> males; and red, Scube3<sup>WT</sup> females). (C) Bone mineral density plotted by body weight (green, Scube3<sup>N294K/N294K</sup> males; yellow, Scube3<sup>N294K/N294K</sup> females; blue, Scube3<sup>WT</sup> males; red, Scube3<sup>WT</sup> females).](#)
**RESULTS**

**Generation of Scube3^{N294K/N294K} mice**

The Scube3^{N294K/N294K} mutation was generated in the large-scale Munich ENU mutagenesis program. The mutant line was identified by screening G3 animals for morphological abnormalities resulting in reduced body size (Figure 1A), a shorter and kinky tail, abnormal digit positioning, and an abnormal posture when hung by the tail. Scube3^{N294K/N294K} variants were crossed to wild-type C3HeB/FeJ (C3H) mice, and none of the offspring showed the characteristic Scube3^{N294K/N294K} phenotypes. However, intercrossing these heterozygous mice resulted in a fraction of about 25% of offspring that showed the characteristic Scube3^{N294K/N294K} phenotypes. Therefore, we considered Scube3^{N294K/N294K} to be a recessive mutant mouse line, and maintained the mutation on a pure C3H genetic background for more than 10 generations.

**Mutation detection**

Rough mapping by an outcross/intercross breeding strategy with C57BL/6j (B6) animals revealed a 12.4 Mb critical region on chromosome 17 between markers D17Mit46 and D17Mit34, with no recombinants near marker D17Mit198. Whole exome sequencing identified a homozygous nonsynonymous sequence variation within the Scube3 gene, the only candidate gene within the critical region on chromosome 17.
A cohort of 15 male and 15 female Phenotypic analysis with a score of 0.99.

In 2012) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), this se-

determination to detect if split ossification centers were detected in T10 and T11 (two asterisks in one vertebra). Sternebrae of Scube3N294K/N294K mice (D) were slightly thicker and longer than ones of wild-type mice (C). Asterisks in (D) indicate hyper-ossified sternebrae. Bones of upper and lower limbs in homoygous mutants (F and H) were also hyper-ossified compared to wild-type (E and G). Arrowhead indicate ossified coracoid process (E). Asterisks in (F) and (H) show representative hyper-ossified metacarpals, metatarsals, phalanx, and tarsals.

Figure 3 Skeletal phenotypes of newborn Scube3N294K/N294K mice. Hyper-ossification of entire vertebrae in homoygous Scube3N294K/N294K mice (B and D) compared to wild-type mice (A and C). Asterisks in (B) indicate remarkable hyper-ossified vertebrae. Note that split ossification centers were detected in T10 and T11 (two asterisks in one vertebra). Sternebrae of Scube3N294K/N294K mice (D) were slightly thicker and longer than ones of wild-type mice (C). Asterisks in (D) indicate hyper-ossified sterna. Bones of upper and lower limbs in homoygous mutants (F and H) were also hyper-ossified compared to wild-type (E and G). Arrowhead indicate ossified coracoid process (E). Asterisks in (F) and (H) show representative hyper-ossified metacarpals, metatarsals, phalanx, and tarsals.

Scube3N294K/N294K mice have defects in bone growth, but not in embryonic patterning: For further investigation of the observed bone abnormalities, we analyzed Scube3N294K/N294K mice at newborn and various embryonic stages by skeletal staining. Multiple hyper-ossifications in the axial and appendicular skeleton were detected. In thoracic, lumbar, and sacral vertebrae of Scube3N294K/N294K mice, ossification center and pedicles were more closed and fused compared to those of wild-type mice (Figure 3B, asterisks indicate the ossification center). Further, in some thoracic vertebrae, ossification centers were split in mutant mice (Figure 3B, two asterisks in one vertebra). In the appendicular skeleton, the coracoid processes showed hyper-ossification, and proximal and middle phalanges in digit II started to be fused (Figure 3F, arrowhead and black asterisks). Furthermore, metacarpal, metatarsal, and tarsal were enlarged due to hyper-ossification (Figure 3F and Figure 3H, white asterisks). These hyper-ossification phenotypes were not detected at embryonic day E15.5 around the time when endochondral ossification starts, but were detected in E17.5 Scube3N294K/N294K embryos (data not shown), suggesting that malformations of Scube3N294K/N294K mice are due to defects in bone growth, but not because of disturbed embryonic patterning as in vertebral segmentation disorders.

Scube3N294K/N294K mice show symptoms pointing to disturbances in renal function: U, CREA, and K levels, as well as α-amylase activities, were increased in plasma of Scube3N294K/N294K mice of both sexes (Table S2B). The findings were consistent for most parameters over

17. A C–A transversion at nucleotide position 882 in exon 8 of Scube3 leads to an asparagine to lysine exchange at protein position 294 (N294K). This nonsynonymous sequence variation cosegregated with the Scube3N294K/N294K phenotype in all 15 tested mutant mice, and was not confirmed in 10 wild-type littermates and other mice of inbred C3H, BALB/c, and B6 strains. Subsequently, this mutation was confirmed in more than 100 mutant Scube3N294K/N294K mice during maintenance breeding and excluded in the equal number of wild-type littermates.

The N294K substitution is located within the Ca-binding EGF-like domain 7 of SCUBE3, which is highly conserved between mouse, human, zebra fish, and chicken (data not shown). Using the protein prediction programs PROVEAN (http://provean.jcvi.org; Choi et al. 2012) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), this sequence variation was classified as deleterious or probably damaging with a score of 0.99.

**Phenotypic analysis**

A cohort of 15 male and 15 female Scube3N294K/N294K mice and the respective number of Scube3WT mice were analyzed in the GMC in the screens for behavior, neurology, nociception, vision and eye, dysmorphism, bone and cartilage, energy metabolism, hematology, clinical chemistry, steroid metabolism, immunology, allergy, cardiovascular system, lung function, molecular phenotyping, and pathology.

**Scube3N294K/N294K mice show skeletal abnormalities and changes in bone metabolism:** In addition to the aforementioned smaller body size, shorter and kinked tails, and abnormal digit positioning, X-ray analysis of the skeleton showed no obvious craniofacial abnormalities but detected malformations of the thoracic and lumbar vertebrae, and shorter femora (Figure 1A and Table S2C) in Scube3N294K/N294K mice that were independent of the body size reduction. Rib fusions occurred with low penetrance (3% of observed animals, data not shown). In DEXA analysis, bone mineral density (BMD) and bone mineral content (BMC) were significantly decreased (Table S2B), but the results might be confounded by body weight (Figure 1, B and C). Several parameters in

**pQCT-measurements supported the findings of the DEXA analysis.** The effects were weaker in 12-month-old than in 9-months-old mice (File S1 and Table S3).

Ca levels at the age of 17 wk were elevated in Scube3N294K/N294K female mice but this finding was not confirmed in older animals. P levels were globally decreased in male mice and ALP activities in mice of both sexes were increased with effects getting weaker with age. At 1 yr of age, Scube3N294K/N294K mice showed a significant increase of the bone resorption marker CTX-1 (Figure 2).

Scube3N294K/N294K mice have defects in bone growth, but not in embryonic patterning: For further investigation of the observed bone abnormalities, we analyzed Scube3N294K/N294K mice at newborn and various embryonic stages by skeletal staining. Multiple hyper-ossifications in the axial and appendicular skeleton were detected. In thoracic, lumbar, and sacral vertebrae of Scube3N294K/N294K mice, ossification center and pedicles were more closed and fused compared to those of wild-type mice (Figure 3B, asterisks indicate the ossification center). Further, in some thoracic vertebrae, ossification centers were split in mutant mice (Figure 3B, two asterisks in one vertebra). In the appendicular skeleton, the coracoid processes showed hyper-ossification, and proximal and middle phalanges in digit II started to be fused (Figure 3F, arrowhead and black asterisks). Furthermore, metacarpal, metatarsal, and tarsal were enlarged due to hyper-ossification (Figure 3F and Figure 3H, white asterisks). These hyper-ossification phenotypes were not detected at embryonic day E15.5 around the time when endochondral ossification starts, but were detected in E17.5 Scube3N294K/N294K embryos (data not shown), suggesting that malformations of Scube3N294K/N294K mice are due to defects in bone growth, but not because of disturbed embryonic patterning as in vertebral segmentation disorders.

**Scube3N294K/N294K mice show symptoms pointing to disturbances in renal function:** U, CREA, and K levels, as well as α-amylase activities, were increased in plasma of Scube3N294K/N294K mice of both sexes (Table S2B). The findings were consistent for most parameters over
the whole life period (measured at 12, 24, 36, and 52 wk of age, Figure 2). In addition, plasma glucose levels, triglyceride, and P, concentrations were decreased in both sexes, whereas total protein, albumin, and cholesterol were decreased only in male mutants (Table S2B). To follow up these findings, we analyzed renal function in metabolic cages. Calculated 24 hr CREA clearance adjusted to body weight was normal in Scube3WT mice, suggesting that a similar amount of plasma was filtered per g of body mass by the kidneys of mutant and control mice within 24 hr (Table 1). However, water uptake and urine production in relation to body mass (Table 1) as well as food consumption (2.85 ± 0.23 vs. 2.02 ± 0.35 and 3.18 ± 0.57 vs. 2.64 ± 0.47 g/24 hr in mutant vs. control males and females, respectively) were significantly higher in Scube3WT mice. Urinary concentrations of electrolytes and U were comparable, while CREA, protein (total protein and albumin), and glucose concentrations were slightly lower, and uric acid level was significantly lower, in Scube3N294K/N294K mice (data not shown). Consequently, calculated 24 hr excretion values per 25 g body mass and fractional excretion rates were significantly increased in Scube3N294K/N294K mice for the electrolytes Na, K, Cl, as well as Ca, U, total protein, albumin, and glucose. Uric acid and P, excretion adjusted to body mass was found to be highly variable and did not significantly differ between genotypes (Table 1).

**Transcriptome analysis suggests a role of Scube3 in reabsorption and/or excretion of urine components:** We performed transcriptome analysis of kidney samples from Scube3WT/N294K mice. Statistical analysis of gene expression patterns identified 138 differentially expressed genes functionally classified by the following overrepresented terms: cellular development, movement, and proliferation, as well as cardiovascular system function, developmental disorder, and renal and urological disease (Table 2). Additionally, literature-based research revealed several genes associated with expression in proximal tubules (Havcr1, Has2, Met, Mep1h, Mme, and SLC29A8), reabsorption of electrolytes (proximal tubule epithelium: Cldn10 and Slc12a1), and maintenance of salt/water balance of blood (renal medulla: Acta2, Adamts1, Ehd3, Kcnj15, and Umod). All these genes were down-regulated in kidney.

**Scube3N294K/N294K mice have hearing deficits, alterations in the inner and middle ear, and show behavioral abnormalities:** Hearing sensitivity was assessed by auditory brainstem response (ABR) to different auditory stimuli. There were significant differences at clicks and all tested frequencies; thresholds were increased in Scube3N294K/N294K mice of both sexes, with female Scube3N294K/N294K mice more severely affected (Figure 4A). Further analysis of this finding revealed alterations in inner and middle ear preparations. The inner and middle ears appeared smaller and the ossicles of the middle ear had decreased size and altered shape. The incus was smaller, and the body and head of the malleus were especially decreased in size (Figure 4B). The middle ear cavity, the bulla, part of the temporal bone, had an irregular shape. The incus was smaller, and the body and head of the malleus were especially decreased in size (Figure 4B). The middle ear cavity, the bulla, part of the temporal bone, had an irregular shape.

### Table 1 Renal function analysis

| Parameter                        | Males (Scube3WT) | Males (Scube3N294K/N294K) | Females (Scube3WT) | Females (Scube3N294K/N294K) | 2-Way ANOVA (P Value) |
|----------------------------------|-----------------|--------------------------|-------------------|---------------------------|-----------------------|
|                                  | Scube3WT n = 12 | Scube3N294K/N294K n = 12 | Scube3WT n = 10   | Scube3N294K/N294K n = 12  | Genotype Sex Genotype × Sex |
| Water uptake/25 g BW (g/24 hr)   | 3.68 ± 0.81     | 6.01 ± 1.54              | 4.65 ± 1.88       | 6.88 ± 1.53               | <0.001 0.039 0.910    |
| Urine excretion/25 g BW (g/24 hr)| 0.59 ± 0.31     | 1.1 ± 0.43               | 0.89 ± 0.57       | 1.2 ± 0.55                | 0.004 0.201 0.412     |
| Creatinine clearance/25 g BW (µg/24 hr) | 583 ± 305       | 548 ± 265                | 812 ± 564         | 606 ± 191                 | 0.246 0.168 0.409     |
| Na/25 g BW (µmol/24 hr)          | 102 ± 50        | 208 ± 59                 | 181 ± 105         | 205 ± 102                 | <0.001 0.004 0.803    |
| K/25 g BW (µmol/24 hr)           | 228 ± 109       | 444 ± 131                | 392 ± 237         | 539 ± 192                 | <0.001 0.014 0.510    |
| Cl/25 g BW (µmol/24 hr)          | 125 ± 62        | 262 ± 71                 | 245 ± 154         | 364 ± 116                 | <0.001 <0.001 0.758   |
| Ca/25 g BW (µmol/24 hr)          | 1.2 ± 0.6       | 2.3 ± 0.8                | 2.7 ± 1.5         | 4.1 ± 1.4                 | <0.001 <0.001 0.611   |
| Urea/25 g BW (mg/24 hr)          | 60 ± 28.6       | 112 ± 31.9               | 111 ± 65.3        | 155 ± 44                  | 0.099 <0.001 0.671    |
| Total protein/25 g BW (mg/24 hr) | 8.2 ± 3.5       | 11.8 ± 3.3               | 2.8 ± 1.9         | 3.7 ± 1.9                 | 0.009 <0.001 0.107    |
| Albumin/25 g BW (µg/24 hr)       | 98 ± 39         | 122 ± 31                 | 108 ± 44          | 153 ± 50                  | 0.008 0.109 0.411     |
| Glucose/25 g BW (µg/24 hr)       | 210 ± 90        | 310 ± 94                 | 427 ± 204         | 501 ± 121                 | 0.030 <0.001 0.742    |
| FE Na (%)                        | 0.13 ± 0.05     | 0.30 ± 0.10              | 0.18 ± 0.05       | 0.33 ± 0.08               | <0.001 0.149 0.656    |
| FE K (%)                         | 9.7 ± 3.1       | 20.4 ± 6.2               | 13.2 ± 3.3        | 21.7 ± 4.8                | <0.001 0.082 0.422    |
| FE Cl (%)                        | 0.22 ± 0.08     | 0.52 ± 0.17              | 0.31 ± 0.08       | 0.57 ± 0.13               | <0.001 0.061 0.672    |
| FE Ca (%)                        | 0.10 ± 0.03     | 0.20 ± 0.07              | 0.15 ± 0.02       | 0.28 ± 0.08               | <0.001 <0.001 0.343   |
| FE urea (%)                      | 19 ± 4.9        | 34 ± 10.4                | 28 ± 6.6          | 41 ± 10.5                 | <0.001 0.002 0.708    |
| FE total protein (%)             | 0.007 ± 0.003   | 0.012 ± 0.006            | 0.027 ± 0.009     | 0.045 ± 0.012             | <0.001 <0.001 0.016   |
| FE albumin (%)                   | 0.00006 ± 0     | 0.00009 ± 0              | 0.00007 ± 0      | 0.00010 ± 0               | <0.001 0.334 0.596    |
| FE glucose (%)                   | 0.029 ± 0.009   | 0.043 ± 0.010            | 0.019 ± 0.007     | 0.031 ± 0.010             | <0.001 <0.001 0.720   |

ANOVA, analysis of variance; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; FE, calculated fractional excretion rate.
Table 2 Functional classification of regulated genes in kidney

| Biological Functions and Disease | Genes | # Genes |
|---------------------------------|-------|---------|
| Cellular development            | Blk, Casp3, Ccr1, Chek1, Commd3-Bmi1, Cxcl12, Eif4g2, Foxc1, Gabpa, Has2, Havcr1, Hif1a, Hmxo1, Hoxa10, Id4, Igf2, Jdp2, Met, Rasgrf1, Scel, Srp2, Tcf21, Tert, Tnn | 25 |
| Cellular movement               | Adams1, Arhgap35, Ccr1, Cxcl12, Ereg, Foxc1, Fpr3, Has2, Hif1a, Hmxo1, Igf2, Ltc4s, Mep1b, Met, Mme, Plec, Ppt2, Rasgrf1, Sema6d, Slc12a1, Srp2, Tgfbr3, Tnn, Trpc1, Usp8, Vav3 | 25 |
| Cardiovascular system function  | A4gnt, Adams1, Casp3, Ccr1, Cxcl12, Ehd3, Foxc1, Has2, Hif1a, Hmxo1, Hoxa10, If57, Igf2, Lefty2, Met, Plec, Srp2, Tcf21, Tert, Tgfbr3, Tnn, Trpc1, Usp8, Vav3 | 24 |
| Nervous system function         | Arhgap35, Casp3, Ccm, Commd3-Bmi1, Cxcl12, Fa2h, Faim2, Gpr37, Hif1a, Hoxa10, If57, Igf2, Madd, Met, Plec, Ppt2, Rgs7, Sh3gl2, Sltkr6, Vav3 | 20 |
| Cell death and survival         | Adams1, C8orf44-Sgk3/Sgk3, Casp3, Ccr1, Chek1, Commd3-Bmi1, Cxcl12, Fa2h, Hif1a, Hoxa10, Id4, Igf2, Jdp2, Met, Tert, Top1, Umod, Usp8 | 18 |
| Neurological disease            | Casp3, Commd3-Bmi1, Gnat1, Gng7, Grik1, Hmxo1, Id4, Igf2, Kcnip2, Man1a1, Mdh1, Ppp1cb, Ppt2, Ranbp1, Sgtb, Sh3gl2, Top1 | 17 |
| Cancer                          | Acta2, Bdh1, Gpr37, Havcr1, Hif1a, Hmxo1, Id4, Igf2, Lefty2, Mdh1, Met, Tert, Tgfbr3 | 13 |
| Cell cycle                      | Chek1, Commd3-Bmi1, Has2, Havcr1, Hoxa10, Id4, Igf2, Met, Sibp, Tert, Trpc1, Usp8 | 13 |
| Cellular growth and proliferation| Chek1, Cxcl12, Ereg, Ghrhr, Has2, Hif1a, Hoxa10, Id4, Lmo3, Met, Tert, Top1 | 12 |
| Developmental disorder          | Ccm, Commd3-Bmi1, Eif4g2, Has2, Hif1a, Hmxo1, If57, Igf2, Ranbp1, Sh3gl2, Slc12a1, Usp8 | 12 |
| Renal and urological disease    | Ccr1, Ereg, Havcr1, Hif1a, Igf2, Lmo3, Met, Mme, Slc39a8, Tert | 10 |

Shown are significantly (P < 0.05) enriched terms from the “biological functions and disease” analysis in Ingenuity Pathway Analysis.

grip strength, which was also influenced by the body mass reduction (Table S2, B and C and Figure 5).

**Scube3N294K/N294K** mice have altered function in energy metabolism: Scube3N294K/N294K mice had significantly reduced body mass, and considerably lower absolute fat and lean mass (Table S2C). They also showed a significant shift in body composition, particularly with regards to decreased lean mass when adjusted to body mass (Figure 6). Interestingly, the relations between body mass and fat content, as well as body mass and lean mass, were significantly different between genotypes (Table S2C), indicating a systematic effect on body composition. Scube3N294K/N294K mice had significantly lower glucose, triglyceride, NEFA, and glycerol concentrations in plasma (Table S2B). In the glucose tolerance test, basal fasting glucose levels were significantly decreased in Scube3N294K/N294K mice. AUC (area under the curve) values were decreased, which indicates an improved

![Figure 4](image-url) (A) Mild conductive hearing loss; asterisks mark genotype effect of both sexes together. *P < 0.05, **P < 0.01, ***P < 0.001. (B) Abnormalities in inner ear development. Upper panel: depiction of smaller and malformed ossicles. The ambos (incus) has a different shape and the body of the hammer (malleus) is narrowed (arrow) and the head surface decreased. Lower panel: the whole inner and middle ear was reduced in size and, besides the alterations of the ossicles (arrow pointing toward the hammer, malleus), the auditory cavity (bulla) has an irregular shape (arrows). WT, wild-type.
glucose tolerance in Scube3N294K/N294K mice (Table S2B). We applied indirect calorimetry to investigate energy expenditure, substrate use, and locomotor activity under home-cage conditions, and found rearing behavior decreased during the 21 hr test phase. No major effects on energy turnover could be detected when VO2 was adjusted to body mass (Table S2C). In a separate test, food consumption and gastro-intestinal functions of Scube3N294K/N294K mice were monitored over 5 d in single caged animals. We could not detect considerable effects on food intake, energy assimilation, and the efficiency of energy extraction from food.

Scube3N294K/N294K mice show mild alterations in cardiovascular parameters, but no hints of deficits in heart performance or conduction: Echocardiography and electrocardiography revealed several mild alterations in Scube3N294K/N294K mice (decreased interventricular septum width, decreased left ventricular posterior wall thickness, decreased left ventricular mass, and prolonged QRS interval duration mainly in male Scube3N294K/N294K mice, as well as decreased diastolic ventricular dimension and decreased respiration rate in female Scube3N294K/N294K mice, Table S2A). We found a significantly lower

Figure 5 Analysis of Scube3N294K/N294K mice in the Open Field test and grip strength analysis. Data for distance traveled (A), number of rears (B), and percent time spent in center (C) are shown over the 20 min test interval. Grip strength (four paw measurement) is plotted vs. body weight (D) (green, Scube3N294K/N294K males; yellow, Scube3N294K/N294K females; blue, Scube3WT males; and red, Scube3WT females).
heart weight of Scube3N294K/N294K mice as compared to sex-matched controls (also when normalized to tibia length). However, no alterations were found in heart performance or conduction, and thus there was no clear hint for a physiologically relevant constraint of cardiovascular function due to the mutation. Further in line, the observed alterations may be confounded by the reduced body weight of Scube3N294K/N294K mice.

**Further tested organ functions are normal in Scube3N294K/N294K mice:** The analysis of immunologically relevant parameters revealed no major changes. We observed tendencies toward decreased frequency of L-selectin expressing cells within T cell populations and increased frequency of CD11b-expressing cells within the NK cell compartment (Table S2A). We did not detect any changes in hematological parameters. Eye morphology and vision were as expected for the genetic background (C3HeB/FeJ). There were no major genotype effects on antibody responses. No differences were detected for rotarod performance. The analysis of transdermal water loss (TEWL) from the skin of the mice did not reveal genotype-specific differences, while hair structure and coat appeared normal. There were no alterations in lung function. Scube3N294K/N294K mice at the age of 21 wk did not present additional histopathological alterations.

**DISCUSSION**

We identified an ENU-derived mouse line carrying a missense mutation in Scube3 (NM_001004366.1:c.882C > A, NP_001004366.1:p.Asn294Lys), and characterized the Scube3N294K/N294K mutant mouse in detail in the GMC (Hrabé de Angelis et al. 2005; Fuchs et al. 2012). As shown previously in a Scube3 reporter mouse line, targeted replacement of exons 2 and 3 by a lacZ-cassette demonstrated early expression of the gene in craniofacial, limb, and neural tube tissues (Xavier et al. 2010). However, these mice, as well as SCUBE3 loss-of-function mice, developed normally and did not show overt phenotypic alterations (Xavier et al. 2013, 2010) whereas Scube3N294K/N294K mice exhibited alterations in several organ systems. One reason for this discrepancy might be owed to the different genetic backgrounds of the mice in these studies. However, a more likely explanation is the redundancy of Scube family members, which might compensate the loss of SCUBE3 in the null mutant (Xavier et al. 2013), and the nature of the mutation in the Scube3 mouse model described here. The amino acid exchange in Scube3N294K/N294K mice lies within the EGF-like domain 7 that follows the consensus sequence D/N-X-D/N-E/Q-Xm-D/N-Xn-Y/F for Ca-binding EGF-like domains (cbEGFs, Figure 7). These domains are known to mediate protein–protein and protein–carbohydrate interactions in a Ca-dependent manner (Downing et al. 1996). They are also structurally important for several cellular processes such as extracellular matrix architecture or specification of cell fates (Downing et al. 1996). For example, missense mutation of conserved amino acids in cbEGFs of fibrillin 1 (FBN1) are causative for Marfan syndrome due to Ca-dependent misfolding of the protein (Whiteman et al. 2007). Likewise, the amino acid exchange in the cbEGF domain 7 in Scube3N294K/N294K mice might lead to a reduced Ca-binding capacity and/or to conformational changes, which in turn could negatively interfere with both homo- and heteromeric interaction of SCUBE proteins (Wu et al. 2004; Yang et al. 2002), posttranslational processing (Wu et al. 2011), and interaction with transforming growth factor-β and hedgehog signaling (Johnson et al. 2012; Wu et al. 2011).

Scube3 is expressed in the cartilaginous primordia of the skeleton and regions of intramembranous bone formation in the developing craniofacial region (Haworth et al. 2007). Nevertheless, besides middle ear abnormalities, Scube3N294K/N294K mice did not show any further craniofacial abnormalities, suggesting that SCUBE3 is dispensable for craniofacial development (Xavier et al. 2013) or that the respective functions may be assigned to regions upstream of cbEGF7 of SCUBE3. However, the Scube3N294K/N294K mutation directly affects bone morphology and bone metabolism. Scube3N294K/N294K mice had a significant reduction in body size and weight. Interestingly, GWAS found SCUBE3 SNPs among adult and pediatric height-associated loci (Gudbjartsson et al. 2008; Zhao et al. 2010), as well as in pigs, associated with body height, body length, and rump circumference (Wang et al. 2014). The influence of the Scube3 mutation on bone metabolism was expressed by increased ALP activities and CTX-1 values in plasma, as well as by decreased BMD and BMC in DEXA and pQCT analysis. These data are supported by the finding that SCUBE3 is expressed in...
Figure 7 Scube3N294K/N294K mice are homozygous for a (C) to (A) point mutation at nucleotide position 882 that leads to an asparagine to lysine exchange at protein position 294 (N294K) in exon 8 of Scube3 (A). The mutation affects the calcium-binding EGF-like domain VII (B), which might have effects on the capabilities to form homo- or heterodimers and block TGFβ/Hedgehog signaling (C), which causes phenotypic alterations in bone development and homeostasis, hearing ability, renal function, energy metabolism, neurological functions, and behavior. EGF, epidermal growth factor; TGFβ, transforming growth factor β.

early osteoblasts and long bones (Wu et al. 2004). Interestingly, the human SCUBE3 gene is located on chromosome 6p21.3, which was discussed to be associated with Paget disease of bone 1 (PDB1) (Wu et al. 2004; Fotino et al. 1977; Tilyssey et al. 1982; Good et al. 2002). Although PDB is characterized by late onset and mostly focal bone abnormalities due to increased bone turnover (Ralston et al. 2008), mouse models for genes known to be mutated in PDB, such as Tnfrsf11a for PDB2 (OMIM 603499) and Sgstm1 for PDB3 (OMIM 601530), have bone abnormalities already at birth (Dougal et al. 1999; Li et al. 2000) or not until adulthood (Kapur et al. 2004; Durán et al. 2004; Daroszewska et al. 2011). Interestingly, cases of PDB were described to be associated with raised ALP activities and deafness (Tan et al. 2014). Therefore, we suggest Scube3N294K/N294K mice as a new model for further studies on PDB1.

SCUBE3 maps to a region that was associated with progressive bilateral hearing loss of the mid and high frequencies (DFNA31, OMIM %608645) (Snoecks et al. 2004). The results of the ABR-hearing assessment and the morphological analysis of the middle ears of Scube3N294K/N294K animals indicate conductive hearing loss. In Scube3N294K/N294K mice, hearing loss might be a consequence of retarded development of middle ear cavity and osseous malformations. By contrast, Xavier et al. (2013) did not observe any gross abnormalities in the (inner) ears of Scube3-/- mice at the age of E17.5. Since background-specific genetic modifiers play an important role in the development of inner ears (Kierman et al. 2007), the differences in the genetic background could be the reason for the different observations in the two studies.

The kidneys play a central role in the regulation of mineral homeostasis and chronic kidney disease (CKD) is associated with bone metabolic disease. However, the regulatory pathways involved are still not fully understood (reviewed in Hu et al. 2013; Peacock 2010; Razzaque 2011; Rowe 2012). Elevated plasma U and CREA levels found in Scube3N294K/N294K mice hint toward possible effects on renal function. We found increased electrolyte and water excretion, but no alteration of CREA clearance. These findings could be a sign of altered renal tubular function or might be a consequence of increased electrolyte consumption due to higher food intake causing an upregulation of excretory mechanisms. The differential expressions of differently expressed genes in kidney with reabsorption of electrolytes and salt/water balance indicate effects on tubular reabsorption. Acta2 (Tanabe et al. 2012), Admats1 (Schrimpf et al. 2011), Ehd3 (George et al. 2011), Kcnj5 (Derst et al. 1998), and Umod (Rampoldi et al. 2011) are annotated with expression in renal medulla and salt/water balance, and Cldn10 (Krug et al. 2012), Havcr1 (Adiyanti and Loho 2012), Has2 (Michael et al. 2011), Mep1b (Oneda et al. 2008), Met (Zhou et al. 2013), Mme (Van der Hauwaert et al. 2013), Slc12a1 (Yang et al. 1996), and Slc39a8 (Nebert et al. 2012) are associated with electrolyte reabsorption in the proximal tubules. Together these findings hint toward a possible role of Scube3 in kidney development and function, supported by the expression of Scube3 in the developing kidney (Haworth et al. 2007), or might be related to secondary effects on the regulation of kidney function.

The human SCUBE3 protein is involved in hedgehog and TGF-β1 signaling (Xavier et al. 2013; Haworth et al. 2007; Yang et al. 2007; Johnson et al. 2012) but, so far, nothing is known about SCUBE3 signaling in this network in mice. Transcriptome profiling analysis of kidney from Scube3N294K/N294K mice identified potential down-stream targets of SCUBE3 that are annotated to TGF-β1 signaling [Clgtnf3 (Hofmann et al. 2011), Foxc1 (Xu et al. 2012), Hus2 (Davies et al. 2005), Jdp2 (Mito et al. 2013), Kcnip2 (Kaur et al. 2013), Lefp2 (Sajioh et al. 2000; Arganaraz et al. 2012), Tgfbr3 (Walker et al. 2011), and Tnn (Alejandre Alcázar et al. 2011)]. Reduced expression of all these genes gives evidence for a supportive role of SCUBE3 within the TGF-β1 signaling pathway by binding to transforming growth factor β receptor 2 (TGFBR2) (Yang et al. 2007).

Although we did not observe structural defects of muscle tissue in the pathological examination, the repeated findings of reduced locomotor and rearing activity as well as movement velocity might be indications for functional defects, which might also be associated with an abnormal muscle energy metabolism. Indeed, very recent findings from detailed analysis of zebrafish scube3 suggest that its gene function is crucial for fast-fiber myogenesis (Tu et al. 2014). Still, decreased activity and movement velocity could also be due to the described early developmental expression of Scube3 in the neural tube (Haworth et al. 2007). SCUBE3 expression was observed in human cultured coronary smooth muscle cells and at low levels in the heart (Wu et al. 2004), and its overexpression led to cardiac hypertrophy in transgenic mice (Yang et al. 2007). Whereas young Scube3 transgenic mice appeared...
phenotypically normal but showed abnormal repolarization ECG patterns as well as thickening of left-ventricular septum and posterior wall thickness when analyzed at 8 months of age (Yang et al. 2007). Scube3<sup>fl/fl</sup> mice showed only very mild alterations in cardiac parameters of our phenotypic analysis. Possibly, they were too young for manifestation of a severe cardiac dysfunction.

In this study, we characterized the first Scube3 mutant mouse line with phenotypic alterations, suggesting a role of Scube3 in bone metabolism and morphology, hearing, and renal function. The observed morphological abnormalities of the skeleton, impaired bone metabolism, and hearing impairments correlate with the rare metabolic bone disorder PDB, associated with the chromosomal region of human SCUBE3. Further phenotypic alterations were observed in energy metabolism parameters, in behavior, and neurological functions (Figure 7). This new mouse model will help us to better understand SCUBE3 function and diseases related to Scube3 gene mutations.

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**LITERATURE CITED**

Adiyanti, S. S., and T. Loho, 2012  Acute kidney injury (AKI) biomarker. Acta Med. Indones. 44: 246–255.

Aigner, B., R. Rathkolb, M. Klempt, S. Wagner, D. Michel et al., 2011  Generation of N-ethyl-N-nitrosourea-induced mouse mutants with deviations in hematological parameters. Mammm. Genome 22: 495–505.

Alejandra Alcázar, M. A., R. E. Morty, L. Lendzian, C. Vohlen, I. Oestreicher et al., 2011  Inhibition of TGF-beta signaling and decreased apoptosis in IUGR-associated lung disease in rats. PLoS One 6: e26371.

Argararaz, M. E., S. A. Apichela, and D. C. Micel, 2012  LEFTY2 expression and localization in rat uterine during early pregnancy. Zygote 20: 53–60.

Choi, Y., G. E. Sims, S. Murphy, J. R. Miller, and A. P. Chan, 2012  Predicting the functional effect of amino acid substitutions and indels. PLoS One 7: e66888.

Davies, M., M. Robinson, E. Smith, S. Huntley, S. Prime et al., 2005  Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-beta1 involves MAPK, Smad and AP-1 signalling pathways. J. Cell. Biochem. 95: 918–931.

Dersch, C., E. Wischmeyer, R. Preisig-Müller, A. Spauschus, M. Konrad et al., 1998  A hyperprostaglandin E2 syndrome mutation in Kir1.1 (renal outer medullary potassium) channels reveals a crucial residue for channel function in Kir1.3 channels. J. Biol. Chem. 273: 23884–23891.

Dowdall, W. C., M. Glaccum, K. Charrier, K. Rohrbach, K. Brasel et al., 1999  RANK is essential for osteoclast and lymph node development. Genes Dev. 13: 2412–2424.

Downing, A. K., V. Knott, J. M. Werner, C. M. Cardy, I. D. Campbell et al., 1996  Solution structure of a pair of calcium-binding epidermal growth factor-like domains: implications for the Marfan syndrome and other genetic disorders. Cell 85: 597–605.

Durán, A., M. Serrano, M. Leites, J. M. Flores, S. Picard et al., 2004  The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. Dev. Cell 6: 303–309.

Fotino, M., A. Haymovits, and C. T. Falk, 1977  Evidence for linkage between HLA and Paget’s disease. Transplant. Proc. 9: 1867–1868.

Fuchs, H., K. Schughart, E. Wolf, R. Balling, and M. Hrabé de Angelis, 2000  Screening for dysmorphological abnormalities—a powerful tool to isolate new mouse mutants. Mamm. Genome 11: 528–530.

Fuchs, H., V. Gailus-Durner, T. Adler, J. A. Aguilar-Pimentel, L. Becker et al., 2011  Mouse phenotyping. Methods 53: 120–135.

Fuchs, H., V. Gailus-Durner, S. Neschen, T. Adler, L. C. Afonso et al., 2012  Innovations in phenotyping of mouse models in the German mouse clinic. Mamm. Genome 23: 611–622.

Gailus-Durner, V., H. Fuchs, L. Becker, I. Bolle, M. Brielmeier et al., 2005  Introducing the German mouse clinic: open access platform for standardized phenotyping. Nat. Methods 2: 403–404.

George, M., M. A. Rainey, M. Naramura, K. W. Foster, M. S. Holzapfel et al., 2011  Renal thrombotic microangiopathy in mice with combined deletion of endocytic recycling regulators EHD3 and EHD4. PLoS One 6: e17838.

Good, D. A., F. Bushfield, B. H. Fletcher, D. L. Duffy, J. B. Kesting et al., 2002  Linkage of paget disease of bone to a novel region of human chromosome 18q23. Am. J. Hum. Genet. 70: 517–525.

Gudbjartsson, D. F., G. B. Walters, G. Thorleifsson, H. Stefansson, B. V. Gulbrandsen et al., 2008  Many sequence variants affecting diversity of adult human height. Nat. Genet. 40: 609–615.

Haworth, K. F., S. Smith, M. Zoupa, M. Seppala, P. T. Sharpe et al., 2007  Expression of the Scube3 epidermal growth factor-related gene during early embryonic development in the mouse. Gene Expr. Patterns 7: 630–634.

Hofmann, C., N. Chen, F. Obermeier, G. Paul, C. Büchler et al., 2011  C1q/TNF-related protein-3 (CTRP-3) is secreted by visceral adipose tissue and exerts antiinflammatory and antiinflammatory effects in primary human colon fibroblasts. Inflamm. Bowel Dis. 17: 2462–2471.

Horsch, M., S. Schädler, V. Gailus-Durner, H. Fuchs, H. Meyer et al., 2008  Systematic gene expression profiling of mouse model series reveals coexpressed genes. Proteomics 8: 1248–1256.

Hrabé de Angelis, M., M. Flaswinkel, H. Fuchs, B. Rathkolb, D. Soewarto et al., 2000  Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat. Genet. 25: 444–447.

Hrabé de Angelis, M., G. Nicholson, M. Selloum, I. K. White, H. Morgan et al., 2015  Analysis of mammalian gene function through broad-based phenotypic screens across a consortium of mouse clinics. Nat. Genet. 47: 969–978.

Hu, M. C., M. Kuro-o, and O. W. Moe, 2013  Renal and extrarenal actions of fibroblast growth factor-like domains: implications for the Marfan syndrome and other genetic disorders. Cell 85: 597–605.

Kaur, K., M. Zarzoso, D. Ponce-Balbuena, G. Guerrero-Serna, L. Hou et al., 2013  TGF-beta1, released by myofibroblasts, differentially regulates transcription and function of sodium and potassium channels in adult rat ventricular myocytes. PLoS One 8: e55391.
