A Mutation in the Mouse Chd2 Chromatin Remodeling Enzyme Results in a Complex Renal Phenotype

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Key Words

Chd2 · Chromatin remodeling enzyme · Glomerular disease · Proteinuria · Anemia

Abstract

Background and Aims: Glomerular diseases are the third leading cause of kidney failure worldwide, behind only diabetes and hypertension. The molecular mechanisms underlying the cause of glomerular diseases are still largely unknown. The identification and characterization of new molecules associated with glomerular function should provide new insights into understanding the diverse group of glomerular diseases. The Chd2 protein belongs to a family of enzymes involved in ATP-dependent chromatin remodeling, suggesting that it likely functions as an epigenetic regulator of gene expression via the modification of chromatin structure. **Methods:** In this study, we present a detailed histomorphologic characterization of mice containing a mutation in the chromodomain helicase DNA-binding protein 2 (Chd2). **Results:** We show that Chd2-mutant mice present with glomerulopathy, proteinuria, and significantly impaired kidney function. Additionally, serum analysis revealed de-

creased hemoglobin and hematocrit levels in Chd2-mutant mice, suggesting that the glomerulopathy observed in these mice is associated with anemia. **Conclusion:** Collectively, the data suggest a role for the Chd2 protein in the maintenance of kidney function.

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Introduction

Kidney disease has emerged as a worldwide health problem. It is estimated that nearly 1.5 million individuals in the developed world have chronic kidney disease (CKD) that has progressed to end-stage kidney disease (ESKD), the point where dialysis or kidney transplantation is required [1]. In the United States, 20 million people have some form of kidney disease ranging from mild renal failure to end-stage renal failure (ESRF) [2]. Each year, 80 thousand people die of kidney disease, making it the 9th leading cause of death in the United States [2, 3]. Strikingly, it has been estimated that the amount of ESRF cases in 2010 will exceed 2 million worldwide [4, 5]. The continued growth of the chronic renal disease (CRD)

and ESRD populations poses a challenge in understanding the genetic components of kidney disease.

CRD is caused by the progressive loss of viable nephrons [6, 7]. The nephron is the functional unit of the kidney, responsible for the purification and filtration of the blood. It consists of a glomerulus, the proximal and distal convoluted tubules, Henle's loop and the collecting duct. The glomerulus is a specialized filtration barrier in the renal cortex that regulates the passage of macromolecules from the bloodstream into the urinary space. The three cell types that comprise this complex structure include highly specialized visceral epithelial cells called podocytes, modified smooth muscle cells termed mesangial cells, and endothelial cells [8, 9]. As blood passes through the glomeruli, the filtered water and metabolic wastes pass into the lumen of the Bowman's capsule and drain into the tubules where reabsorption occurs. The glomerular filtration barrier itself consists of podocytes, fenestrated glomerular endothelial cells, and an intervening glomerular basement membrane [8, 10]. Of the nephron's components, the glomerular filtration barrier is often the site of injury in kidney diseases [7].

While glomerular structure is well understood, the underlying molecular events that regulate glomerular structure and function are not as well defined. Initial progress has been made with the discovery of specific genetic mutations in some congenital kidney diseases. Mutations in nephrin (*NPHS1*) and podocin (*NPHS2*) cause genetic defects in the congenital Finnish nephrotic syndrome, inherited as an autosomal-recessive trait, and in the steroid-resistant autosomal-recessive nephrotic syndrome in children, respectively [11, 12]. Mutations in podocin have also been detected in congenital sporadic focal segmental glomerulosclerosis (FSGS) [13, 14]. Both of these genes are exclusively expressed in podocytes and are predominantly localized to the slit diaphragm [13, 15, 16]. Like nephrin and podocin, other genes known to be mutated in kidney diseases have been found to encode proteins that affect the integrity of the glomerulus and its constituent cells [7, 9, 10, 13, 17].

To a lesser extent, regulatory protein mutations or deletions have also been implicated in kidney disease. Examples of these genes include *GDNF* (glial cell line-derived neurotrophic factor) and its receptor *RET* (receptor tyrosine kinase), *PAX2* (paired-box 2), *WT1* (Wilms' tumor suppressor 1), and components of the renin-angiotensin pathway [17–19]. The identification and characterization of additional molecules controlling glomerular function may provide new insights into potential targets for the treatment of kidney diseases.

We recently reported that a *Chd2*-mutant mouse model showed an increased susceptibility to non-neoplastic lesions affecting multiple organs. In particular, approximately 85% of the mutant mice exhibited glomerular abnormalities [20]. *Chd2* is a putative chromatin remodeling enzyme that belongs to the chromodomain helicase DNA binding (CHD) protein subfamily of ATP-dependent enzymes [21, 22]. The signature sequence motifs of this class of enzymes include paired chromodomains in the N-terminal region and a SNF2-like ATPase domain located in the central portion of the protein structure [22–24]. The CHD family is divided into subfamilies based on the presence or absence of additional functional domains. *Chd2* and the closely related *Chd1* protein, for example, also contain a DNA-binding domain located in the C-terminal portion of the protein structure [22, 24]. The mutant mouse strain contains a gene trap encoding a lacZ-neo gene fusion inserted in the middle of the *Chd2* DNA-binding domain and expresses a stable *Chd2*- β gal-neo fusion protein [20, 25]. Based on studies of the analogous *Chd1* DNA-binding domain, the insertion is predicted to abrogate DNA binding by the mutant *Chd2* protein [26].

CHD enzymes have been implicated in multiple cellular functions, including chromatin assembly and remodeling, development and differentiation, and transcriptional regulation [22]. Several of these enzymes, including CHD3, CHD4, CHD5, and CHD7, have been implicated in human disease processes [22, 27–29]. Recently, a female patient with a balanced translocation between the CHD2 locus and a region of the X chromosome apparently devoid of predicted genes was described [30]. This patient had scoliosis, learning and developmental problems, as well as other abnormalities. These findings indicate a relationship between dysfunction of CHD proteins and human disease. In this study, we specifically examined the connection between murine *Chd2* and kidney disease. We investigated the renal lesions and the functional consequences of renal damage in the *Chd2*-mutant mice. Histopathology revealed widespread glomerulopathy associated with tubular and interstitial changes. Proteinuria was also detected in these mice, indicating alteration of the glomerular filtration barrier. We also detected decreased hemoglobin and hematocrit levels, indicative of anemia. Collectively, these data provide genetic evidence of an in vivo role for *Chd2* in kidney disease.

Methods

Generation of *Chd2* Mice

Mice used for experimental analysis in these studies were previously described [20, 25]. Briefly, a gene-trap method created embryonic stem (ES) cells containing an insertional mutation in the *Chd2* locus. The targeted ES cells were purchased from BayGenomics and used to generate *Chd2* mutant mice. All animals were maintained and used in accordance with the UMass Medical School Animal Care and Use Committee.

Histological Analysis

Kidneys of age- and sex-matched *Chd2*^{+/+} and *Chd2*^{+/mut} mice were dissected, fixed in 10% formalin, embedded in paraffin, and sectioned. For histopathological evaluation, sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and trichrome-C. Trichrome-C sections were also counterstained with hematoxylin. Independent and blinded diagnostic pathology analyses were performed by C.G.A.M. and D.S.G.

Urinalysis

Urine samples were collected from 1-, 4- and 8-month-old *Chd2* adult mice (n = 3 for *Chd2*^{+/+} mice of all ages analyzed, n = 5 for *Chd2*^{+/mut} mice at 1 and 8 months of age, and n = 8 for *Chd2*^{+/mut} mice at 4 months of age) for urinalysis using Multistix 10SG reagent strips (Bayer). These dipsticks were used to detect the presence or absence of protein and red blood cells in the urine. Glucose levels in the urine were also evaluated by this method. The standard colorimetric assay was performed according to the manufacturer's instructions.

In addition, 4 μ l of urine from representative *Chd2*^{+/+} and *Chd2*^{+/mut} mice were mixed with sample buffer, boiled, and resolved on a 10% SDS-PAGE gel. 4 μ g of bovine serum albumin (BSA) was used as a positive control. Protein was visualized by staining with Coomassie brilliant blue.

Serum Chemistries

The left femoral artery was catheterized to monitor physiology. Mice were anesthetized with isoflurane (5% for induction, 2% for surgery, 1.2% for maintenance) in room air. PE-10 polyethylene tubing was inserted into the femoral artery for monitoring of mean arterial blood pressure and for obtaining blood samples to measure blood gases, electrolytes, and plasma glucose from 6- to 8-month-old *Chd2* wild-type and heterozygous mice. These parameters were measured using CG8+ variety blood gas cartridges and recorded with an I-stat instrument (Abbot, East Windsor, N.J., USA).

Plasma samples were also obtained with capillary tubes (Fisherbrand) via retro-orbital bleed and placed in serum separator tubes (BD Diagnostics) for monitoring of albumin, total protein, creatinine, and urea nitrogen. Serum glucose, potassium, and sodium were also evaluated via this method. These values were measured with a VetScan chemistry analyzer (abaxis.com). For this analysis, n = 7 for both genotypes.

RNA Isolation and Quantitative RT-PCR

Total RNA was isolated from adult *Chd2*^{+/+} and *Chd2*^{+/mut} kidneys with Trizol (Invitrogen) according to the manufacturer's instructions. RT-PCR was performed as previously described [20]. Quantification of erythropoietin (EPO) and the EF1-alpha

cDNAs was performed with primers: EPO (forward) 5'-TCC TAT GTG GGT GAC GAG GC-3', EPO (reverse): 5'-TAC ATG GCT GGG GTG TTG AA-3'. EF1-alpha primers were previously described [20]. Amplifications were performed in a DNA Engine Opticon System (MJ Research), quantified and normalized to EF1-alpha mRNA levels. The data are a representation of the mean plus/minus standard deviation of seven independent experiments.

Statistical Analysis

The hematological values detected in the *Chd2*^{+/+} and *Chd2*^{+/mut} mice were analyzed by the t test. Values that were not normally distributed were evaluated by the two-tailed Mann-Whitney test. Values that were p < 0.05 were determined to be statistically significant.

Results

Chd2 Mutation Results in Widespread Glomerular, Tubular, and Interstitial Lesions

Chd2 is a putative chromatin remodeling enzyme with important functions for mammalian development and survival. A mouse model of *Chd2* has been generated by gene-trap mutagenesis, resulting in a *Chd2*- β geo fusion protein that lacks a portion of the DNA-binding domain [20, 25]. Homozygous mutation of the *Chd2* locus in mice resulted in perinatal lethality, whereas heterozygous mice developed multiple abnormalities, the most prevalent of which involved the kidneys. Macroscopically, the kidneys of these moribund heterozygous animals exhibited a roughened surface and tan discolorization. Histological analyses characterized the *Chd2* heterozygous kidney lesions collectively as overt bilateral glomerulopathy [20].

To assess the link between *Chd2* and kidney disease, we performed additional histological analyses of the previously described *Chd2*-mutant mouse model [20]. Kidney sections of terminally ill *Chd2*^{+/mut} mice (n = 25), ranging from 5 to 9 months of age, were compared to their wild-type littermates. HE stained sections revealed a spectrum of kidney lesions in *Chd2*^{+/mut} mice. These alterations were not related to gender or to age (data not shown). Moderate to severe glomerular enlargement, primarily due to segmental to global matrix deposition, was detected in the kidney sections of 80% of the *Chd2*^{+/mut} animals (fig. 1b). These glomerular changes were not seen in age- and sex-matched wild-type littermates (fig. 1a).

In addition to the glomerular changes, tubular lesions were noted in the HE stained kidney sections from 60% of the *Chd2*^{+/mut} mice (fig. 1d, f). Moderate-to-severe tubular ectasia or dilation was observed in the renal cortex

of *Chd2*^{+/-mut} mice, in comparison to the normal architecture seen in the renal cortex of *Chd2*^{+/-+} mice (fig. 1c–f). Moreover, representative lesions in the *Chd2*^{+/-mut} kidney sections included tubular atrophy and tubular necrosis (fig. 1f). Indications of tubular changes were not detected in the *Chd2*^{+/-+} littermates (fig. 1e). In addition, subacute to moderate interstitial nephritis (inflammation) accompanied the glomerular and tubular changes displayed in the *Chd2*^{+/-mut} mice. Areas of lymphoplasmatic inflammation and the presence of mononucleated infiltrate, seen in the cortical interstitium or within the lumens of the tubules, were noted in 32% of the *Chd2*^{+/-mut} mice (fig. 1b, d, f). This pathology was also not observed in the *Chd2*^{+/-+} kidney sections.

Examination of the renal medulla revealed marked scarring and atrophy of the papilla in 36% of the heterozygous mice, whereas no similar structural changes involving the papilla were observed in the wild-type kidney sections (fig. 1g, h). Additional observations included occasional (n = 4) attenuation of the *Chd2*^{+/-mut} cortex, whereas *Chd2*^{+/-+} mice displayed normal cortices (fig. 1i, j).

To further examine the glomerular changes detected in *Chd2*^{+/-mut} mice, we performed additional histological analyses. PAS stain accentuated basement membrane constituents while trichrome stains identified interstitial and intraglomerular collagen matrices. In contrast to the *Chd2*^{+/-+} mice, kidney sections from *Chd2*^{+/-mut} mice revealed segmental to global thickening of the basement membrane due to deposition of PAS-positive matrix (pink stain) (fig. 2a, b). Additionally, trichrome staining demonstrated that the heterozygous glomerulus and periglomerular regions displayed increased collagen deposition (light blue stain) in 80% of the *Chd2*^{+/-mut} mice, which was not seen in wild-type littermates (fig. 2c, d). Overall, the structural alterations in the kidney sections of the *Chd2*^{+/-mut} mice suggest the development of glomerular disease associated with tubulointerstitial changes.

Since immune cell infiltration was observed in only about one-third of the *Chd2*^{+/-mut} heterozygous kidneys examined, we do not believe that the primary defect is due to a deficiency in the immune system in the *Chd2*^{+/-mut} mice.

Mutation of Chd2 Induces Kidney Dysfunction

We next examined the consequences of the kidney alterations seen in the heterozygous mice. Glomerular diseases are often associated with leakage of proteins across the glomerular filtration barrier into the urine [31] that can alter the structure and function of the renal tubulointerstitial microenvironment [32]. HE staining and light microscopy revealed protein casts within the Bowman's space in most *Chd2*^{+/-mut} mice (fig. 3b). Furthermore, accumulation of proteinaceous material was also found within the dilated tubules of *Chd2*^{+/-mut} mice (fig. 3b, d). Importantly, this pathology was not observed in the kidney sections of wild-type mice (fig. 3a, c). Together, these data suggest that the glomerular filtration barrier was altered, thereby allowing macromolecules such as proteins to leak into the urine. A compromised glomerular filtration barrier can also result in collagen deposition and fibrosis, consistent with the trichrome staining in figure 2d.

To confirm these histological findings, urinalysis of *Chd2*^{+/-+} and *Chd2*^{+/-mut} age- and sex-matched littermates was performed at 1, 4, and 8 months of age. During this analysis, we observed a significant decrease in the total urine output collected from *Chd2*^{+/-mut} mice in comparison to their wild-type littermates (data not shown). SDS-PAGE analysis identified albumin, measuring approximately 66 kDa, in the urine of *Chd2*^{+/-mut} mice, confirming that proteinuria existed in the afflicted animals (fig. 3e). Low-molecular-weight proteins (20 kDa) normally found in mouse urine were observed in both genotypes. Moreover, significantly decreased levels of serum albumin were also detected in the analyzed *Chd2*^{+/-mut}

Fig. 1. Structural alterations in kidneys of *Chd2*^{+/-mut} mice. HE-stained kidney sections from terminally ill, end-stage *Chd2*^{+/-mut} mice ranging from 5 to 9 months of age and their age- and sex-matched wild-type (WT) littermates. **a, b** The cortex of the heterozygous glomerulus (arrow) is enlarged due to global matrix deposition, which is not seen in the WT glomerulus. Note the inflammatory cells (*) present in the cortical interstitium of *Chd2*^{+/-mut} mice. **c, d** In contrast to the normal tubules in the renal cortex of WT mice, tubular dilatation (TD) is observed in *Chd2*^{+/-mut} mice. **e, f** Tubulointerstitial lesions with dilation of

tubules, tubular atrophy, and interstitial nephritis are seen in *Chd2*^{+/-mut} kidney sections, but not in the WT littermates. Note the inflammatory cells within the tubular lumens of *Chd2*^{+/-mut} kidney. **g, h** Marked atrophy of the papilla (*) is observed in the renal medulla of the *Chd2*^{+/-mut} kidney compared with the WT control. **i, j** The capsule of the *Chd2*^{+/-mut} kidney is irregular in shape (arrow) compared to the normal capsule (arrow) of the WT control mice. Images were taken with the objective lens magnification denoted in each panel.

Table 1. Urinalysis of *Chd2* wild-type and mutant mice

	1 month		4 months		8 months	
	<i>Chd2</i> ^{+/+}	<i>Chd2</i> ^{+/<i>mut</i>}	<i>Chd2</i> ^{+/+}	<i>Chd2</i> ^{+/<i>mut</i>}	<i>Chd2</i> ^{+/+}	<i>Chd2</i> ^{+/<i>mut</i>}
Protein						
Negative	3 of 3	0 of 5	3 of 3	0 of 8	3 of 3	0 of 5
Trace		2 of 5		0 of 8		1 of 5
30 mg/dl		2 of 5		6 of 8		2 of 5
100 mg/dl		1 of 5		1 of 8		1 of 5
300 mg/dl		0 of 5		1 of 8		1 of 5
Blood						
Negative	3 of 3	5 of 5	3 of 3	8 of 8	3 of 3	4 of 5
Trace						1 of 5
Leucocytes						
Negative	3 of 3	4 of 5	3 of 3	6 of 8	3 of 3	4 of 5
Trace		1 of 5		1 of 8		1 of 5
125 Leu/ μ l				1 of 8		

Fig. 2. *Chd2*^{+/*mut*} mice present with glomerulopathy. Images display representative kidney sections from terminally ill, end-stage *Chd2*^{+/*mut*} mice and their age- and sex-matched WT littermates stained with periodic acid-Schiff (PAS) and trichrome. **a, b** PAS staining reveals that the *Chd2* heterozygous glomerulus shows segmental thickening of basement membrane regions (arrow) due to PAS positive matrix deposition, which is not seen in the WT glomerulus. **c, d** Increased collagen deposition (light blue) within the glomerulus and regions surrounding the glomerulus is observed in the *Chd2*^{+/*mut*} kidney stained with trichrome compared to the WT control. Images were taken with the objective lens magnification denoted in each panel.

Fig. 3. Proteinuria in *Chd2*^{+/*mut*} mice. Representative images of HE-stained kidney sections from terminally ill, end-stage *Chd2*^{+/*mut*} mice and their age- and sex-matched WT littermates. Protein was not observed in either the Bowman's space (**a**) or the tubules (**c**) of the WT mice. Prominent protein casts (*) were present within the Bowman's space (**b**) and within dilated tubules (**d**) of the *Chd2*^{+/*mut*} mice. Images were taken with the objective lens denoted in each panel. **e** SDS-PAGE analysis of urinary protein in WT and mutant mice. An equal volume of urine from each mouse (lanes 1–3) and purified bovine serum albumin (BSA) (lane 4)

were electrophoresed on an SDS polyacrylamide gel, and protein bands were visualized by staining with Coomassie brilliant blue. Various amounts of albumin (66 kDa) were observed in *Chd2*^{+/*mut*} mice. Low-molecular-weight proteins (~20 kDa) were present in all urine samples and serve as a loading control. **f** Decreased levels of serum albumin were detected in *Chd2*^{+/*mut*} mice in comparison to their *Chd2*^{+/+} littermates. For these analyses, *n* = 7 for both genotypes. **g** In contrast to *Chd2*^{+/+} mice, decreased levels of total serum protein were detected in the *Chd2*^{+/*mut*} mice (*n* = 7 for both genotypes).

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Fig. 4. *Chd2*^{+/mut} mice present with azotemia. Elevated levels of serum creatinine (a) and urea nitrogen (b) are observed in terminally ill, end-stage *Chd2*^{+/mut} mice. (*) indicates an outlier obtained from a *Chd2*^{+/mut} mouse. For these analyses, n = 7 for both genotypes.

mice (fig. 3f). Upon examination of total serum protein, we also observed significantly lower levels of protein in the *Chd2*^{+/mut} mice (fig. 3g). The presence of urine albumin together with the decreased levels of serum albumin and total protein are suggestive of protein-losing glomerulopathy.

The incidence of proteinuria was qualitatively measured with urine dipsticks. Thirty mg/dl of protein was defined as nephrotic range proteinuria. Urinalysis of *Chd2*^{+/+} and *Chd2*^{+/mut} littermates was performed at various ages. At 1 month of age, 3 of 5 *Chd2*^{+/mut} mice presented with proteinuria, and the other 2 *Chd2*^{+/mut} mice contained traces of protein (table 1). At 4 months of age, all of the 8 analyzed *Chd2*^{+/mut} mice presented with nephrotic range proteinuria (table 1). Of the *Chd2*^{+/mut} mice analyzed at 8 months of age, 4 of 5 displayed proteinuria, and the other one showed traces of protein in the urine sample (table 1). Importantly, no protein was detected in the urine samples of the age-matched *Chd2*^{+/+} littermates at any point, and there was no evidence for hematuria in any of these analyses (table 1). Consistent with the kidney histopathology, the urinalyses and serum analyses suggest impairment of the structural integrity of the glomerular filtration barrier.

This prompted us to further evaluate kidney function in *Chd2*^{+/mut} mice. Previously, we reported that the median survival for *Chd2*^{+/mut} mice was 8 months [20]. Therefore, we analyzed physiological parameters that are maintained by the kidneys in 6- to 8-month-old *Chd2*^{+/+} and *Chd2*^{+/mut} mice. The pathologic changes observed in the *Chd2*^{+/mut} mice resulted in few alterations in the levels of serum markers. Levels of bicarbonate, the main form of CO₂ in the body and a measurement of the metabolic

Table 2. Serum chemistries of *Chd2*-mutant mice

Physiological parameters	<i>Chd2</i> ^{+/+} (n = 3)	<i>Chd2</i> ^{+/mut} (n = 5)
pH	7.4 ± 0.0	7.3 ± 0.1
PaCO ₂ , mm Hg	36.8 ± 9.8	33.8 ± 2.9
PaO ₂ , mm Hg	77.3 ± 10.7	57.4 ± 14.9
TCO ₂ , mmol/l	22.7 ± 2.1	17.2 ± 1.8*
SO ₂	92.3 ± 7.2	83.0 ± 12.1
HCO ₃ ⁻	21.5 ± 1.4	16.2 ± 2.0
Na ⁺ , mmol/l	141.7 ± 0.6	148.2 ± 4.1
K ⁺ , mmol/l	4.6 ± 0.1	6.0 ± 1.0*

* p < 0.05.

component of the acid-base balance, were significantly decreased in *Chd2*^{+/mut} mice (table 2). Bicarbonate is excreted and reabsorbed by the kidneys in response to pH imbalances. The kidneys also maintain the balance of other electrolytes, such as potassium and sodium, in the body. At the time of analysis, the potassium levels were significantly higher in the *Chd2*^{+/mut} mice, whereas the levels of sodium were not affected compared to their wild-type littermates (table 2).

To determine if *Chd2*-mutant mice present with kidney failure, we further analyzed the serum chemistries of *Chd2*^{+/+} and *Chd2*^{+/mut} mice. Mild but elevated plasma creatinine (5 of 7) and urea nitrogen (4 of 7) levels were seen in the analyzed *Chd2*^{+/mut} mice compared to their wild-type littermates (fig. 4). Although not all *Chd2*^{+/mut}

Fig. 5. *Chd2*^{+/mut} mice are anemic. **a** Decreased levels of erythropoietin (EPO) are observed in *Chd2*^{+/mut} mice in comparison to the *Chd2*^{+/+} mice. For these analyses, *n* = 7 for both genotypes. EPO transcript levels were normalized to EF1- α mRNA levels,

and the wild-type values were set to 1.0. Differences in hemoglobin (Hb) levels (**b**) and hematocrit (Hct) levels (**c**) show that *Chd2*^{+/mut} are anemic. For these analyses, *n* = 3 for *Chd2*^{+/+} mice and *n* = 5 for *Chd2*^{+/mut} mice.

mice demonstrated an increase in either creatinine or urea nitrogen, the findings were statistically significant. The damaged kidneys of the *Chd2*^{+/mut} mice resulted in azotemia, abnormal levels of body waste products in the blood, which is consistent with decreased glomerular filtration.

Kidney Alterations and Malfunctions in Chd2^{+/mut} Mice Are Linked to Anemia

CRD is often associated with other disease states, such as diabetes mellitus, hypertension, and anemia [2, 33–35]. Diabetes mellitus is a condition characterized by high blood glucose resulting from the body's inability to use glucose efficiently. Urinalysis via both urine dipsticks and serum chemistries did not reveal a significant change in the glucose levels of *Chd2*^{+/mut} mice as compared to wild-type controls (data not shown). This suggests that the kidney disease seen in the *Chd2*^{+/mut} mice may not be associated with diabetes mellitus.

Hypertension, defined as raised arterial pressure, is also a major risk factor for kidney disease [33]. Previously, we reported that *Chd2*^{+/mut} mice became progressively lethargic and tachypneic until collapse [20]. To determine if these mice were hypertensive, we analyzed the blood pressure in *Chd2*^{+/mut} mice and their wild-type littermates. No significant difference in the mean arterial blood pressure was detected between the *Chd2*^{+/mut} and *Chd2*^{+/+} mice (data not shown), but measurements of blood gas levels revealed low levels of CO₂ in the *Chd2*^{+/mut} mice (table 2). These data suggest that hypertension may not be coupled to the kidney disease seen in *Chd2*^{+/mut} mice.

A decline in kidney function can progress to chronic renal disease, which in turn may result in decrease production of EPO, a hormone primarily made in the kidneys. Impairment of the production of EPO results in the development of anemia [36, 37]. We observed a significant decrease of EPO mRNA in *Chd2*^{+/mut} kidney samples as determined by quantitative RT-PCR (fig. 5a). Moreover, decreased hemoglobin and hematocrit levels were detected in blood analyses of *Chd2*^{+/mut} mice, whereas normal values were obtained from *Chd2*^{+/+} mice (fig. 5b, c). Taken together, these data indicate that anemia is linked to the renal failure seen in *Chd2*^{+/mut} mice.

Discussion

The Chd subfamily of SNF2 ATPases is characterized by the presence of tandem chromodomains and a SNF2-like helicase [21–24]. Members of this family have been implicated in a variety of cellular processes and have also been correlated with human diseases [22]. CHD3 and CHD4 have been implicated in dermatomyositis, a connective tissue disease involving the inflammation of both the skin and muscular systems [22, 27–29]. Mutations of CHD5 have been found in patients with neuroblastoma, a malignant neoplasm of the peripheral nervous system [38, 39]. Mutations in CHD7 have been observed in patients afflicted with CHARGE syndrome, an acronym for a specific group of coexistent congenital anomalies [40]. Finally, a patient with a balanced translocation between the CHD2 locus on chromosome 15 and the X chromo-

some presented with scoliosis and multiple other abnormalities [30]. Though this patient had no reported kidney abnormalities, the differences between the observed phenotypes and those described for the mutant *Chd2* mouse strains could be due to the differences in the alterations in the respective *Chd2* loci, as the balanced 15:X translocation in humans is genetically distinct from the hypomorphic allele created by the insertion in the mouse *Chd2* locus. Regardless, the data collectively suggest a link between the CHD proteins and normal development.

Our previous characterization of *Chd2* mutant mice demonstrated gross alterations and histological abnormalities in the kidneys of heterozygous mice [20], a finding that was recently independently confirmed [30]. Here, we further investigated the phenotypes of the kidneys in end-stage adult mutant mice. Histopathological analyses revealed that the morbidity and mortality of *Chd2*^{+/*mut*} mice was associated with significant kidney disease affecting the glomerulus in most animals and the tubules in more than half of the animals examined. In particular, the changes in the glomerulus were characterized by increased thickening of the basement membrane, including an increased deposition of collagen (fig. 1, 2). These changes correlated with the loss of normal kidney function. It remains unclear why a small percentage (~15%) of the heterozygous mutant mice did not present with any obvious kidney phenotypes, though we suspect that variations due to the mixed genetic background of these mice are responsible.

Generally, glomerular diseases interfere with the normal function of the glomerular filtration barrier, thus permitting protein loss. Histological analysis showed prominent protein casts within the Bowman's space and within the dilated tubules (fig. 3a, b). As detailed in table 1, protein was detected in the urine samples of *Chd2* heterozygous mice. Additionally, urinary albumin excretion was also observed in *Chd2*^{+/*mut*} mice (fig. 3c). Collectively, these data implicate that *Chd2* is necessary to obtain a normal renal phenotype and maintain normal renal excretory activities.

Proteinuria can trigger the progression of CRD. We measured the balance of serum electrolytes, as they are often affected by kidney disease. Normally, the kidneys regulate fluid absorption and excretion and maintain a narrow range of electrolyte fluctuation. The three most common serum electrolytes are bicarbonate, sodium, and, potassium. Bicarbonate is the major buffer in the body that helps to maintain the proper blood pH. Decreased levels of serum bicarbonate, coupled with an acidic pH, implies metabolic acidosis and kidney dys-

function in the analyzed heterozygous *Chd2* mice (table 2). Measurements of blood gases in *Chd2*^{+/*mut*} mice showed decreased levels of CO₂ (table 2). Together with acidic pH and decreased levels of bicarbonate, the abnormal breathing patterns initially described in the *Chd2*^{+/*mut*} mice [20] was likely due to partial respiratory compensation through hyperventilation.

Imbalances of sodium and potassium are also suggestive of poor kidney function. We did not detect significant differences in the level of sodium; however, we did detect a significant increase in the potassium levels in *Chd2*^{+/*mut*} mice in comparison to the wild-type controls (table 2). Furthermore, the significant elevation of blood urea nitrogen (BUN) and serum creatinine suggests a decrease in renal function in *Chd2*^{+/*mut*} mice (fig. 4). Collectively, these data demonstrate that the decrease in renal function is a consequence of the pathologic abnormalities observed in the *Chd2*^{+/*mut*} mice.

Kidney diseases are frequently associated with a number of systemic diseases. Diabetic nephropathy is the primary cause of kidney disease [2]. An elevated level of blood glucose is the central feature of diabetes. We did not observe elevated blood glucose, the central feature of diabetes mellitus, nor did we observe elevated urine glucose levels in *Chd2*^{+/*mut*} mice, suggesting that diabetic nephropathy was not a consequence of the *Chd2* mutation (data not shown). Hypertension is also frequently associated with kidney disease [2, 33]; however, we did not observe any indications of arterial hypertension in the *Chd2*^{+/*mut*} mice (data not shown). Further examination would be needed to completely rule out the association of diabetes and/or hypertension with the kidney defects observed in the *Chd2*^{+/*mut*} mice.

Anemia is a common subsequent symptom in patients with CKD [41, 42]. The decrease in EPO mRNA in the *Chd2*^{+/*mut*} kidney samples, which correlates with the decreased levels of hemoglobin and hematocrit, can explain the anemia seen in the *Chd2*^{+/*mut*} mice (fig. 5). Thus, the anemia in *Chd2*^{+/*mut*} mice might be a secondary consequence of renal failure caused by the destruction of the kidneys. Alternatively, the anemia may be due to an intrinsic defect in erythropoiesis or to a combination of kidney and erythropoietic dysfunction. Further studies will be required to understand the precise role of *Chd2* in anemia.

We did not directly address the requirement for *Chd2* during embryonic and postnatal development, although examination of kidneys from one litter taken at postnatal day 21 indicated that the kidneys from the three heterozygous individuals present were discolored and pale, as

observed in the adults [20]. Microscopic examination indicated a disorganized tissue structure (data not shown). However, the authors of a recently published report describing mice with the same insertional mutation in the *Chd2* locus indicated that no kidney abnormalities were observed in the neonates. This difference may be due to differences in genetic background, or may indicate that the first indications of kidney abnormalities occur between birth and day 21. Additional studies will be needed to address this issue.

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