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Autosomal Dominant Mutation in the Signal Peptide of *Renin* in a Kindred with Anemia, Hyperuricemia, and CKD

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Abstract

Homozygous or compound heterozygous *Renin* (*REN*) mutations cause renal tubular dysgenesis (RTD), which is characterized by death *in utero* due to renal failure and pulmonary hypoplasia. The phenotype resembles the fetopathy caused by angiotensin-converting enzyme inhibitor or angiotensin receptor blocker intake during pregnancy. Recently, heterozygous *REN* mutations were shown to result in early-onset hyperuricemia, anemia and chronic renal failure. So far, only three different heterozygous *REN* mutations were reported.

We performed mutation analysis of the *REN* gene in 39 kindreds with hyperuricemia and chronic kidney disease (CKD) previously tested negative for mutations in the *UMOD* and *HNF1β* genes. We identified one kindred with a novel c.28T>C (p.W10R) *REN* mutation in the signal sequence, concluding that *REN* mutations are rare events in CKD patients. Affected individuals over four generations were identified carrying the novel *REN* mutation and were characterized by significant anemia, hyperuricemia and CKD. Anemia was severe and disproportional to the degree of renal impairment. Moreover all heterozygous *REN* mutations are localized in the signal sequence. Therefore, screening of the *REN* gene for CKD patients with hyperuricemia and anemia may be focusing on exon 1 sequencing, which encodes the signal peptide.

Introduction

Renin is synthesized as a preproprotein called preprorenin. Preprorenin is targeted to the endoplasmatic reticulum (ER) by a signal sequence where the protein undergoes glycosylation and proteolytic processing, resulting in prorenin (1). Further processing results in storage of renin in electro-dense granules in the juxtaglomerular cells, where renin is

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secreted upon stimulation by lower blood pressures (baroreceptor reflex) or decreased chloride delivery to the macula densa (2,3).

Mutations in the *Renin* (*REN*) gene were reported in patients with renal tubular dysgenesis (RTD) (MIM 267430) (4). Homozygous or compound heterozygous *REN* mutations were reported in neonates or fetuses, who had died *in utero* or soon after birth due to anuria and pulmonary hypoplasia (5). The phenotype resembles the fetopathy caused by angiotensin-converting-enzyme (ACE) inhibitor or angiotensin II receptor antagonists (ARB) intake during pregnancy (6,7).

Recently, three different heterozygous *REN* mutations were found in four European kindreds with autosomal dominant inheritance of anemia, hyperuricemia and chronic kidney disease (CKD) (8,9). With the exception of the severe anemia and low blood pressures this phenotype is almost indistinguishable from patients with *Uromodulin* (*UMOD*) mutations causing: (i) familial juvenile hyperuricemia (FJHN) (MIM 162000), (ii) medullary cystic kidney disease type 2 (MCKD2) (MIM 603860) and (iii) glomerulocystic kidney disease (GCKD) (MIM 609886). These disorders have been summarized under the term *Uromodulin* associated kidney disease (UAKD). Infrequently, mutations in the hepatocyte nuclear factor 1 β (*HNF1 β*) were reported with an UAKD phenotype (10).

We performed mutation analysis of the *REN* gene in 42 individuals from 39 different kindreds with a history of hyperuricemia and CKD, all previously tested negative for *UMOD* and *HNF1 β* mutations.

Case Report

We detected a novel heterozygous *REN* mutation in one out of 39 kindreds (for more information about patients and methods see Supplementary Information S1), indicating that heterozygous *REN* mutations are a rare cause of CKD with hyperuricemia and anemia. The novel *REN* mutation c.28T>C (p.W10R) is located in exon 1 of the *REN* gene, which encodes the signal peptide.

The family carrying the c.28T>C (p.W10R) mutation is of Caucasian descent and has affected individuals over four generations (Fig. 1A). Patient IV-1 is a 3 year old female, who was seen at the age of eleven months due to impaired renal function and significant anemia (Table 1). Her potassium was at the upper level of the reference range with 5.0 mEq/L (5 mmol/L). At the age of 3 years renal function remained stable with a glomerular filtration rate (GFR) of 61 ml/min/1.73m² and a creatinine of 0.81 mg/dL (71.6 μ mol/L). Her uric acid was elevated at 8.3 mg/dL (493.6 μ mol/L) and her hemoglobin remained low at 9.2 g/dL (92 g/L). A renal ultrasound showed increased echogenicity but normal kidney size. She has not required any treatment. Her non-identical twin brother (IV-2 in Fig. 1A) is clinically not affected. Her mother (III-2) is a 35 year old female who presented at the age of 16 years with fatigue, impaired renal function, hyperuricemia and anemia (see Table 1). Her blood pressure was low at 90/70 mmHg. She is treated for her anemia with darbopoietin. The maternal grandmother (II-2) is 57 years old and presented at the age of 11 years with fatigue, anemia, and impaired kidney function (Table 1). A kidney biopsy showed tubular atrophy, interstitial fibrosis, and a thickened basement membrane of the Bowman's capsule, with normal glomeruli. Uric acid was 10.2 mg/dL (606.7 μ mol/L) at the age of 52 years. At the age of 55 years, she received a preemptive renal transplant. Her older brother (II-3) presented at the age of 15 years with anemia and fatigue. His renal function was reportedly impaired indicated by an elevated BUN. 15 years after initial presentation he was found to be in end-stage renal disease (ESRD) (Table 1). He was treated with allopurinol. A renal ultrasound 25 years after presentation showed bilaterally small kidneys. He started dialysis

in his 40's. 31 years after initial presentation he received a kidney transplant. The maternal great-grandfather (I-1) presented at the age of 28 years with fainting episodes and was found to be anemic. Due to anemia and impaired kidney function in his daughter (II-2) and son (II-3) he was evaluated at the age of 50 years, showing impaired kidney function, hyperuricemia (Table 1) and hypertension (156/80 mmHg). At the age of 55 years he died due to ESRD and congestive heart failure. Post mortem examination showed small kidneys (size 8 cm bilaterally), small cysts in the renal cortex, and a thickened basement membrane of the Bowman's capsule.

The novel heterozygous *REN* mutation (c.28T>C, p.W10R) is located in exon 1, which encodes for the signal peptide (Fig. 1B). The mutation segregates with all affected individuals of the kindred. We did not find the *REN* mutation (c.28T>C, p.W10R) in 100 ethnically matched healthy control individuals. The non-polar amino acid tryptophan is exchanged to the basic amino acid arginine. The tryptophan at position 10 of *REN* is highly evolutionary conserved in mammals (Fig. 1C). Analysis using the Polyphen-2 software (11) resulted in a significant score of 0.94 (with a maximum score of 1.0) indicating that this mutation is "probably damaging" (sensitivity 0.75, specificity 0.92). We also used the SignalP 3.0 (12) software in order to rule out that the p.W10R mutation would change the cleavage site for the Renin signal sequence. The hydrophobicity score was calculated by using the Kyte and Doolittle method and did not change significantly (13).

Discussion

The occurrence of anemia is surprising because kidney function in our patients was initially not profoundly impaired. Early onset of anemia has also been described by Zivná et al. with a one year old affected child (8). Interestingly, once children advance to adolescence anemia improves with normal hemoglobin levels as long as renal function is not too impaired (9). Increasing testosterone synthesis has been hypothesized to compensate for the childhood anemia (14). Anemia responded well to erythropoietin treatment but not to iron treatment (8,9). Anemia can also be found in patients treated with ACE inhibitors or ARBs (15,16). The RAS influences erythropoiesis mostly via angiotensin II (ANG II). AT1 receptors were identified on erythroid progenitor cells. Treatment of cultured erythroid progenitor cells with ANG II resulted in an increase of blood-forming units, which was blocked by the ARB losartan (17). ANG II also seems to act as a secretagogue for erythropoietin (EPO). Patients with renin-mediated hypertension were found to have higher serum EPO levels than patients with non-renin-mediated hypertension (18,19). This effect is most likely caused by ANG II-mediated vasoconstriction of the renal vessels, thus decreasing postglomerular peritubular blood flow and oxygen delivery to the renal tubulointerstitium (20,21). Moreover, ANG II has a stimulatory effect on proximal tubule sodium reabsorption, which increases oxygen requirement of tubular cells (22). Both events will trigger increased EPO production. Finally, ANG II has been shown to stimulate EPO secretion by direct interaction with hypoxia-inducible-factor 1 (HIF1), which is the master regulator of EPO production signaling hypoxia as a key regulator of erythropoiesis. In cell culture HIF1 expression was increased by adding ANG II (23).

Hyperuricemia is another key characteristic of patients with heterozygous *REN* mutations and was also previously reported (8,9). Low serum renin and aldosterone levels may result in relative hypotension causing increased proximal tubule sodium reabsorption, which subsequently may result in increased urate reabsorption (24). Impaired kidney function was found as early as three and four years (8,9). Renal function deteriorates very slowly over many years in most patients with progression to ESRD in the fifth or sixth decade. The slow decline may reflect intrinsic blockade of the RAS secondary to the *REN* mutation.

Computer-based analysis of the novel *REN*_{p.W10R} did not show any alteration in regard of the signal peptide cleavage site or the hydrophobicity score. It is very likely that the p.W10R mutation is also impairing ER targeting as has been shown for the other heterozygous *REN* mutations (8,9). Over time this may result in chronic ER stress, triggering apoptosis and inflammation resulting in juxtaglomerular cell loss and nephron loss.

In conclusion, we describe a pedigree in which patients manifested anemia, hyperuricemia, and slowly evolving CKD in association with a heterozygous *REN* mutation. The incidence of *REN* mutations is likely to be low in CKD patients with hyperuricemia based on our study of 39 families. Screening of exon 1 may be most efficient as all heterozygous *REN* mutations are located in exon 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

A. Pedgree of the affected kindred with the novel c.28T>C (p.W10R) *REN* mutation. Males and females are affected in an autosomal dominant pattern over four generations. Affected individuals are indicated by black symbols whereas unaffected individuals are shown with white symbols. Males are indicated by a square, whereas females are indicated by circles.

B. The nucleotide sequence encoding the first 16 amino acids of Renin is shown. The upper sequence shows the heterozygous nucleotide exchange c.28T>C of individual IV-1, resulting in the amino acid exchange from tryptophan to arginine at position 10. Normal reference sequence is shown below. The nucleotide triplets encoding the corresponding amino acids are shown on top of the sequence. The mutation is shown in red and indicated by a red arrow. The corresponding amino acids are shown at the bottom of each sequence.

C. Evolutionary conservation of the signal peptide of Renin is shown. The different species are outlined on the left margin. Strong evolutionary conservation is shown for the affected amino acid at position 10 (p.10W) down to frog (red arrow). At the very end leucine 16 is shown which is affected by two different published mutations which alter the penta-leucine motif (8).

Table 1

Synopsis of the patients' clinical information

	I-1	II-2	II-3	III-2	IV-1
Age at presentation	28 yo	11 yo	15 yo	16 yo	11 mos
gender	M	F	M	F	F
Onset of ESRD (years)	55 yo	~ 55 yo	~ 30 yo	NA	NA
Serum creatinine (mg/dl)	ND	1.55	4.6*	2.2	0.55
BUN (mg/dL)	46 [#]	62	106*	46	49
GFR (ml/min/1.73m ²)	ND	ND	ND	37	66
Serum Uric Acid (mg/dL)	8.0 [#]	10.2 ^{\$}	Tx with allopurinol	9.2	8.3 [%]
Hemoglobin (g/dL)	↓ ^{\$}	10.0	9.5*	10.0	9.1
Pathological findings	Post mortem: small kidneys B/L, small cortical cysts, thickened Bowman's capsule	Bx: TA, IF, TBM	NA	NA	NA
Imaging	Angio: small kidneys	ND	US: small kidneys B/L	ND	US: hyperechogenic kidneys, normal kidney size

Angio, Angiogram; B/L, bilaterally; Bx, kidney biopsy; F, female; IF, interstitial fibrosis; M, male; ND, no data; NA, not applicable; TA, tubular atrophy; TBM, thickened basement membrane; Tx, treatment; US, renal ultrasound;

[#] these lab results were obtained at the first documented lab data at the age of 50 years;

^{\$} uric acid was first documented at the age of 52 years;

^{*} these laboratory results are the first documented lab data at the age of 30 years;

[%] data obtained by history;

laboratory data obtained at age of 3 years
%

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