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Bi-allelic *REN* Mutations and Undetectable Plasma Renin Activity in a Patient With Progressive CKD

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INTRODUCTION

Renin is a component of the renin-angiotensin system and plays an important role in the regulation of embryonic kidney development, blood pressure, kidney perfusion, and potassium and acid-base balance.[1] Identification and characterization of renin mutations provide unique insight into the physiology of renin and its organ-specific functionality and regulation. Total loss of renin production because of bi-allelic loss-of-function *REN* mutations results in autosomal recessive renal tubular dysgenesis and death within the first week of life.[2] Heterozygous *REN* mutations affecting synthesis and leading to intracellular deposition of mutant renin result in autosomal dominant tubulointerstitial kidney disease, with an age of onset and clinical presentation dependent on protein domain–specific mutations.[3]

Here, we describe a patient with bi-allelic *REN* mutations and loss of systemic renin activity but functional kidney development. This case illustrates that bi-allelic mutations of *REN* and other renin-angiotensin system genes[4] may present as slowly progressive chronic kidney disease (CKD). Clinical presentation of the patient provides additional insight into *REN* genetic disorders and renin function in embryonic kidney development.

CASE PRESENTATION

The index case (II.1) is a 38-year-old white female. She has had a history of hypotension, with systolic blood pressure in the 80 to 90 mmHg range. At the age of 16 years, she received a diagnosis of anemia and CKD. She denied a history of gout or polyuria. The patient had an episode of acute kidney disease at 33 years from dehydration, which resolved with intravenous fluids. When examined at the age of 38 years for future pregnancy planning, her blood pressure was 90/56 mmHg, hemoglobin level 10.9 g/dl, blood urea 71 mg/dl, serum creatinine 1.44 mg/dl (eGFR: 49.2 ml/min per 1.73 m^2), serum potassium 5.6 mEq/l, and serum urate 9 mg/dl, and she had persistently low urine specific gravity. The plasma renin level was high at 59.6 pg/ml (normal <38.7 pg/ml), but the plasma renin activity was undetectable at <0.2 ng/ml per hour (normal 0.5–4 ng/ml per hour).

The serum aldosterone level was low at 12 pg/dl (normal 42–202 pg/dl). Renal ultrasound revealed small kidneys bilaterally (right 8.3 cm and left 9.3 cm) with increased echogenicity and small cysts. The patient was maintained on erythropoietin and received sodium bicarbonate (4–6 pills/d), sodium polystyrene sulfonate (30–90 g/d), and allopurinol. The pregnancy proceeded without complications. She was started on fludrocortisone at the age of 42 years with good response, allowing her to stop all previous medications, except

Table 1. Laboratory results from family 1

Family member/yr/Age (yr)	Father/2016/73	Father/2019/76	Mother/2016/70	Mother/2019/73	Proband/2016/38	Proband/2019/41
REN mutation	p.W10X	p.W10X	p.P113L	p.P113L	p.W10X and p.P113L	p.W10X and p.P113L
Hemoglobin (g/dl); [13.0–17.5 g/dl]	13.9	14	13.8	14.3	10.9	10.7
Hematocrit (%); [40.0%–50.0%]	41.6	42	40.8	43		
Serum creatinine (mg/dl); [0.7–1.3 mg/dl]	1.1	**1.41**	0.55	0.44	1.44	1.7
CKD-EPI (ml/min/1.73 m^2)	66.2	**48**				
Serum potassium (mmol/l); [3.5–5.1 mmol/l]	4.8	5.4	4.5	4.98	5.7	5.7
Serum chloride (mmol/l); [98–107 mmol/l]	103	106	106	105		
Serum uric acid (mg/dl); [3.7–9.2 mg/dl]	8.4		5.7		9	7.4
Urine specific gravity [1015–1025]	**1010**	**1012**	1015	1015	**1008**	**1007**
Microhematuria [<5/μl]	**8.9**	-	-	-		
Serum aldosterone (Quimioluminescence) [Orthostatism: 2.52–39.2 ng/dl Decubitus: 1.76–23.2 ng/dl]	6.9	-	8.4	-	**1.9**	2.4
Plasma renin [<38.7 pg/ml] (Quimioluminescence)					**59.6**	**62**
Plasma renin activity (Radio-imunoassay) [Orthostatism: 0.5–4 ng/ml/h Decubitus: 0.2–1.6 ng/ml/h]	3.1	-	2.3	-	**<0.2**	**0.2**[a]

Reference range is in square bracekts. Bold values are out of physiological range.
[a]Incubation period extended to 2 hours (there was no detectable activity after 1 hour).

for erythropoietin, and providing an improvement in global well-being. Renal function has remained stable.

The patient's mother is asymptomatic with normal laboratory test results, and her father was asymptomatic with normal plasma renin activity and aldosterone levels until the age of 76 years, when he was found to have CKD Stage 3a (Table 1).

Results

Specific clinical presentation prompted *REN* sequencing. It revealed that the proband is compound heterozygote for a truncating mutation (p.W10X) in the signal peptide of preprorenin and a missense mutation (p.P113L) located in the region encoding for mature renin that she inherited from her father and her mother, respectively (Figure 1a). *In silico* analyses revealed that the p.W10X mutation is predicted to affect renin synthesis through nonsense-mediated mRNA decay. The mutation p.P113L is located in mature renin between the amino acid residues D104 that forms part of the renin active site and C117 that constitutes disulfide bridge with the C124 residue (Figure 1b). Both mutations affect residues that are evolutionarily conserved (Figure 1c). The mutation p.P113L may destabilize prosegment and renin domain interactions (Figure 1d). Accordingly, characterization of transiently expressed wild-type and mutated preprorenin in HEK293 showed that the mutation p.W10X abolishes prorenin and renin synthesis. The p.P113L mutation reduces synthesis, secretion, and proteolytic activity of prorenin and renin (Figure 1e), and the P113L protein is abnormally localized in the Golgi and LAMP2 positive lysosomal-like structures (Figure 1f). See supplement for Supplementary Methods.

DISCUSSION

In this report, we describe clinical outcomes of bi-allelic *REN* mutations, resulting in autosomal recessive slowly progressive CKD. Compared with cases with

Table 2. Teaching points

Diseases associated with *REN* mutations
Autosomal recessive renal tubular dysgenesis
• Type of causal mutations: Biallelic loss-of-function *REN* mutations resulting in total loss of renin production.
• Disease manifestation: Disorder affecting renal tubular development characterized by persistent fetal anuria leading to severe hypotension, oligohydramnios, and the Potter sequence, associated with skull ossification defects. Patients typically died either *in utero* or shortly after birth.
• Treatment: None.
Autosomal dominant tubulointersitital kidney disease
• Type of causal mutations:Heterozygous missense mutations or in-frame deletions in *REN* affecting synthesis and leading to intracellular deposition of mutant renin
• Disease manifestation: All affected individuals have low plasma renin activity. Clinical presentation depends on the type of mutation and the protein domains affected. Mutation in the signal peptide and in the prosegment lead to hypoproliferative anemia, hyperuricemia, hyperkalemia, hypoaldosteronism, and decreased estimated glomerular filtration rate early in life followed by slowly progressive chronic kidney disease. Renal failure after the age of 35 yr. Mutation in mature part of renin lead to hyperuricemia, gout, and mildly progressive chronic kidney disease beginning in the third decade of life. Anemia, hyperkalemia, and acidemia do not occur. Renal failure after the age of 50 yr.
• Treatment: Anemia may be treated with erythropoietin. Treatment with allopurinol normalizes uricemia and prevents gout. Fludrocortisone corrects aldosterone deficiency, hyperkalemia, and acidemia.
Autosomal recessive tubulointersitital kidney disease
• Type of causal mutations: Biallelic *REN* mutations—combination of all types of *REN* mutations resulting in partial synthesis of mutated renin.
• Disease manifestation: Hypoproliferative anemia, hyperuricemia, hyperkalemia, hypoaldosteronism, and decreased estimated glomerular filtration rate early in life followed by slowly progressive chronic kidney disease without gout. High plasma renin concentration with low plasma renin activity. Small kidneys bilaterally with small cysts.
• Treatment: Anemia may be treated with erythropoietin. Treatment with allopurinol normalizes uricemia and prevents gout. Fludrocortisone corrects aldosterone deficiency, hyperkalemia, and acidemia.

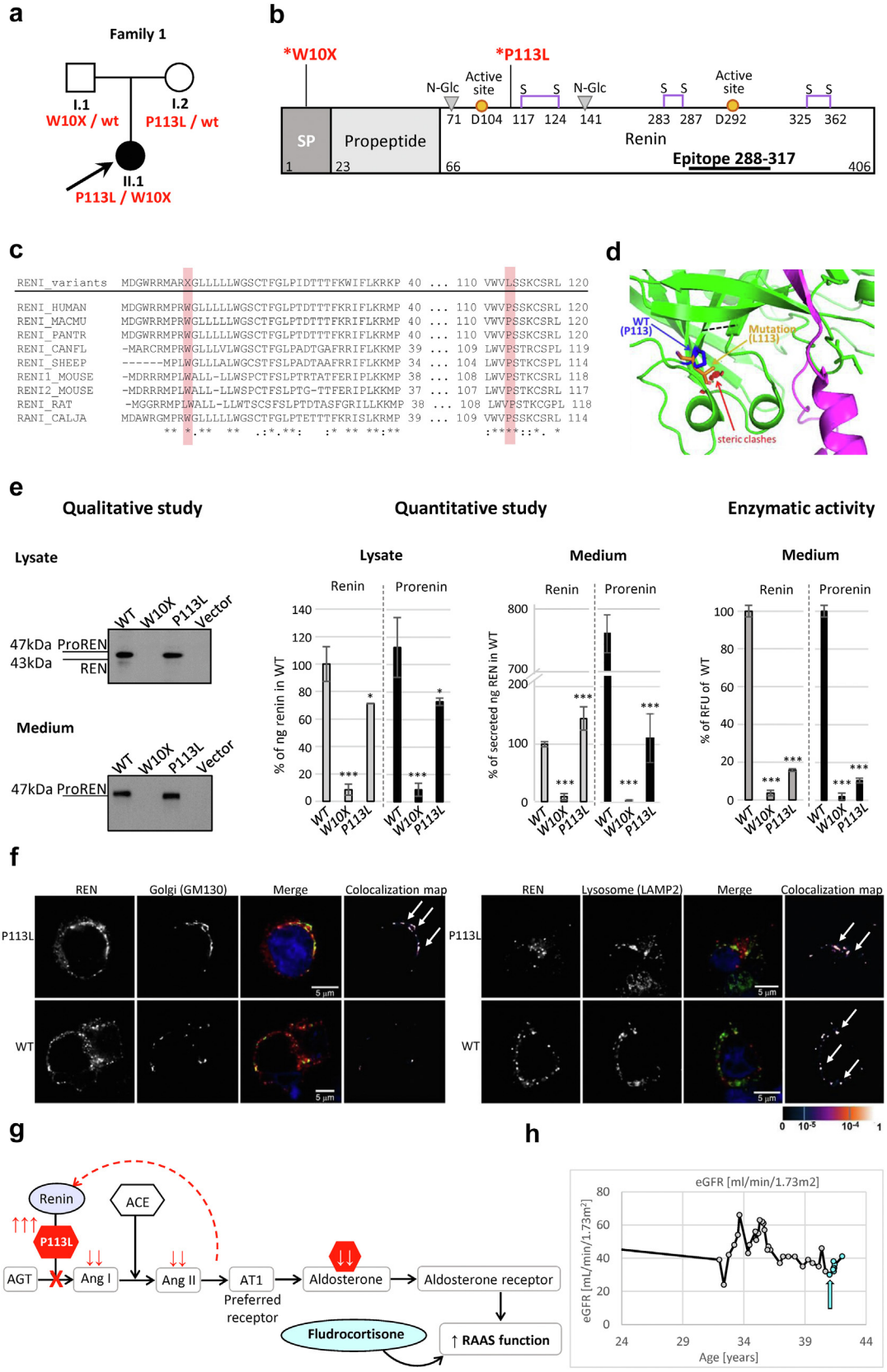

Figure 1. (a) Pedigree of the family. (b) A schematic diagram of the preprorenin, including the following: (i) the signal peptide (SP), essential for targeting and insertion of the synthesized protein into the endoplasmic reticulum (ER) membrane; (ii) the propeptide, which determines the biosynthesis, cellular trafficking, and enzymatic activity; and (iii) the mature enzymatically active renin that is formed upon the proteolytic cleavage of prorenin. Mutations identified in this study are shown in red. Aspartic acid residues 104 and 292 forming the active site of renin are marked by orange circles. N-glycosylation sites at residues 71 and 141 are marked by gray triangles. Disulfide bonds between cysteine residues 117–124, 283–287, and 325–362 are denoted by violet lines. Epitope 288–377 denotes the protein segment recognized by the (continued)

classic bi-allelic truncating *REN* mutations that resulted in an absolute loss of prorenin and renin production and renal tubular dysgenesis, the index patient presented with different clinical findings. Heterozygous carriers of truncating *REN* mutations are usually clinically unaffected. Likewise, the patient's father was asymptomatic with normal plasma renin and aldosterone levels until the age of 76 years, when he was found to have CKD Stage 3a with an eGFR of 48 ml/min. Contribution of the mutation to his kidney dysfunction is unclear. However, according to data from the National Health and Nutrition Examination Survey, 35% of individuals older than 60 years have an eGFR between 30 and 59 ml/min per 1.73 m^2 or eGFR $\geq$ 60 ml/min per 1.73 m^2 and albumin creatinine ratio $\geq$ 30 mg/g.

Heterozygous carriers of missense *REN* mutations with intracellular deposition of mutant renin usually present with anemia, hyperuricemia, and low to low-normal plasma renin and aldosterone levels associated with hypotension and hyperkalemia. The patient's mother is asymptomatic, with normal plasma renin and aldosterone levels and well-preserved kidney function at the age of 73 years. The absence of clinical findings correlates with functional characteristics suggesting that the p.P113L mutation results in blandly decreased production of less enzymatically active renin, with limited intracellular deposition and insignificant dominant negative effect.

The proband thus had a combination of the truncating mutation resulting in no translation, and the missense mutation resulting in production of less enzymatically active renin. How would such a combination of individually clinically insignificant mutations result in CKD in the proband? We propose a model where the absence of prorenin and renin production from the p.W10X allele may be compensated by increased production of the p.P113L protein from the other allele. This would lead to the disproportionately high plasma renin level with undetectable plasma renin activity observed in the proband and to increased intracellular deposition of the abnormal protein resulting in typical autosomal dominant tubulointerstitial kidney disease–REN clinical features

Figure 1. (continued) anti-preprorenin antibody used in this study. (c) Amino acid conservation across affected domains of preprorenin in higher mammals. Asterisks (*) indicate amino acid residues that are absolutely conserved, a colon (:) indicates residues with strong conservation, and a dot (.) indicates residues with weak conservation across species. (d) Mutation P113L destabilizes the beta sheet 110–114 (black dashed line), which may induce destabilization of the whole area responsible for interaction of the propeptide with renin. Wild-type (P113) and mutated (L113) residues are highlighed as blue and orange sticks, respectively. Steric clashes caused by the mutation are shown as red dots. (e) Transient expression of *REN* in HEK293 cells. **Qualitative study**—Western blot analysis of cell lysates and culture media suggested that the mutation p.W10X entirely abolishes prorenin and renin expression, whereas the mutation p.P113L decreases intracellular prorenin and renin expression and limits their secretion. Molecular weights of immunoreactive proteins present in the wild-type (WT) lysates correspond to expected molecular weights of prorenin (ProREN at 47 kDa) and renin (REN at 43 kDa). **Quantitative study**—Immunoradiometric assay (IRMA) of prorenin and renin amounts in cell lysates and culture media using a radiolabeled antibody that specifically recognizes active site of renin confirmed a decrease in expression and secretion of properly folded p.W10X and p.P113L proteins. The concentration of prorenin was calculated as the difference between the renin concentration measured before and after trypsin treatment, which activates renin by proteolytic cleavage of the prosegment from prorenin. Amounts of renin (gray bars) and prorenin (black bars) were normalized to total protein concentration in cell lysates and are shown relative to the amount of WT renin. The values represent means $\pm$ SD. The individual measurements were carried out in triplicate. The statistical significance of the differences between the WT and individual renin variants protein amounts was tested by paired *t* test. *$P \leq 0.05$; ***$P \leq 0.001$. **Enzymatic activity**–Both p.W10X and p.P113L proteins are enzymatically inactive. The values were normalized to transfected cell counts and are shown relative to the WT (100%) and represent means $\pm$ SD of relative fluorescent unit (RFU) generated by renin-mediated cleavage of the 5-FAM and QXL520 conjugated renin substrate. Measurements were performed in 3 independent clones for each of the constructs. The individual measurements were carried out in triplicate. The statistical significance of the differences between activity of the renin (WT) and renin variants was tested by paired *t* test. ***$P \leq 0.001$. (f) **Cell localization study—**Transiently expressed p.P113L and WT proteins were detected using an antibody recognizing the epitope 288–317 of preprorenin and colocalized with markers of ER (PDI protein), endoplasmic reticulum intermediate compartment (ERGIC; ERGIC53 protein), Golgi apparatus (GM130 protein), and lysosome (LAMP2 protein). WT protein was present in coarsely granular structures that were localized in the cytoplasm and LAMP2-positive granules. Mutation p.P113L demonstrated diffuse cytoplasmic staining and abnormally localized in Golgi. The degree of renin colocalization with selected markers is demonstrated by the fluorescent signal overlap coefficient values that range from 0 to 1. The resulting overlap coefficient values are presented as the pseudocolor, the scale of which is shown in corresponding lookup table. Neither p.P113L nor WT protein was detected in the ER or ERGIC (not shown). (g) **Pathogenetic model of the bi-allelic p.W10X/p.P113L constellation—**Only P113L catalytically inactive prorenin and renin are produced. Enzyme deficiency limits conversion of angiotensinogen (AGT) to angiotensin 1 (Ang1) and presumably depresses production of angiotensin 2 (AngII) and its interaction with angiotensin I receptor (AT1) and results in aldosterone deficiency. Low Ang II levels stimulate renin expression leading to increased renin plasma levels and probably also increased intracellular deposition of the abnormal protein. Synthetic aldosterone, fludrocortisone, may replace the missing hormone. (h) **Estimated glomerular filtration rate versus age.** The patient had about of acute kidney injury at the age of 33 years. Blue arrow indicates the start of fludrocortisone therapy in index patient with significant clinical and laboratory improvement. ACE, angiotensin-converting enzyme; AGT, angiotensinogen; Ang1, angiotensin 1; eGFR, estimated glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system; WT, wild-type.

(Figure 1g), with significant clinical and laboratory improvement of the index patient with fludrocortisone (Figure 1h, Supplementary Table S1), similar to what has been reported in other patients.[5]

An interesting aspect of the p.W10X/p.P113L combination is that despite minimal systemic production of "proteolytically active" renin, the patient had normal kidney development and did not develop severe renal tubular dysgenesis, as has been seen in all other patients with biallelic loss-of-function *REN* mutations.[2] There are several possible explanations for this. First, the p.P113L mutation may maintain a very small amount of renin activity, and this may be all that is required for kidney development. A second possibility is that the enzymatic action of renin itself may not be necessary for renin-expressing cell functionality and kidney development. A third possibility is that prorenin is more important in kidney development, and the mutant prorenin molecule was able to engage the prorenin receptor to facilitate local renin-angiotensin system activities and/or induce a variety of intracellular signal cascades.[6]

CONCLUSION

Our results complement recent reports by Fila *et al*.[4] on biallelic mutations in angiotensin-converting enzymes and by Dilliot *et al*.[7] on biallelic mutations in *REN* without renal tubular dysgenesis and suggest that similar findings may be expected in other renin-angiotensin system–related genes.[8] As more of these cases are identified, we propose that for these conditions, similar to recently adopted nomenclature for autosomal dominant tubulointerstitial kidney disease,[9] the term autosomal recessive tubulointerstitial kidney disease with gene suffix, for example, autosomal recessive tubulointerstitial kidney disease-angiotensin-converting enzyme and autosomal recessive tubulointerstitial kidney disease–*REN*, be used (Table 2).

DISCLOSURE

SJ is a member of the advisory board for Lumasiran; she is the clinical director of Hemodialysis clinic, Diaverum, Portugal. EN is a sporadic medical consultant for GSK and Vifor; she is a member of the advisory board for Belimumab and Avacopan. All the other authors declared no competing interests.

PATIENT CONSENT

The authors declare that they have obtained consent from the patients discussed in the report.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Table S1. Clinical findings of the index patient (II.1) in Family 1.

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