Received: 25 December 2018 | Revised: 28 March 2019 | Accepted: 7 April 2019

DOI: 10.1002/mgg3.705

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine Open Access WILEY

A familial case of pseudohypoaldosteronism type II (PHA2) with a novel mutation (D564N) in the acidic motif in *WNK4*

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Abstract

Background: There have been still few case reports of pseudohypoaldosteronism type II (PHA2), also known as Gordon's syndrome, genetically diagnosed, and this is the first report of familial PHA2 case in Japan with a novel D564N mutation in *WNK4*.

Methods: A 29-year-old woman was admitted to our hospital due to hyperkalemia (serum potassium: 6.4 mmol/L). She had mild hypertension (135/91 mm Hg), a bicarbonate level at the lower limit of the normal range (HCO_3: 22 mmol/L) with a normal anion gap, low plasma renin activity (0.2 ng ml^{-1} hr^{-1}), and high urinary calcium excretion (505.4 mg/g Cre). A hereditary condition was suspected because her mother also had the same symptoms. We performed a comprehensive genetic analysis for major inherited kidney diseases with next-generation sequencing including the genes responsible for PHA2 (*WNK1*, *WNK4*, *KLHL3*, and *CUL3*).

Results: Genetic analysis revealed that the patient and her mother had a novel missense mutation (D564N) in the acidic motif in *WNK4*, which leads to the diagnosis of PHA2. Administration of trichlormethiazide (1 mg/day) effectively ameliorated her blood pressure (114/69 mm Hg), plasma bicarbonate (25 mmol/L), serum potassium (4.3 mmol/L), and urinary calcium excretion (27.2 mg/g Cre).

Conclusion: We report the first Japanese familial case of PHA2 with *WNK4* mutation. D564N mutation in *WNK4* is a novel genetic cause of PHA2 with a relatively mild phenotype.

KEYWORDS

D564N, familial case, pseudohypoaldosteronism type II, *WNK4*

1 | INTRODUCTION

Pseudohypoaldosteronism type II (PHA2), also known as Gordon's syndrome, is a rare autosomal dominant condition with hypertension, metabolic acidosis, and hyperkalemia (Gordon, Geddes, Pawsey, & O'Halloran, 1970). To date, four genes have been reported as causing PHA2, which are *With-no-lysine kinase 1* (*WNK1*), *4* (*WNK4*), *kelch like 3* (*KLHL3*), and *cullin3* (*CUL3*) (OMIM: 605232, 601844, 605775, and 603136)(Boyden et al., 2012; Louis-Dit-Picard et al., 2012; Wilson et al., 2001). KLHL3 and CUL3 physiologically constitute E3 ligase complex and regulates protein degradation of WNK kinases. Mutations in one of the four genes result in the impaired degradation and increase the protein abundance of WNK kinases. That causes constitutive activation of WNK phosphorylation cascade and aberrantly increased sodium reabsorption via Na-Cl cotransporter (NCC) in distal convoluted tubule (DCT), leading to salt sensitive hypertension (Wakabayashi et al., 2013). The pathological condition also reduces potassium excretion via the renal outer medullary potassium channel (ROMK) (Wakabayashi et al., 2013; Yang et al., 2007). PHA2 has only been genetically diagnosed in a few patients, and this is the first report of familial PHA2 case in Japan with a novel D564N mutation in *WNK4*.

2 | CASE PRESENTATION

A 29-year-old woman with mild hypertension (135/91 mm Hg) and short stature (height: 147 cm, foot length: 19 cm) was transferred to our hospital for treatment of hyperkalemia (potassium: 6.4 mmol/L). She was not on medications and

TABLE 1 Summary of laboratory data

Plasma chemistry tests			Blood cells			Urinalysis		
TP	7.1	g/dL	Hb	11.3	g/dL	Gravity	1.012	
Albumin	4.2	g/dL	Plt	28.5×10^3	/μL	pH	7.0	
AST	19	IU/L	WBC	6,500	/μL	WBC	1–4	
ALT	17	IU/L				RBC	–	
LDH	150	IU/L	Venous gas analysis (RA)			Ketone	–	
ALP	204	IU/L	pH	7.35		Na	238	mmol/g Cre
γGT	21	IU/L	pCO_2	41	Torr	K	39	mmol/g Cre
UN	7	mg/dL	pO_2	59	Torr	Cl	225	mmol/g Cre
Cre	0.59	mg/dL	HCO_3	22	mmol/L	Ca	505.4	mg/g Cre
eGFR	97.1	mL/min/ 1.73 m^2	ABE	−3.0	mmol/L	Protein	0.07	g/g Cre
UA	2.6	mg/dL				NAG	3.6	IU/g Cre
Glucose	89	mg/dL	Endocrine tests			α1MG	1.96	mg/g Cre
HbA1c	5.7	%	PRA	0.2	ng $mL^{-1}hr^{-1}$	β2MG	0.1	mg/g Cre
Na	137	mmol/L	PAC	50.7	ng/dL			
K	6.4	mmol/L						
Cl	108	mmol/L						
Ca	9.1	mg/dL						
IP	3.2	mg/dL						
T.Bil	0.5	mg/dL						
TSH	1.113	μIU/mL						
fT4	0.87	ng/dL						
TG	49	mg/dL						
LDL-C	78	mg/dL						
HDL-C	71	mg/dL						

Abbreviations: ABE, actual base excess; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, calcium; Cl, chlorine; Cre, creatine; eGFR, estimated glomerular filtration rate; fT4, free thyroxine; Hb, hemoglobin; Hb, hemoglobin; HCO_3, bicarbonate ion; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphorus; K, potassium; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Na, sodium; NAG, *N*-acetyl-beta-D-glucosaminidase; PAC, plasma aldosterone concentration; pCO_2, partial pressure of carbon dioxide; pH, hydrogen ion concentration; plt, platelets; pO_2, partial pressure of oxygen; PRA, plasma renin activity; T.Bil, total bilirubin; TG, triglyceride; TP, total protein; TSH, thyroid-stimulating hormone; UA, uric acid; UN, urinary nitrogen; WBC, white blood cells; α1MG, alpha1-microglobulin; β2MG, beta2-microglobulin; γGT, gamma-glutamyltransferase.

had no relevant past medical history. Table 1 shows laboratory data obtained at the first visit, revealing bicarbonate at the lower limit of the normal range (HCO_3: 22 mmol/L) with a normal anion gap and low plasma renin activity (PRA) (0.2 ng ml^{-1} hr^{-1}). Since ultrasound revealed no abnormalities of her kidneys and estimated glomerular filtration rate (eGFR) and sodium excretion were within the reference ranges (eGFR: 97.1 ml/min/1.73 m^2, sodium excretion: 238 mmol/g Cre), we suspected PHA2. Urinary excretion of calcium was significantly elevated (505.4 mg/g Cre) and the plasma aldosterone concentration (PAC) was also significantly increased (50.7 ng/dl), which was uncommon among reported cases of PHA2. To investigate the reason for the extraordinary elevation of PAC, we restricted potassium intake under 40 milliequivalent per day without sodium restriction to reduce her serum potassium level and repeated the laboratory tests. PAC was normalized in association with milder hyperkalemia (PAC: 13.7 ng/dl, potassium: 5.4 mmol/L), while metabolic acidosis persisted along with suppression of PRA (HCO_3: 21 mmol/L, PRA: 0.2 ng ml^{-1} hr^{-1}). Urinary calcium excretion was also increased (309.7 mg/g Cre). The pedigree of this family was presented in Figure 1. The proband's mother (height: 151 cm, foot length: 21 cm) was aged 57 and she was taking amlodipine for hypertension, with her blood pressure being controlled at 130/80 mm Hg. She also showed hyperkalemia and her bicarbonate level was at the lower limit of normal with low PRA (potassium: 5.7 mmol/L, HCO_3: 23 mmol/L, PRA: 0.4 ng ml^{-1} hr^{-1}). Interestingly, her PAC level was not as high as that of her daughter (18.6 ng/dl). In addition, the patient's older sister (height: 153 cm) had history of detection of metabolic acidosis with hyperkalemia (potassium: 6.0 mmol/L) and low PRA (0.2 ng ml^{-1} hr^{-1}) at another hospital when she was aged 15. Marked elevation of PAC was not observed (16.0 ng/dl). Her blood pressure was normal (120/70 mm Hg) at that time, but it increased to 140/93 mm Hg in August when she was 28. Only limited laboratory data were available because she died suddenly of subarachnoid hemorrhage in November at the same age. The patient's father was of normal height for a Japanese man (165 cm). Similar clinical findings of hypertension, hyperkalemia, and low PRA in the three family female members strongly suggested that they shared a hereditary mutation, which led us to perform genetic analysis. After we obtained the ethics committee and patient's approval, comprehensive genetic analysis for major inherited kidney diseases was performed with next-generation sequencing according to the method reported previously (Mori et al., 2017). Briefly, the exons and splicing regions in *WNK1, WNK4, KLHL3,* and *CUL3* responsible for PHA2 were targeted using biotin-labeled custom RNA probes (Agilent Technologies, Inc.) and then were sequenced simultaneously. We first excluded SNVs with allele frequencies >0.01 in any population within the Exome Aggregation Consortium (ExAC v.0.3) Browser (http://exac.broadinstitute.org), NHLBI Exome Sequencing Project Exome Variant Server dataset ESP6500 (http://evs.gs.washington.edu/EVS/), 1,000 Genomes (Abecasis, et al., Nature, 2010), HGVD (Japanese) (http://www.hgvd.genome.med.kyoto-u.ac.jp), and JGVD (Japanese) (https://ijgvd.megabank.tohoku.ac.jp). We also excluded SNVs with a "low" impact according to the definition in GEMINI, which includes the following functional predictions: "synonymous_coding," "intergenic," "upstream," "UTR," "intron," etc. As a result, a novel heterozygous missense mutation in the acidic motif in *WNK4* (NM_032387: c.G1690A: p.D564N) was detected both in the mother and in the proband. The mutation was validated with conventional Sanger sequencing. The single nucleotide variant detected, G1690A in *WNK4*, was not registered in the major allele frequency databases (ExAC, 1000 Genome, ToMMo2K), suggesting that it is an extremely rare variant. It has already been reported that other alterations of the same amino acid 564 (D564A (Wilson et al., 2001) and D564H (Golbang et al., 2005)) can cause PHA2. The mutation is located in the acidic motif in *WNK4*, where it physiologically binds to KLHL3, one of the E3 ubiquitin ligase complex proteins that degrade WNK4. The *WNK4* mutation results in the disruption of the binding, leading to the increased protein amount of WNK4 (Wakabayashi et al., 2013). The cosegregation is reasonable even though genetic testing was unavailable for the deceased older sister. High values in in silico pathogenicity predicting scores (SIFT (Kumar, Henikoff, & Ng, 2009), PolyPhen-2 (Adzhubei et al., 2010), and CADD (Kircher et al., 2014)) also support the evidence. Taken together, the variant we detected was classified as "likely pathogenic" based on 3 points in the ACMG guidelines indicating moderate evidences of pathogenicity (Richards et al., 2015). Administration of trichlormethiazide (1 mg/day) effectively reduced her blood pressure (114/69 mm Hg) and

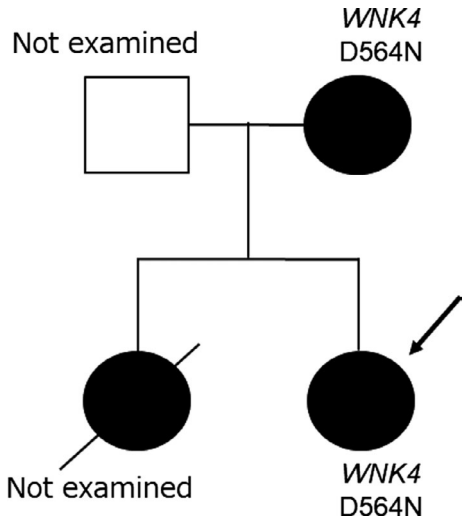

FIGURE 1 Family tree. ● indicates an affected woman; □ indicates an unaffected man; / indicates a deceased individual. The proband is indicated by an arrow

serum potassium level (4.3 mmol/L), while normalizing PRA (1.1 ng ml^{-1} hr^{-1}). Hypercalciuria also responded to trichlormethiazide and urinary calcium excretion became normal (27.2 mg/g Cre). Good response to the thiazide diuretics therapy and phenotypic amelioration also support the diagnosis.

3 | DISCUSSION

We report the first Japanese familial case of PHA2 genetically confirmed. Recent intensive physiological and genetic analyses reveal molecular and pathophysiological mechanisms of PHA2 (Boyden et al., 2012; Louis-Dit-Picard et al., 2012; Wakabayashi et al., 2013; Wilson et al., 2001; Yang et al., 2007). On the other hand, the clinical features of PHA2 have not yet been fully elucidated presumably because of the small number of genetically diagnosed patients. PHA2 patients generally have a normal or low PAC (Brautbar et al., 1978), while our patient's PAC level was high. We assume that a high serum potassium level was the underlying cause of PAC elevation, because PAC returned to normal after hyperkalemia was improved by restricting the patient's potassium intake without sodium restriction. Achard et al. stated that aldosterone secretion would be reduced because of low renin and would be insufficient to control the potassium level, although stimulated by hyperkalemia (Achard, Disse-Nicodeme, Fiquet-Kempf, & Jeunemaitre, 2001). However, Mayan et al. reported somewhat higher PAC levels in individuals from a kindred with the Q565E mutation of *WNK4* (Mayan et al., 2004). Considering the previously reported cases, significant increase in PAC observed in our case appears to be atypical. It seems likely that PAC varies according to the serum potassium level, and the PHA2 phenotype does not have any specific effect on PAC. Boyden et al. reported that the HCO_3 level varies depending on the causative gene of PHA2, and suggested that *WNK4* mutation is associated with less severe metabolic acidosis compared to other responsible genes (Boyden et al., 2012). They also reported that the age at diagnosis was older in patients with mutations causing a smaller decrease in HCO_3, while the HCO_3 level varied among different *WNK4* mutations, even though the specific reason is still not clear (Boyden et al., 2012). Golbang et al. reported a father and son with the D564H mutation of *WNK4*, who had HCO_3 levels of 16.2 and 13.0 mmol/L, respectively (Golbang et al., 2005), which were more severe than in our cases with the D564N mutation. Based on these limited information, the effect of the amino acid, Asparagine (N), is likely weaker than that of Histidine (H) for the binding disruption. However, basic studies for further validation would be necessary. Patients with such milder mutation might be diagnosed later in life as were our cases. Differences of the causative gene may also influence urinary calcium excretion, which has ranged from normal (Sanjad, Mansour, Hernandez, & Hill, 1982) to high (Semmekrot et al., 1987) in the patients reported previously. It was reported that the Q565E and Q562E mutations of *WNK4* lead to hypercalciuria (Lalioti et al., 2006; Mayan et al., 2004, 2002). On the other hand, Golbang et al. did not report on urinary calcium excretion in their patient with D564H (Golbang et al., 2005), possibly because hypercalciuria was not recognized as a diagnostic marker at that time. Urinary calcium excretion is one of the characteristics of PHA2, and it was in fact reduced by administration of trichlormethiazide in both our patients. Yang SS et al. reported increased expression of TRPV6 and CBP-D28k was observed in Wnk4(D561A/+) knock-in mice, indicating that decreased Ca(2+) reabsorption in the upstream nephron, especially in the thick ascending loops of Henle might be involved in the hypercalciuria of PHA2 (Yang et al., 2010). Besides, it may also be related to increased glomerular calcium filtration secondary to elevation of the serum level of free ionic calcium by metabolic acidosis, or to reduced Ca^{2+} reabsorption because of impairment of $3Na^{+}$-Ca^{2+} antiporter function under highly sodium reabsorbed condition via NCC (Mahnensmith, Thier, Cooke, Broadus, & DeFronzo, 1979). *WNK4* mutation leads to downregulation of the transient receptor potential V5 channel (TRPV5), which is involved in Ca^{2+} reabsorption from the DCT (Jiang, Ferguson, & Peng, 2007), and this could be another reason. In any case, hypercalciuria is a useful marker for suggesting the diagnosis of PHA2. Interestingly, despite excessive urinary calcium excretion, both the patient and her mother had no history of renal calculi. The neutral pH of the urine in our patient may be one of the reasons, since variation in urine pH is generally thought to be essential for stone formation, such as alkaluria in type 1 renal tubular acidosis (RTA) patients. In summary, we reported a familial case of PHA2 with a novel mutation of *WNK4*. In addition to the commonly reported characteristics of PHA2 such as hypertension, hyperkalemia, and low PRA, our patient with the D564N mutation of *WNK4* showed mild metabolic acidosis together with prominent hypercalciuria. PAC ranged from high to low depending on dietary potassium amount and therefore PAC in PHA2 patients may be easily varied regardless of the constitutively increased NCC activity. These findings in our patient may help to improve understanding of the pathophysiology of PHA2, and may contribute to establish the phenotype-genotype correlation in PHA2.

ACKNOWLEDGMENTS

This work was partly supported by a grant from the Okinaka Memorial Institute for Medical Research. The manuscript was checked by a native English–speaking medical editor from Yamada Translation Bureau, Inc. (Tokyo, Japan).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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How to cite this article: Sakoh T, Sekine A, Mori T, et al. A familial case of pseudohypoaldosteronism type II (PHA2) with a novel mutation (D564N) in the acidic motif in *WNK4*. *Mol Genet Genomic Med*. 2019;7:e705. https://doi.org/10.1002/mgg3.705