Endocr (2008) 33:230–234
DOI 10.1007/s12020-008-9084-8

ORIGINAL PAPER

A patient with pseudohypoaldosteronism type II caused by a novel mutation in *WNK4* gene

Hui Gong · Zhengyi Tang · Yang Yang · Lihao Sun · Wei Zhang · Weiqing Wang · Bin Cui · Guang Ning

Received: 18 April 2008 / Accepted: 28 July 2008 / Published online: 18 November 2008

Abstract Pseudohypoaldosteronism Type II (PHAII) is a very rare disorder characterized by hyperkalemia, hypertension, and slight hyper-chloremic metabolic acidosis. The index patient showed typical features of PHAII, including elevated blood pressure (140–150/90–100 mmHg), hyperkalemia in the range of 5.30–5.60 mmol/l (normal range is 3.50–5.10 mmol/l), accompanied by hyperchloremia of 109.5–112.0 mmol/l (normal 95.0–108.0 mmol/l) and acidosis with bicarbonate levels of 19.5–20.1 mmol/l (normal 22.0–27.0), GFR was 98.95 ml/min (normal > 90). However, these features were absent in his parents. Sequencing analysis found the patient with a *WNK4* gene mutation, 1682 C > T in Exon 7, which resulted a missense mutation at codon 561 (P561L). The variation in codon 561 was not found in his parents and 100 unrelated control subjects. The identified WNK4 mutation which has not been described previously is the probable cause of PHAII.

H. Gong · Z. Tang (✉) · Y. Yang · L. Sun · W. Zhang · W. Wang · B. Cui · G. Ning
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrinology and Metabolism, Ruijin Hospital, Shanghai JiaoTong University School of Medicine, 197 RuiJin Er Lu, Shanghai 200025, People's Republic of China
e-mail: zhypty@sh163.net

B. Cui · G. Ning
Laboratory Endocrine & Metabolic Diseases of Institute of Health Science, Shanghai JiaoTong University School of Medicine and Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 225 South ChongQing Road, Shanghai 200025, People's Republic of China

G. Ning
Division of Endocrine and Metabolic Diseases, E-Institute of Shanghai Universities, Ruijin Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, People's Republic of China

Keywords Pseudohypoaldosteronism type II · *WNK4* gene · Mutation

Introduction

Pseudohypoaldosteronism Type II (PHAII), characterized by hyperkalemia, hypertension, slight hyper-chloremic metabolic acidosis, and otherwise normal renal function, also known as Gordon's syndrome or familial hyperkalemia and hypertension (FHH), is a very rare disorder. These abnormalities could be ameliorated by small doses of thiazide diuretics which are specific antagonists of the Na^+–Cl^-co-transporter [1].

In 1964, Paver and Pauline [2] initially reported this disorder in an Australian young man with Hypertension, hyperkalemia, and normal renal function. Then in 1978, Farfel et al. [3, 4] described an affected Ashkenazi Jewish family in which some members had hyperkalemia in childhood and hypertension that developed later in life. Pseudohypoaldosteronism type II was firstly used by Schambelan et al. [5] to define this syndrome of chronic mineralocorticoid-resistant hyperkalemia with hypertension. Then, Gordon reviewed clinical details of several sporadic cases and pedigrees with PHAII, and pointed out that PHAII is associated with volume-dependent hypertension, hyperkalemia, acidemia, and low renin levels [6]. Up to now, three different genetic loci have been identified for this syndrome. A whole-genome scan was performed in the first eight kindreds, which demonstrated the genetic heterogeneity, and identified two associated loci, chromosome 1 and 17 [7]. Disse-Nicodeme et al. [8] analyzed a large French family and identified the third locus on Chr.12p13. Recently, *WNK1* and *WNK4* genes were determined responsible for PHAII disease in different families [9]. Until

now, there have been only five mutations to be reported in *WNK4* gene, E562K, D564A, D564H, Q565E, and R1185C [9, 10]. Two deletion mutations in *WNK1* gene have been found in two different kindreds, 41 kb and 22 kb deletion in intron 1[9].

Here we reported a 17-year-old Chinese boy who developed hypertension 2 years ago, and metoprolol, nifedipine and lisinopril failed to control his hypertension. Because hyperkalemia and hypertension were found, he was suspected to have PHAII. Genetics analysis further revealed that he carries a novel mutation (P561L) in *WNK4* gene.

Results

Clinical characteristics

The patient's blood pressure fluctuated between 140 and 150/90–100 mmHg without treatment in hospital. The major results of his blood chemical values on admission are shown in Table 1. GFR was 98.95 ml/min (normal > 90 ml/min) and serum creatinine was 75 μmol/l (normal range 53–115 μmol/l), 24-h urine albumin was 9.09 mg/24-h, and the renal imaging examination did not find any abnormality. Hyperchloremia and low bicarbonate levels suggested mild metabolic acidosis, and it was confirmed by the repeated measurements. Plasma rennin activity and aldosterone concentration were in normal range, 0.26 ng/ml per hour (normal range 0.10–5.50) and 74.9 ng/ml (normal range 29.4–161.5), respectively. Serum potassium was higher than normal (5.30–5.60 mmol/l, normal range is 3.50–5.10 mmol/l) confirmed by several tests. Finally the clinical diagnosis of PHAII was made. His parents had no history of hypertension and hyperkalemia, and their blood potassium was also normal.

Table 1 Blood chemical values of the patient when hospitalized

Variable	First hospital day	Eighth hospital day	Normal range
K^+(mmol/l)	5.40	5.60	3.50–5.10
Cl^-(mmol/l)	112.0	109.5	95.0–108.0
Na^+(mmol/l)	138.9	135.6	130.0–147.0
Ca^{2+}(mmol/l)	2.37	2.22	2.0–2.75
PO_4^{3-}(mmol/l)	1.43	1.54	0.8–1.6
PH(artery)	7.30	7.31	7.35–7.45
BE(artery, mmol/l)	−5.8	−4.8	−3.0–3.0
HCO_3^-(artery, mmol/l)	19.5	20.1	22.0–27.0
Creatinine (μmol/l)	75	–	53–115

BE, base excess

Genetic screening

To screen the *WNK4* gene, all protein-coding exons were PCR amplified individually and the products were directly sequenced. A nucleotide substitution was detected in Exon 7 (1682 C > T), which was not found in his unaffected parents and 100 normal Chinese unrelated subjects. The substitution causes a missense mutation at codon 561, the substitution of Leu for Pro (P561L), as shown in Fig. 1. Further subclone sequencing of WNK4 gene for the patient showed a monoallelic mutation 1682 C > T, which demonstrated only one of the two alleles harboring the mutation. This P561L mutation has not been reported previously. No variations of other exons of *WNK4* gene have been identified.

Discussion

We here reported a patient presenting with the sporadic form of PHAII, who carried a heterozygous mutation in Exon 7 (1682 C > T), which causes a missense mutation P561L. This mutation lies within the acid motif (^{557}EPEEPEADQH^{566}), which is highly conserved among all members of the WNK family in human [9]. Previously reported four *WNK4* gene mutations (E562K, D564A, D564H, Q565E) were also located in this negatively charged 10 amino acid segment [9, 10], and our patient had similar clinical phenotypes with previous reported mutations, as the same domain in WNK4 protein was in dysfunction.

The acid motif may play an important structural and functional role in a protein molecule. Recently, several functional studies observed the effects of the mutants altering amino acid within the acid motif on the functions of the co-expressed ion channels or co-transporters, including thiazide-sensitive Na^+–Cl^- co-transporter(TSC) [11], apical secretory K^+ channel (renal outer medullary potassium channel, ROMK) [12], epithelial Na^+ channel (ENaC) [13] in Xenopus oocytes, or paracellular ion flux [14] through tight junctions in epithelial cells. All these experiments had shown the same results even with different WNK4 gene mutations. After the mutated WNK4 gene transfer, TSC and ENaC activity increased while ROMK activity suppressed, which resulted in more Na^+ reabsorption and less K^+ secretion. These findings established that WNK4 protein is a multifunctional regulator of diverse ion transporters; it could balance the Na^+ reabsorption and K^+ secretion, whereas PHAII-mutant WNK4 could have effects, such as decreasing the inhibition to TSC and ENaC, increasing the inhibition to ROMK, and then influence ion transportation expressed as increasing renal NaCl reabsorption and inhibiting K^+ secretion simultaneously on those ion channels [15].

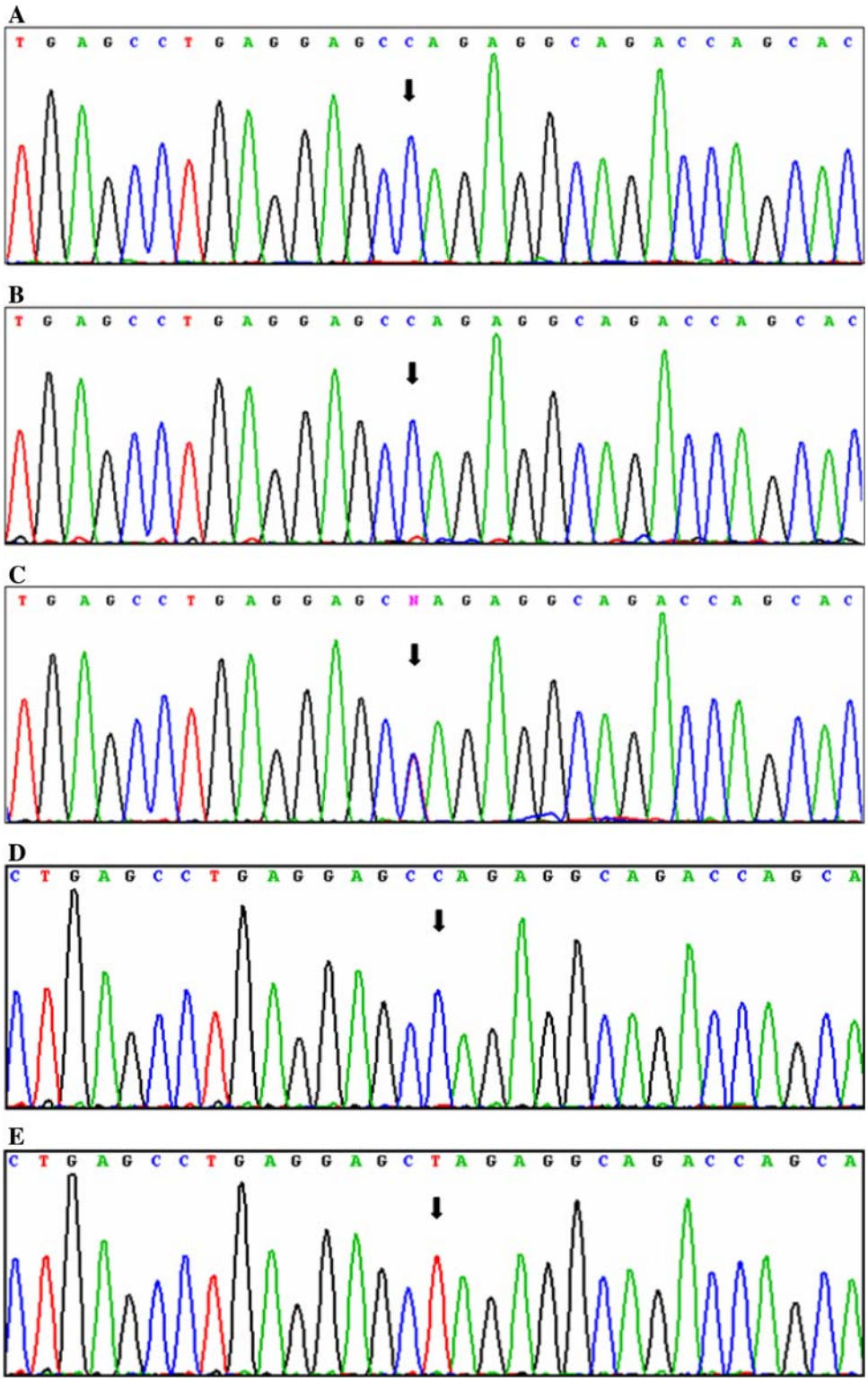

Fig. 1 Identification of mutation by PCR direct sequencing and subclone sequencing of the *WNK4* gene. Section **a** stands for the direct sequencing result of normal mother's Exon 7, **b** for his normal father, **c** for the patient's PCR direct sequencing result, found a red line(T) overlapped the blue line(**c**), **d** for subclone sequencing of the patient's normal mono-allele, and **e** for the patient's mutational mono-allele. The arrow indicated the variation nucleotide. The patient parents showed a normal nucleotide C, while the patient was detected with a C/T heterozygote. This mutational T would change Proline to Leucine in the 561 amino acid of WNK4 protein (P561L)

A number of disorders are considered as the reasons of hyperkalemia, but renal insufficiency is the most common. PHAII will be suspected firstly when patients are presented with hyperkalemia and consistently normal GFR. Other disorders associated with hyperkalemia such as Addison's disease, isolated hypoaldosteronism, and pseudohypoaldosteronism are the result of the sodium wasting and subsequent volume contraction.

It was noted that the patient presented hyperkalemia, whereas normal renal function was evidenced by his

Table 2 Primers for *WNK4* gene amplification

Exon	Sequences	Fragment size (bp)	Annealing temperature (°C)
1	5′>GGGCGGTGACTAAGGTGA<3′	1003	60
	5′>CAGGGCAAAGACAGCAGAG<3′		
2	5′>TTACGTGCGGGACTGTTG<3′	532	58
	5′>ACTGGAGTGCTGGGTGGA<3′		
3,4	5′>GAGAATGCTGGCAGAAGATG<3′	1085	60
	5′>CTCCCGCTGTTTGTTCAT<3′		
5,6	5′>ATGAACAAACAGCGGGAGAC<3′	778	60
	5′>CGTGCGGGTAAGTTAGGG<3′		
7	5′>CTCTGGGCTTGGTCTGTG<3′	520	60
	5′>GGTAGCCATCAGTCTCGC<3′		
8	5′>CAGTACGTGAACGGGTTG<3′	629	60
	5′>GGAGATAGGTCATAGGCTTG<3′		
9,10	5′>ATTGCGAGACTGATGGCTAC<3′	895	60
	5′>GATTCAAAGTCAGCAGAGGG<3′		
11	5′>CCCCTCTGCTGACTTTGA<3′	560	58
	5′>ATGCCTTTGCTTATGCTGT<3′		
12	5′>TGTTGACCCTCGCAAGGTATA<3′	188	58
	5′>ATTTCCCCTGGAAGTGTCCA<3′		
13,14,15	5′>CCACCTTAGCGTCATCATTG<3′	1289	60
	5′>CTGCGGCTTTCCCTCTGT<3′		
16	5′>TCCTCGTCGCCCTACAGA<3′	454	60
	5′>CTCACTCCTCCATCCTCCC<3′		
17,18	5′>CAGCTTGTTGGGCGTTTC<3′	1335	60
	5′>AGGCTTGGGTGGGATTGC<3′		

normal urine albumin level, serum creatinine level, GFR, and renal ultrasound imaging. These features were considered as the main phenotype of PHAII. Unfortunately, the early manifestation of chronic hyperkalemia in PHAII was always asymptomatic. Most patients would not see the doctor until hypertension occurred. Even though the hypertension was diagnosed, these patients were always treated for essential hypertension initially. PHAII would be suspected when the hypertension was poorly controlled or hyperkalemia was found. A similar situation happened in this patient. He was initially diagnosed for essential hypertension and treated with several anti-hypertension medications such as metoprolol, nifedipine, and lisinopril; however, the blood pressure was not controlled ideally. PHAII was not suspected until serum potassium was measured. When hydrochlorothiazide (25 mg bid) was given, the normal blood pressure was achieved 1 week after prescription, and his hyperkalemia and slight hyperchloremic metabolic acidosis were also corrected. Then hyperkalemia is the key point for differential diagnosis of PHAII to other monogenic hypertensive syndromes. It is therefore important to make early diagnosis for PHAII that once the diagnosis of hypertension is established, blood electrolytes should be examined to find mild hyperkalemia.

The clinical severity of the presentations such as hyperkalemia and hypertension varied widely among affected individuals even with same gene mutation. Besides hyperkalemia and hypertension, some patients had other inconstant clinical manifestations, for example, patients with Q565E mutation in WNK4 protein showed marked hypercalciuria [16]. In some cases, short stature, intellectual impairment, and dental abnormalities have also been noted [6]. However, this patient had not been observed to have other symptoms.

Subjects and methods

Subjects

The index patient, a 17-year-old boy, was hospitalized because of hypertension for almost 2 years and hyperkalemia (5.60 mmol/l, normal range of 3.50–5.10) found 1 week ago. No hypertension and hyperkalemia family history was available. He was found with elevated blood pressure (140/90 mmHg) at home incidentally 2 years ago. His maximal blood pressure was about 150/110 mmHg when off treatment. Several anti-hypertension agents such

as metoprolol, nifedipine, and lisinopril failed to control his blood pressure to normal level. Hyperkalemia was found when lisinopril was ceased 3 months later. The patient had never presented with paroxysmal severe headaches, palpitations, diaphoresis, or periodic paralysis.

When he was hospitalized, his blood biochemical parameters and hormones were measured. Plasma electrolyte levels were measured twice, 1 week apart. His glomerular filtration rate (GFR) was measured with ^{99m}Tc-DTPA renal dynamic imaging. His parents' clinical information and blood electrolyte levels were also collected.

We obtained informed consent from all subjects participating in the study, and the Hospital Ethical Board approved this study.

PCR and sequencing of WNK4 gene

Genomic DNA was extracted from peripheral blood cells by the classical phenol chloroform protocols. PCR Reactions were performed with 100 ng of genomic DNA in 20 μl volume containing standard PCR 100× buffer 2 μl, each specific primer (Table 2) 1 μl, 25 mM dNTP 0.4 μl, 5 U/μl *Taq* polymerase 0.2 μl (Takara, Shiga, Japan). The PCR conditions were as follows: an initial denaturing cycle at 94°C for 45 s, followed by 35 cycles of: 94°C for 30 s, annealing (annealing temperature for different primers shown in Table 2) for 30 s and extension at 72°C for 1 min. A final extension step at 72°C for 10 min was used. PCR products were purified by QIAGEN PCR purification kits (Qiagen, Hilden, Germany) and then sequenced in both sense and antisense directions by ABI PRISM 3700 Genetic Analyzer (Applied Biosystems PerkinElmer, Foster City, CA). To determine that P561L substitution was not at the polymorphic site, we screened the Exon 7, which included codon 561, in his unaffected family members and 100 unrelated normal Chinese subjects by direct sequencing of PCR products.

Subclone sequencing

The purified PCR product of mutant exon was ligated into pGEM-T easy vector (Promega, Madison, WI, USA) and then transformed to *E. coli* strain DH-5α. Positive subclones were sequenced by ABI 3700 Genetic Analyzer (Applied Biosystems PerkinElmer, Foster City, CA).

Acknowledgments We thank the patient and his family members for their participation in this study. We are also grateful to Libin Liu (Department of endocrinology and metabolism, Union Hospital affiliated to Fujian Medical University, Fujian, China) for collecting samples from the patient's family. This study was supported by the grant from Shanghai Leading Academic Discipline Project (project number: Y0204).

References

1. H. Mayan, I. Vered, M. Mouallem et al., J. Clin. Endocrinol. Metab. **87**, 3248–3254 (2002)
2. W.K.A. Paver, G.J. Pauline, Med. J. Aust. **2**, 305–306 (1964)
3. Z. Farfel, A. Iaina, T. Rosenthal et al., Arch. Intern. Med. **138**, 1828–1832 (1978)
4. Z. Farfel, A. Iaina, J. Levi et al., Arch. Intern. Med. **138**, 1837–1840 (1978)
5. M. Schambelan, A. Sebastian, F.C. Rector Jr., Kidney Int. **19**, 716–727 (1981)
6. R.D. Gordon, Hypertension **8**, 93–102 (1986)
7. T.A. Mansfield, D.B. Simon, Z. Farfel et al., Nat. Genet. **16**, 202–205 (1997)
8. S. Disse-Nicodeme, J.M. Achard, I. Desitter et al., Am. J. Hum. Genet. **67**, 302–310 (2000)
9. F. Wilson, S. Disse-Nicodeme, K. Choate et al., Science **293**, 1107–1112 (2001)
10. A.P. Golbang, M. Murthy, A. Hamad et al., Hypertension **46**, 295–300 (2005)
11. F.H. Wilson, K.T. Kahle, E. Sabath et al., Proc. Natl. Acad. Sci. USA. **100**, 680–684 (2003)
12. K.T. Kahle, F.H. Wilson, Q. Leng et al., Nat. Genet. **35**, 372–376 (2003)
13. A.M. Ring, S.X. Cheng, Q. Leng et al., Proc. Natl. Acad. Sci. USA. **104**, 4020–4024 (2007)
14. K.T. Kahle, G.G. MacGregor, F.H. Wilson et al., Proc. Natl. Acad. Sci. USA. **101**, 14877–14882 (2004)
15. G. Gamba, Am. J. Physiol. Renal Physiol. **288**, F245–F252 (2005)
16. H. Mayan, G. Munter, M. Shaharabany et al., J. Clin. Endocr. Metab. **89**, 4025–4030 (2004)