Clinical and molecular analysis of six Japanese patients with a renal form of pseudohypoaldosteronism type 1

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Abstract. Pseudohypoaldosteronism type 1 (PHA1) is a rare condition characterized by neonatal salt loss with elevated plasma aldosterone and renin levels. Two types of PHA1 have been described: an autosomal recessive systemic form and an autosomal dominant renal form, in which the target organ defect is confined to the renal tubules. The dominant renal form of PHA1 is caused by heterozygous mutations in the NR3C2 gene, which encodes the mineralocorticoid receptor (MR). We determined clinical and biochemical parameters in two familial and four sporadic Japanese patient and analyzed the status of the NR3C2 gene. Failure to thrive was noted in five of the six patients. In one of the familial cases, the mother had an episode of failure to thrive when she was a toddler, but received no medical treatment. NaCl supplementation was discontinued in four of the six patients after they reached one year of age and they have grown normally thereafter. However, in one patient, 9 g/day of salt has been required to maintain serum Na concentration after 1 year of age. Analysis of NR3C2 identified three novel mutations [c. C1951T (p.R651X), c.304_305delGC (p.A102fsX103), c.del 603A (p.T201fsX34)] and one previously reported mutation [c.A2839G (p.947X)]. p.R651X was identified in one familial case and one unrelated sporadic patient. The patient who has been supplemented with large amount of salt was heterozygous for c.del 603A in exon 2. In conclusion, our study expands the spectrum of phenotypes, and characterized mutations of NR3C2 in the renal form of PHA1.

Key words: Pseudohypoaldosteronism, NR3C2, Mutation, Phenotypic variability

Pseudohypoaldosteronism type 1 (PHA1) is characterized by congenital resistance to aldosterone of the kidney and/or other mineralocorticoid target tissues, resulting in excessive salt wasting [1, 2]. Two models of inheritance of PHA1, an autosomal recessive (systemic) form and an autosomal dominant renal form, have been described [1-4]. The autosomal recessive form manifests as severe life-long salt wasting resulting from mineralocorticoid resistance in multiple target tissues such as the sweat glands, salivary glands, colonic epithelium and lung [3, 5]. A pulmonary phenotype has been reported in some patients with recessive PHA1 who present with recurrent respiratory infections in the weeks or months after birth [1, 2, 5, 6]. This disease is caused by mutations of the amiloride-sensitive luminal sodium channel (ENaC) gene, which encodes a protein responsible for sodium reabsorption [3, 5, 6]. On the other hand, in the renal form of PHA1, mineralocorticoid resistance is restricted to the kidney [1, 2, 4, 7]. Clinical symptoms gradually improve with advancing age, whereas aldosterone and plasma renin activity (PRA) may remain high. In addition, patients respond well to salt supplementation and sodium supplementation becomes generally unnecessary by 1-3 years of age, as renal salt conservation abilities mature [2, 7]. It has been also reported that phenotypic vari-
ability is present even in familial cases [7-10].

Autosomal dominant and sporadic PHA1 cases are caused by loss-of-function mutations in the human mineralocorticoid receptor (hMR) gene \( NR3C2 \) [4, 7-14]. \( NR3C2 \) is located on chromosome 4q31.1 and consists of 10 exons, encoding a protein of 984 amino acids [4]. The hMR protein consists of an amino-terminal region including a ligand-independent transactivation function (AF1), a DNA-binding domain, and a C-terminal domain responsible for ligand binding and ligand-dependent transactivation (AF2) [15, 16]. The hMR acts as a ligand-activated transcription factor and induces or represses specific target genes, including those encoding the amiloride-sensitive epithelial sodium channel, serum and glucocorticoid regulated kinase, channel-inducing factor, and different protooncogenes [17].

In this study, we assessed the clinical and biochemical parameters and \( NR3C2 \) status of two familial and four sporadic Japanese cases of PHA1.

**Subjects and Methods**

**Subjects**

Clinical symptoms, age at diagnosis and biochemical data are summarized in Table 1. Patients 1 through 5 showed failure to thrive, and biochemical evaluation showed low serum Na concentration, high plasma aldosterone level and high PRA. Consequently, they were suspected to have a renal form of PHA1. All patients received and responded to NaCl supplementation. NaCl supplementation of patients 1, 2, 3 and 4 was withdrawn at the age of 1 year.

Patients 1 and 2 were siblings. In an interview, their mother related an episode of vomiting when she was a toddler for which she received no treatment. Since her family history indicated the renal form of PHA1, endocrinological evaluation was carried out. Her plasma aldosterone (38.9 ng/dL, normal range 7.1-10.1 ng/dL for adult) and plasma renin activity (PRA) (4.3 ng/mL/hr, normal range 0.87-1.14 ng/mL/hr for adult) were elevated, whereas her serum Na and K were in the normal range. Patient 1 is now 7 years old, and his plasma aldosterone level and PRA are elevated (563 ng/dL and 3.1 ng/mL/hr, respectively). Patient 2 is now 4 years old, and his plasma aldosterone level and PRA are also elevated (889 ng/dL and 4.2 ng/mL/hr, respectively).

Briefly mentioning clinical course of patient 5 and 6, patient 5 was the second child and born at term after an uneventful pregnancy with a birth weight of 2820 g (-0.7SD for Japanese boy). He was admitted to hospital at 29 days of age with poor weight gain (3020 g, -2.2SD for Japanese boy) and failure to thrive. At that time hyponatremia (121 mEq/L) and hyperkalemia (7.1 mEq/L) were noticed. Urinary Na (10 mEq/L) and K (13 mEq/L) were within the normal range. Sodium concentration was elevated in sweat (123 mEq/L, normal range 9-72 mEq/L) and in saliva (86 mEq/L, normal range 7-22 mEq/L). Plasma aldosterone was ele-
NR3C2 mutation and phenotype

Results

Heterozygous mutations of NR3C2 were identified in all six patients (Fig. 1). Siblings of patient 1 and 2 had the nucleotide change C→T at position 1951 (c. C1951T), introducing the nonsense substitution (p.R651X) in exon 4. p.R651X was also present in the mother of these siblings and in patient 3, who was unrelated to patient 1 and 2. Patient 4 had a heterozygous mutation (A→G) at position 2839 (c. A2839G), resulting in a stop codon (p.R947X), which was previously reported in several patients of different ethnic origin [10, 14]. In patient 5, deletion of A at position 603 (c.603delA) caused a frame shift, generating a premature stop codon at codon 201 in exon 2 (p.T201fsX34). There was no mutation in the SCNN1A, SCNN1B, and SCNN1G genes of patient 5. Patient 6 had a heterozygous two base deletion (c.304_305delGC), introduced premature stop codons in exon 2 (p.A102fsX103). In patients 4 and 5, neither parents had the mutations, suggesting de novo mutation. DNA from the parents of patient 3 and 6 was not available for analysis.

Discussion

In this study we have identified four mutations in NR3C2, three of which are novel and one of which has been previously reported. The p.R651X mutation, which was identified in one family and one unrelated patient corresponds to codon 651, which harbors a CpG dinucleotide (CGA). Deamination of 5-methylcytosine to thymine at CpG sites is thought to be an important mechanism for generating point mutations in humans, accounting for more than 20% of all base substitutions in genetic disease [18]. Two novel exon 2 mutations, c.304_305delGC and c.603delA, introduced premature stop codons in exon 2 (p.A102fsX103 and p.T201fsX34, respectively). Although we did not determine the functional consequences of these mutants in vitro, mRNA containing these two mutations may be subject to nonsense-mediated mRNA decay [19].

The p.R947X mutation is present in the C-terminal ligand binding domain of the hMR [14]. Therefore, it is plausible that impaired ligand-binding capacity associated with p.R947X may contribute to the disease. It is also interesting that p.R947X mutation has been identified in patients from Turkey and Brazil [14]. As our patient is Japanese, the founder effect is unlikely and, as has been previously reported, this region of the

NR3C2 analysis

Genomic DNA was extracted from peripheral blood leukocytes using the Nucleospin Blood kit (Takara, Tokyo, Japan). All translated exons (2 to 9) of NR3C2 and the exon/intron boundaries were sequenced as previously described [13]. PCR products were sequenced directly using a commercial kit (ABI Prism Big Dye; PE Applied Biosystems, Foster City, CA) and analyzed with an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems). In one patient (patient 5), since the Na concentration in sweat and saliva was elevated, the human amiloride sensitive ENaC subunit genes SCNN1A, SCNN1B, and SCNN1G were amplified according to a previous report [3, 6] and the PCR products were sequenced directly.

NR3C2 mutation and phenotype
NR3C2 gene is a mutational hot spot.

Several studies have shown that the renal form of PHA1 has a broad phenotypic range [7-10]. Patients 1 and 2 showed failure to thrive at one month of age and required salt supplementation. Their mother had an identical mutation, but did not develop severe symptoms during childhood. In patient 6, prior to genetic diagnosis, NaCl supplementation was stopped after her serum Na and K returned to normal and she has grown normally. These results are agreement with previous studies.

In general, elevated sodium concentration in sweat and saliva is a useful tool for the diagnosis of systemic form of PHA1 [1, 2]. Recently, a patient harboring compound heterozygous mutations (S166X and W806X) in the NR3C2 has been reported [20]. This patient showed elevated Na concentration in a sweat test at the age of 3 years [21]. Since hMR expression was found in the sweat glands [22], defective hMR may affect sodium reabsorption. In patient 5 in this study, saliva and sweat sodium levels were also elevated at diagnosis. Moreover, his NaCl dose is much higher than those of other patients in our study, and at 1 year of age 9g/day of NaCl is still required to maintain serum sodium levels. This continuing requirement of NaCl in this patient 5 may be related to loss of salt.

![Fig. 1](image-url)
from sweat and saliva gland.

Patients with the renal form of PHA1 respond well to salt supplementation and it generally becomes unnecessary by 1-3 years of age [1, 2, 7]. Martinerie et al. [23] showed that during human embryogenesis, hMR mRNA expression was observed between 15 and 24 weeks of gestation, however immunoreactive hMR protein expression was not found in late gestation, nor in neonatal kidneys. Moreover while hMR protein expression was not observed at 10 months of age, it was observed at 11 months in human kidney [23]. These findings indicate that the increased hMR expression with advancing age may compensate for the haploinsufficiency of hMR, allowing for withdrawal of salt supplementation in patients with the renal form of PHA1. While the exact regulatory mechanisms governing hMR expression are currently unknown, it is plausible that these mechanisms partly influence the phenotypic variability of the renal form of PHA1.

In conclusion, our study expands the spectrum of phenotypes, and characterized mutations of NR3C2, in the renal form of PHA1.

References

17. Fernandes-Rosa FL, Hubert EL, Fagart J, Tchitchek


