Identification of a Novel Mutation in the Human Mineralocorticoid Receptor Gene in a German Family with Autosomal-Dominant Pseudohypoaldosteronism Type 1: Further Evidence for Marked Interindividual Clinical Heterogeneity

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Pseudohypoaldosteronism (PHA) type 1 presents in infancy with potential life-threatening salt wasting and failure to thrive. Plasma renin activity and aldosterone levels are markedly elevated. PHA1 is inherited in either an autosomal recessive or autosomal dominant trait. The autosomal dominant form manifests with renal salt loss in infancy and a gradual improvement with advancing age.

We report the case of a renal form of PHA1 in a German family. PCR and direct sequencing revealed a heterozygous point mutation, c.488C>G (S163X), leading to a premature stop codon in the index patient. The segregation analysis revealed the identical mutation in the patient’s father, who showed no symptoms of PHA at the time of investigation or before. The family was screened for amino acid polymorphisms in the amiloride-sensitive epithelial sodium channel, which could be a cause for phenotypic differences within the family. PCR and direct sequencing revealed identical epithelial sodium channel haplotypes in the patient and his father. These polymorphisms can, therefore, not be responsible for the difference in clinical presentation. It remains to be elucidated whether other defects or polymorphisms in genes coding for regulatory proteins participating in sodium homeostasis are a cause of the heterogeneity of the clinical manifestations in autosomal dominant PHA1.

Type 1: Further Evidence for Marked Interindividual Clinical Heterogeneity

PSEU.DOHYPOALDOSTERONISM TYPE I (PHA1) is a rare congenital disease characterized by neonatal salt loss resistant to mineralocorticoids. Cheek and Perry (1) were the first to describe the disease as early as 1958. At least two forms of PHA1 exist (2-7). The autosomal recessive form is characterized by severe multiple target organ resistance to aldosterone, including the kidneys, colon, and sweat and salivary glands. Autosomal recessive PHA1 usually persists into adulthood (5, 6). It is caused by mutations of the amiloride-sensitive luminal sodium channel (ENaC) gene responsible for sodium reabsorption (8).

In contrast, the autosomal dominant form shows a renal resistance to aldosterone, characterized by renal salt loss, hyperkalemia, metabolic acidosis, failure to thrive, elevated plasma renin activity (PRA), and elevated aldosterone levels in infancy. Patients can be treated with oral salt supplementation (9). Patients with the autosomal dominant form of PHA1 typically show a gradual clinical improvement with regard to renal salt loss during childhood. To date, the reason for this clinical improvement is not known. Some patients are clinically asymptomatic but may have elevated PRA and aldosterone levels (5). The autosomal dominant form, at least in some patients, is caused by mutations of the human mineralocorticoid receptor (MR) gene. The MR gene consists of nine exons, with a coding region spanning from exons 2–9, encoding for 984 amino acids (10). To date, six mutations associated with PHA1 have been described (Fig. 1; Refs. 11–13). Geller et al. (11) identified four mutations in the human MR gene: two frameshift mutations and one nonsense mutation in exon 2 and an intron 5 splice mutation. One missense mutation in exon 8 was found by Tajima et al. (12). Our group recently described another new frameshift mutation in exon 9 of the MR gene (13). The clinical improvement after infancy cannot be explained on the molecular level. Arai et al. (14) recently proposed that polymorphisms within the three ENaC gene subunits α, β, and γ may contribute to or deterriorate dominant PHA1 due to a negative influence on the salt conservation mechanisms.

Here, a novel MR gene mutation in a German family with autosomal dominant PHA1 is presented, resulting in a variable phenotype within the affected family members. The hypothesis concerning polymorphisms in the ENaC gene aggravating the renal salt loss was investigated in this family and reviewed. Here, we provide further evidence for a marked interindividual clinical heterogeneity of PHA1, suggesting the involvement of previously unidentified determinants in the etiology of this life-threatening disease usually manifesting in early infancy.
Patients and Methods

The male index patient was born from nonconsanguineous parents after an uneventful pregnancy by spontaneous vaginal delivery at term with a birth weight of 2500 g (–2.8 SD score). He was breast-fed until 4 months of age. Early psychomotor development was adequate. Failure to thrive was noticed at 4 months of age. He presented with a weight of 9.0 kg (–5.0 SD score) and a length of 57 cm (–3.0 SD score). No episodes of vomiting or diarrhea were observed. On examination, he had hypokalemia (127 mmol/liter) and hyperkalemia (5.8 mmol/liter), but no metabolic acidosis. Multitest-oxid analysis revealed elevated plasma aldosterone (1.73 ng/ml; normal reference range for age, 0.60–0.92 ng/ml) and precursor levels in the upper normal range. All other steroids including progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol, and cortisone were normal. PRA was also elevated (20.5 ng/ml/h; normal reference range for age, 1.5–10.2 ng/ml/h). Other parameters, including ACTH and plasma creatinine, were in the normal range. After acute therapy with iv saline substitution and fludrocortisone, the infant showed marked catch-up growth with an oral NaCl supplementation of 2.4 mmol/kg/d [after 17 months: length, 80 cm (–0.6 SD score); weight, 10.5 kg; body mass index, 16.4 kg/m2 (–0.6 SD score)]. Fludrocortisone administration was stopped after the mineralocorticoid receptor mutation was detected. No clinical deterioration was noticed after the discontinuation of mineralocorticoids. Plasma electrolytes remained normal under oral NaCl therapy. Accordingly, plasma aldosterone and PRA have normalized, to date. Both parents were clinically free of symptoms and had normal aldosterone and PRA levels. No events of prior electrolyte disturbances or inpatient treatments were recorded or collected by the parents. They both presented with a height within the normal limits for the German population (father, 176 cm; –0.6 SD; mother, 164 cm, –0.6 SD).

Analysis of plasma steroids was performed by RIA after extraction and HPLC, as described previously (15). ACTH levels and PRA were measured using standard procedures (Lumitest ACTH, Brahms Diagnostics, Berlin, Germany; and PRA RIA, Immuno-Biological Laboratories, Hamburg, Germany).

Blood samples for molecular genetic analysis were taken after informed consent. Genomic DNA was extracted from peripheral blood leukocytes, and the MR gene was amplified using 19 primer pairs as described previously (11). PCR was performed using the following conditions: initial denaturation at 94 C for 5 min; 35 cycles of denaturation at 94 C for 40 sec, annealing at 58 C or 65 C for 40 sec, and extension at 72 C for 40 sec; final extension at 72 C for 5 min. For the screening of ENaC polymorphisms, five segments of the human ENaC were amplified using five primer pairs as described previously (8). PCR conditions were: initial denaturation at 94 C for 5 min; 35 cycles of denaturation at 94 C for 30 sec, annealing at 58 C or 66 C for 30 sec, and extension at 72 C for 2 min; final extension at 72 C for 7 min. Before sequencing, PCR products were purified using the QiAquick-spin PCR purification kit (QIAGEN, Hilden, Germany). The nucleotide sequences of both strands of the PCR products were directly determined using an automated fluorescent sequencer (ABI Prism 310 or 373 Genetic Analyzer, Perkin Elmer Corp., Wellesley, MA). MR sequencing included all translated exons (2–9) of the MR gene and the exon/intron boundaries. PCR restriction analysis of a 251-bp fragment of exon 2 of the MR gene including the mutation was performed to verify the mutant MR allele. The primer pairs were described previously (11). A restriction enzyme digestion with Sau3AI (Roche, Mannheim, Germany) was carried out at 37 C for 2 h. ENaC sequencing included codons 663 of the α ENaC-subunit; codon 336 of the β ENaC-subunit; and codons 178, 502, and 614 of the γ ENaC-subunit and the according adjacent areas.

Results

Direct sequencing of PCR products of the ENaC gene revealed a nonconservative nucleotide substitution, c488C>G (S163X), in exon 2 (Fig. 2), according to the nucleotide numbering of Arriza et al. (10). This mutation leads to a premature stop codon, resulting in a truncated protein. The father, although he had no measurable salt loss, showed the identical heterozygous mutation of the MR gene as the index patient. The analysis of the maternal MR gene revealed a wild-type allele on both chromosomes. The mutant allele was detected by restriction enzyme digestion with Sau3AI (Fig. 2). Sau3AI digested the wild-type MR PCR fragment to a 221-bp and a 30-bp fragment. The Sau3AI restriction site was lost in the mutant allele (wild type, 221-bp and 30-bp fragments; mutant, 251-bp fragment).

Sequencing of PCR products of the ENaC gene revealed a nonconservative nucleotide substitution, 2086A>G (T663A), in the α ENaC-subunit in the homozygous state in the index patient and his father. The mother showed a heterozygous substitution, 2086A>G (T663A). The nucleotide substitutions 1006C>G (P336A) in the β ENaC-subunit, 554T>G (W178R), 1526C>G (P502A), and 1862T>G (S614A) in the γ ENaC-subunit were identified in the homozygous state in the index patient and both parents (Fig. 2). Both subjects carrying a mutation of the MR gene showed an identical ENaC haplotype regarding the previously reported polymorphisms (14).

Discussion

A novel nonsense mutation (S163X) in the MR gene in a German patient with a renal form of PHA1 was identified. The nonsense mutation is localized in the aminoterminal region of the MR gene in exon 2 (Fig. 1). Due to this localization, the complete DNA- and hormone-binding domain is lost (16) and, therefore, the phenotype of the index patient is identical to the phenotype of the patients reported by Geller et al. (11), with frameshift or nonsense mutations in exon 2.

The segregation analysis of the allele carrying the mutation S163X revealed the father as heterozygous for the identical nonsense mutation. However, he was clinically free of symptoms and had balanced plasma electrolytes and normal aldosterone and PRA levels. He has had no prior occasions of renal salt loss, in particular during infancy and childhood, and has never received inpatient treatment. Arai et al. (14) recently reported a significantly increased co-occurrence of MR and ENaC gene polymorphisms, neither of which alone causes PHA1. Thus, a possible explanation for the inconsistency of the clinical symptoms in the father and the patient may be a different configuration of polymorphisms of the ENaC gene within the family. Therefore, we screened for the
described ENaC polymorphisms that have been found to be associated with PHA1 (14). All described polymorphisms were detected in the homozygous state in the \(/H9251\), \(/H9252\), and \(/H9253\) subunits of ENaC in both the father and the patient. Therefore, in the investigated family these polymorphisms cannot be responsible for the differing phenotype. A digenic heredity of \(MR\) and \(ENaC\) gene variations leading to PHA1 cannot be supported in the cases described. As far as we can judge, the \(MR\) gene and the \(ENaC\) gene are identical in both individuals. It is possible that relevant changes within the promoter region or intronic sequences in the \(MR\) gene may have been missed. Furthermore, we can speculate that as yet unidentified genes could be responsible for the distinct phenotype of father and son. Such genes would code for regulatory factors involved in preserving renal salt homeostasis, independent of MR-mediated effects. These factors still have to be elucidated. A potential candidate may be NEDD4, an ubiquitin protein ligase controlling the number of ENaC at the cell surface (17, 18). The serine-threonine kinases WNK1 (19) and WNK4 (20), located in the distal nephron, may also contribute to the phenotype. Changes in \(PRSS8\) (prostasin; Ref. 21) and \(TMPSRSS2\) (22) expression may play a role in \(ENaC\) regulation in PHA1 (23).

In conclusion, our findings support the hypothesis that PHA1 is a genetically markedly heterogeneous disorder in which other, still unknown modifiers are probably involved (5, 13, 24–26). We are still far from a complete understanding of the pathophysiology of renal salt conservation and potential alternative rescue mechanisms of sodium homeostasis.

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