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Clinical Research Article

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A Unique Genotype of Pseudohypoaldosteronism Type 1b in a Highly Consanguineous Population

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Abbreviations: ENaC, epithelial sodium channel; KFSHRC, King Faisal Specialist Hospital & Research Centre; PCR, polymerase chain reaction; PHA, pseudohypoaldosteronism.

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Abstract

Context: Pseudohypoaldosteronism (PHA) is a condition in which serum aldosterone level is normal or elevated but its action is deficient.

Objective: This study describes the molecular genetics of PHA 1b in the highly consanguineous population of 2 Arabian Gulf countries, Saudi Arabia and Oman.

Methods: This study enrolled 22 patients from 13 unrelated families (2 families with 5 patients from Oman and 11 families with 17 patients from Saudi Arabia). All of these patients had presented within the first 10 days of life with nausea and vomiting, hyponatremia, hyperkalemia, and hypotension. We isolated DNA from peripheral blood and PCR-sequenced all exons and exon-intron boundaries of *SCNN1A* and, if negative, *SCNN1B* and *SCNN1G* using the Dideoxy Chain termination method.

Results: We found a total of 8 mutations in 13 families as follows: 6 mutations in *SCNN1A*, 1 in *SCNN1B*, and 1 in *SCNN1G*. All of these mutations were novel except one. *SCNN1A* mutations were: c.1496A>G, p.Q499R (novel) in 1 patient; c.1453C>T, p.Q485X (novel) in 1 patient; c.1322_1322delA, p.N441Tfs*41 (novel) in 2 patients of 1 family; c.876 + 2 delGAGT (novel) in 3 patients of 1 family; c.203_204 delTC, p.I68Tfs*76 (a known mutation) in 8 patients of 5 families; and whole *SCNN1A* gene deletion (novel) in 2 patients of 2 families. In addition, a nonsense *SCNN1B* mutation c.1694C>A, p.S565X (novel) was

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found in 3 siblings from 1 Omani family, and an *SCNN1G* deletion mutation c.527_528 delCA, p.T176Rfs*9 (novel) in 2 siblings from another Omani family.
Conclusion: We characterized a unique genotype of PHA 1b with several novel gene structure–disrupting mutations in *SCNN1A*, *SCNN1B*, and *SCNN1G* in a highly consanguineous population.

Key Words: pseudohypoaldosteronism, PHA, sodium epithelial channel, ENaC, *SCNN1A*, *SCNN1B*, *SCNN1G*

Aldosterone plays a critical role in sodium, potassium, and hydrogen ion homeostasis [1-3]. It acts at the renal distal convoluted tubule and collecting ducts through a complex chain of tubular cell channels and transporters [1-3]. Collectively, this action results in the reabsorption of sodium and excretion of potassium and hydrogen ions [1-3]. As a consequence of these actions, hyperaldosteronism typically presents with hypertension and hypokalemic metabolic alkalosis. Conversely, hypoaldosteronism is associated with sodium loss, volume depletion, hypotension, and hyperkalemic metabolic acidosis.

Pseudohypoaldosteronism (PHA) is a condition in which the clinical presentation suggests aldosterone deficiency, but serum aldosterone level is normal or frequently elevated [1, 4]. This is due to the loss of action of aldosterone rather than its synthesis [1, 5]. Although acquired forms may occur [6], most cases of PHA are hereditary in nature [1, 5]. There are 2 main variants, PHA 1 and PHA 2 [4, 5]. In turn, PHA 1 has 2 subtypes: PHA 1a (MIM#177735) is an autosomal dominant disorder that results from inactivating mutations of the mineralocorticoid receptor (*NR3C2*) [3, 5]. Mutations in this gene affect only the renal tubules. This disorder usually presents during the neonatal period with hyponatremia, volume depletion, hyperkalemia, and metabolic acidosis [1, 5]. Most patients improve with time and grow out of this condition by 2 years of age [1, 5]. On the other hand, PHA 1b (MIM #264350) is an autosomal recessive disorder secondary to inactivating mutations in any of the 3 subunits (α, β, γ) of the epithelial sodium channel (ENaC) on the luminal membrane of the distal convoluted tubules and collecting ducts [1, 4, 5]. It also presents with similar features to PHA 1a but is usually more severe and tends to have additional systemic manifestations, including cystic fibrosis–like respiratory symptoms and cutaneous manifestations [7-10]. This occurs due to the fact that ENaC is expressed and involved in the regulation of fluid and sodium in many other organs, including the lungs, colon, skin, and sweat and salivary glands [4, 11]. Furthermore, PHA 1b is usually a lifelong disorder and must be managed with salt and fluid supplementation and potassium restriction [5, 12, 13]. PHA 2 results from mutations in the *without-lysine kinase* 1 or 4 (WNK1 and WNK4). Additionally, it is associated with loss of aldosterone action with its usual manifestations of volume depletion, hyponatremia, hyperkalemia, and metabolic acidosis [14, 15].

PHA is rare [7, 12], and literature reports on this subject have been limited. In particular, there have been minimal data reported on the underlying genetics of PHA from Arab populations in whom consanguinity rates are high [16]. Thus, this report describes the largest series of patients with PHA 1b from Saudi Arabia and Oman. It presents their clinical, biochemical, and underlying mutations and provides an analysis of their genotype-phenotype correlations. To the best of our knowledge, this is also the largest series of patients with PHA reported in the literature and the only study involving this highly consanguineous Arab population. These findings show a unique genotype, with several novel mutations of different types, including missense, nonsense, splice-site mutations, and small and gross gene deletions.

Methods

The study was approved by the Institutional Review Board of the King Faisal Specialist Hospital & Research Centre (KFSHRC), Riyadh, Saudi Arabia. KFSHRC is a major tertiary care academic center, to which most cases of PHA and other rare genetic diseases are referred for management. Informed consents were obtained from the patients or their guardians. Five cases from 2 Omani families were diagnosed and managed in Oman, and their genetic testing was performed at KFSHRC.

Patients

This study included 22 patients from 13 families. Five patients came from 2 families from Muscat, Oman, and 17 patients came from 11 families from Saudi Arabia (Table 1). Ten were females, and 12 were males. All parents of the 22 patients were first-degree cousins. The clinical and biochemical features are summarized in Table 1.

Molecular Studies

Genomic DNA was extracted from peripheral leucocytes using the Gentra Puregene blood kit (Catalog #158389,

Table 1: Clinical and biochemical characteristics of 22 patients with PHA 1b

Clinical feature	No/total (%)
Age at presentation, Median (range) days	2.5 (1-10)
Current age, Median (range) years	12.5 (3-31)
Family history of PHA 1b	17/22 (77.3)
Vomiting and dehydration	21/22 (95.5)
Hypotension	11/22 (50)
Respiratory symptoms	16/22 (72.7)
Dermatitis	7/22 (31.8)
Urinary Tract infection	5/22 (22.7)
Polyuria	7/22 (31.8)
Current height below the mean, No. of patients	20/22 (91)
Median height SD below the mean	−1.5 (−0.4 to −3.0)
Current Weight below the mean, No. of patients	17/22 (77.3)
Median Weight SD below the mean	−1.5 (−0.8 to −3.0)
Biochemical values*	**Median (Range)**
Lowest Serum Na (mmol/l)	122 (110-132)
Lowest serum Cl (mmol/l)	88 (81-108)
Lowest serum HCO3 (mmol/l)	15 (10-18)
Highest Serum K (mmol/l)	7.5 (4.2-13)
Serum renin, mU/l	241 (52-832)
Serum aldosterone, pmol/l	7078 (751-11980)

Normal ranges: Na 135-145 mmol/l; Cl 95-105 mmol/l; HCO3 22-26 mmol/l; K 3.5-5.0 mmol/l; Renin 4.4-46 mU/l, Aldosterone 48-643.5 pmol/l.

Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. All exons and exon-intron boundaries of the underlying genes were amplified by polymerase chain reaction (PCR). The primers used had been previously published [10, 17-19]. Amplification was carried out in a total volume of 25 mL, containing 100 ng of genomic DNA, 0.2mM of each deoxynucleotide triphosphates (dNTPs), 1X PCR buffer: 20mM Tris-HCl (pH 8.4) and 50mM KCl, 1.5mM MgCl2, 0.2mM of forward primer, 0.2mM of reverse primer, and 2 units of Taq DNA Polymerase (Catalog #10,966e018, Platinum Taq DNA Polymerase, Invitrogen). Next, the volume was increased to 25 mL using nuclease-free water. The PCR Thermal Cycler (Catalog #4,375,786, Veriti 96-Well Thermal Cycler, Applied Biosystems) was set to appropriate cycling conditions based on the length of the fragment and the Tm of the primers. Beyond this, PCR conditions for different genes have been described in previous studies. The amplicons were resolved by 2% agarose gel (Catalog #16500-500, UltraPure Agarose, Invitrogen). Successfully amplified fragments were directly sequenced in forward and reverse directions using an ABI PRISM BigDye Terminator V3.1 Cycle Sequencing Reaction Kit (Catalog #4337455, Applied Biosystems, Foster City, CA 94404, USA) and an ABI PRISM® 3730XI Genetic Analyzer (Catalog #3730S, Applied Biosystems, Foster City, CA 94404, USA). The sequence variations were analyzed and compared with those in standard databases (eg, ensemble, NCBI, HGMD) to ascertain whether each change was a polymorphism, a novel mutation, or a previously reported mutation. When samples were available for parents and siblings, targeted PCR amplification and sequencing of the exon/intron with potential mutation was carried out to determine mutation heterozygosity in unaffected relatives and homozygosity in other affected relatives. In silico analysis using Polyphen2, Mutation Taster, PROVEAN, and/or SIFT estimated the likely significance of the genetic variant. Next, a search was conducted for detected variants in the Human Gene Mutation and the ClinVar databases. Reported mutations in these databases were considered to be confirmed, and those that were not reported there were considered to be potentially novel. Thus, this study directly assessed the changes these variants produced and their potential pathogenicity depending on amino acid variations and gene and protein location. In addition, this research examined the conservation of the amino acid among species. The American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines were followed in the designation of the novel variants [20].

Results

Clinical and Biochemical Features

As seen in Table 1, patients exhibited symptoms soon after birth. For instance, nausea and vomiting were almost universal among the patients, and hypotension was also common, occurring in 11/22 patients (50%). In addition, respiratory symptoms were prevalent, occurring in 16 patients (72.7%) and dermatological manifestations occurred in 7 cases (31.8%). Growth retardation was quite common; the heights of 20 patients (91%) were below the mean for age with a median standard deviation of −1.5 below the mean (range, −0.4 to −3.0). The heights of the other 2 patients were on the mean for age. Similarly, the weights of 17 patients (77.3%) were below the mean for age with a median standard deviation of −1.5 below the mean (range, −0.8 to −3.0) (Table 1). Five patients had urinary tract infections. Their symptoms of PHA were present before and persisted after they were cured from the infection.

Beyond this, hyponatremia, hyperkalemia, metabolic acidosis, hyperreninemia, and hyperaldosteronism of variable severities were hallmarks of the biochemical profile of these patients (Table 1). The patients' management consisted of generous doses of sodium chloride (NaCl2), sodium bicarbonate ($NaHCO_3$), and oral sodium polystyrene

sulfonate resin (Kayexalate). Except for 1 patient who was able to stop medications at 6 years of age, all of the other patients continue to need active management with large doses of NaCl2, $NaHCO_3$, Kayexalate, and potassium restriction.

Molecular Genetics

This study revealed a total of 8 mutations in the 13 families examined (Table 2 and Fig. 1); all but 1 of these mutations were novel. These mutations included the following: 6 mutations in *SCNN1A*, 1 in *SCNN1B*, and 1 in *SCNN1G*. The *SCNN1A* mutations included a novel missense mutation (c.1496A>G, p.Q499R) in 1 patient; a novel nonsense mutation (c.1453C>T, p.Q485X) in 1 patient; a novel single nucleotide deletion frameshift mutation (c.1322_1322delA, p.N441Tfs*41) in 2 patients of 1 family; a novel 4-base splice-site deletion (c.876 + 2delGAGT [IVS3 + 2delGAGT]) in 3 patients of 1 family; a previously described 2-base deletion frameshift mutation (c.203_204delTC, p.I68Tfs*76) in 8 patients of 5 families; and a novel whole gene deletion mutation (exons 1-12) in 2 patients of 2 unrelated families from the same region of Saudi Arabia (Fig. 2). A novel nonsense *SCNN1B* mutation (c.1694C>A, p.S565X) was found in 3 siblings from 1 Omani family. In addition, another novel deletion *SCNN1G* mutation (c.527_528 delCA, p.T176Rfs*9) was found in 2 siblings from another Omani family (Table 2).

Genotype-Phenotype Correlation

As shown in Tables 1 and 3, all of the patients had severe to very severe illnesses at the time of initial presentation and in the first few years of their lives. As time passed, however, all of them showed stability and improvement in health. Table 3 summarizes the main clinical and biochemical features of the 8 mutations described in this study. As shown, the missense mutation c.1496A>G, p.Q499R was found in 1 patient (A1) who presented with vomiting, dehydration, and polyuria but no respiratory or dermatological manifestations. Although her growth seems to be significantly affected, the electrolyte disturbances were of mild to moderate severity. The nonsense mutation c.1453C>T, p.Q485X also was detected in 1 patient (B1) who had relatively mild clinical and biochemical manifestations, although aldosterone and renin were significantly elevated. The deletion/frameshift mutation c.1322_1322delA, p.N441Tfs*41 was detected in 2 siblings (C1 and C2) and was associated with severe hemodynamic and respiratory symptoms and significant electrolyte and hormonal changes. The splicing site mutation IVS3 + 2delGAGT, c.876 + 2delGAGT was detected in 3 siblings (D1, D2, and D3) and also was associated with severe hemodynamic, respiratory, and dermatological symptoms and significant electrolyte disturbances (Table 3). The c.203_204delTC, p.I68Tfs*76 deletion/frameshift mutation was the most commonly detected mutation occurring in 8 patients from 5 families (E1, E2, E3, F1, G1,G2, H1, L1) who presented with severe hemodynamic, respiratory, and dermatological manifestations and significant electrolyte and hormonal changes. Two patients (J1, K1) had whole *SCNN1A* gene deletion and had severe illness and very severe electrolyte and hormonal changes (Table 3). The single nonsense mutation in *SCNN1B* was detected in 3 siblings (L1, L2, L3) and was associated with moderate symptoms but severe electrolyte changes and the single deletion mutation in *SCNN1G*, c.527_528delCA, p.T176Rfs*184 was present in 2 siblings (M1, M2) and

Table 2. Genetic alterations found in 13 families (22 patients) showing that 7 out of 8 mutations found are novel

Family*	No. of affected siblings	Gene	Mutation (nucleotide)	Mutation (protein)	Novelty	ACMG classification
A	1	*SCNN1A*	c.1496A>G	p.Q499R	Yes	Likely pathogenic
B	1	*SCNN1A*	c.1453C>T	p.Q485X	Yes	Pathogenic
C	2	*SCNN1A*	c.1322_1322delA	N441Tfs*41	Yes	Pathogenic
D	3	*SCNN1A*	IVS3 + 2delGAGT (c.876 + 2delGAGT)		Yes	Pathogenic
E, F, G, H, I	3, 1, 2, 1, 1	*SCNN1A*	c.203_204delTC	p.I68T fs*76	No	Pathogenic
J, K	1, 1	*SCNN1A*	Whole gene deletion		Yes	Pathogenic
L	3	*SCNN1B*	c.1694C>A	S565X	Yes	Pathogenic
M	2	*SCNN1G*	c.527_528delCA	T176Rfs*184	Yes	Pathogenic
Total	22					

Abbreviation: ACMG, American College of Medical Genetics and Genomics.
*All parents of the 13 families are first-degree relatives

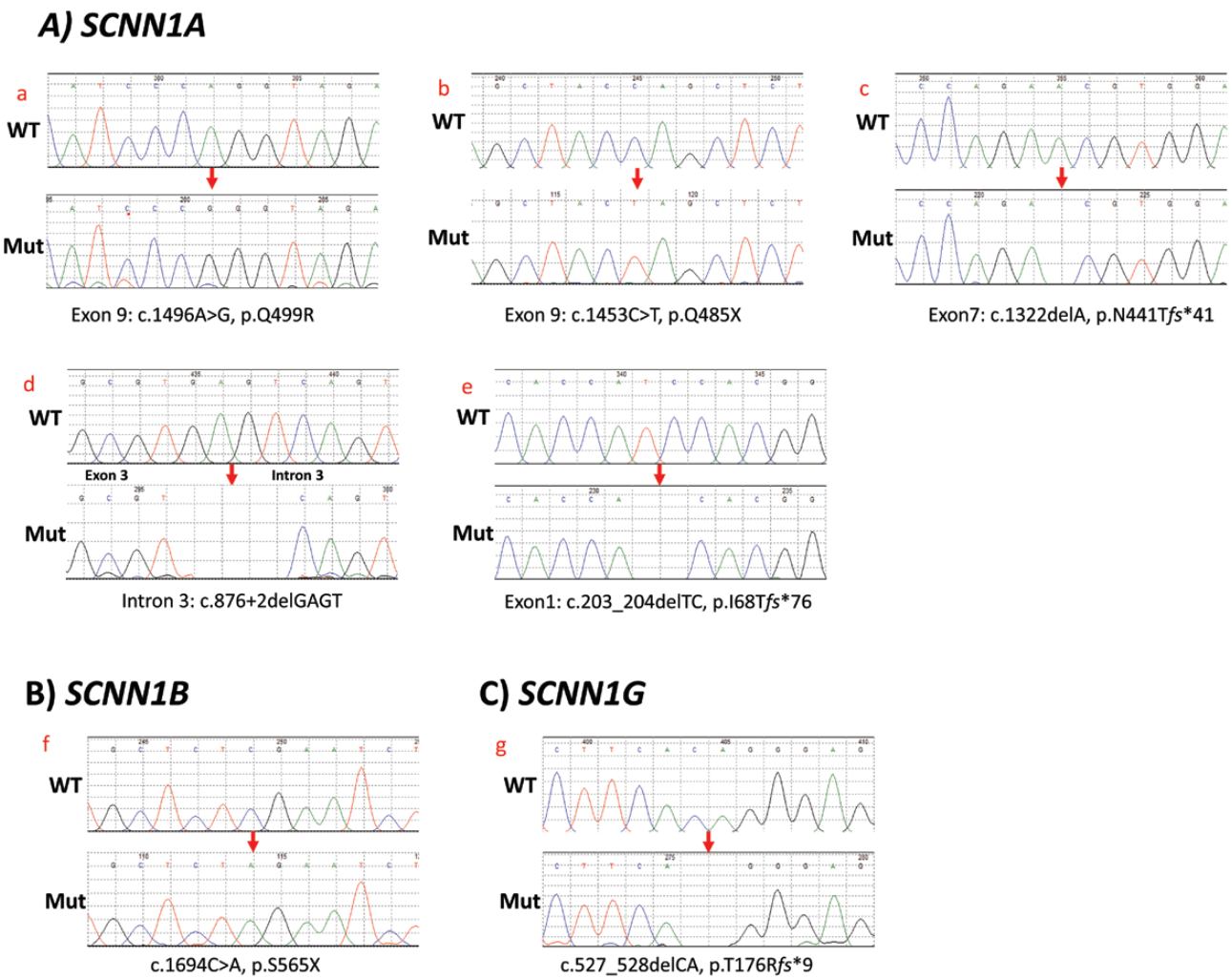

Figure 1. The sequence chromatograms showing different *SCNN1A* mutations found in this study. A) *SCNN1A*: a is a missense mutation found in Family A; b is a nonsense mutation found in Family B; c is a deletion mutation found in Family C; d is a splice-site mutation found in Family D; e is a deletion mutation found in Families E, F, G, H, I. B), f is an *SCNN1B* nonsense mutation in Family L; and C), g is an *SCNN1G* deletion mutation in Family M.

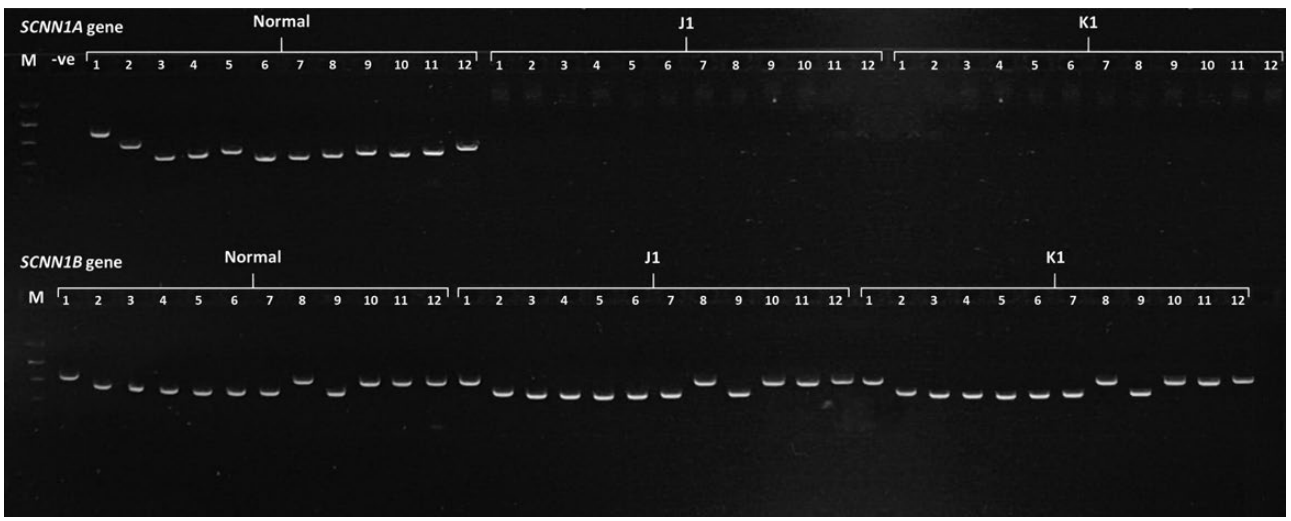

Figure 2. Gel electrophoresis of PCR of exons 1-12 of *SCNN1A* showing successful amplification of the normal control but failure to amplify any of exons 1-12 in patients J1 or K1. A simultaneous PCR using the same master mix for *SCNN1B* successfully amplified all exons in the normal control, as well as patients J1 and K1. This indicates that *SCNN1A* was deleted in patients J1 and K1. This experiment was repeated 3 times.

associated with severe symptoms and moderate electrolyte and hormonal changes (Table 3).

Discussion

This study characterized the molecular genetics of a large series of patients with the rare disorder PHA 1b. To our best knowledge, this is the largest series of patients with PHA 1b reported so far in the literature and the first of its kind from the Arab world. These findings revealed a significant number of novel mutations in *SCNN1A*, *SCNN1B*, and *SCNN1G*, suggesting a unique genotype of this disease in this highly consanguineous population. The most common mutation among these patients was p.I68Tfs*76, which was not novel [17]. This mutation affected 8 patients from 5 unrelated families from one region (Eastern region) of Saudi Arabia (Table 2). Thus, this might be a founder mutation in this region. Interestingly, the first and only report of this mutation was published in 1996 and included 3 Saudi patients carrying this mutation (p.I68T FS*76) from the same region of Saudi Arabia as these current patients [17].

The only missense mutation in this study was c.1496A>G, p.Q499R, found in patient 1 (Table 1); this mutation had not been reported before. It was found in neither the 1000G nor the ExAC databases and not in the Saudi Genome Project, in which the whole-exome sequences of more than 5000 members of the Saudi population are recorded (https://shgp.sa). This mutation was predicted to be disease-causing by the Mutation Taster and probably damaging by PolyPhen2 (score = 0.97). It changes the last amino acid of *SCNN1A* exon 9 from the highly conserved glutamine to arginine (Q499R) and could also interfere with the splicing of exon 9 with exon 10 since it involves the last codon of exon 9. This was the only missense mutation in this series. All of the other patients had mutations that disrupted the gene structures, including deletion with frameshift and truncation, splice-site mutations, and gross whole gene deletion (Tables 1 and 2, Figs. 1 and 2). These gene structure–altering mutations are very likely to interfere with the normal function of the encoded protein subunits of SCNN1A to cause disease. In this respect, these findings were consistent with those of previous studies showing that in PHA 1b, most mutations in the

Table 3. Genotype-phenotype correlation of the 8 mutations found in this series of 22 patients with PHA 1b

Gene mutation	No. of patients/ families	Clinical manifestations	Height (–SD)	Na, mmol/L	CO_2, mmol/L	K, mmol/L	Renin, mU/L	Aldosterone, pmol/L
SCNN1A c.1496A>G, p.Q499R	1/1	Vomiting, dehydration, polyuria	–2.7	129	15	6.2	121	3208
SCNN1A c.1453C>T, p.Q485X	1/1	Vomiting, dehydration	–0.4	130	16	6.0	247	7510
SCNN1A c.1322_1322delA, p.N441Tfs*41	2/1	Vomiting, dehydration, hypotension, respiratory symptoms	–1 to –2.4	121-122	11-16	6.9-7.0	499-832	6669-11 980
SCNN1A IVS3 + 2delGAGT, (c.876 + 2delGAGT)	3/1	Vomiting, dehydration, hypotension, respiratory symptoms, dermatitis, polyuria, UTI	–1 to –1.5	127-129	17-18	7.5-8.0	53-170	5580-8450
SCNN1A c.203_204delTC, p.I68Tfs*76	8/5	Vomiting, dehydration, hypotension, respiratory symptoms, and dermatitis	0 to –2.0	110-130	11-15	6-13	74-274	751-10 740
SCNN1A Whole gene deletion	2/2	Vomiting, dehydration, hypotension, respiratory symptoms, arrhythmias,	–1.8 to –2.5	115-118	14-16	8.7-11	530	2068-7500
SCNN1B c.1694C>A, p.S565X	3/1	Vomiting, dehydration, dermatitis	0 to –3	116-132	15-18	4.8-8.5	50-184	2340-3300
SCNN1G c.527_528delCA, p.T176Rfs*184	2/1	Vomiting, dehydration, respiratory symptoms, and dermatitis	–1 to –1.5	129-131	10-18	5.8-5.9	118-208	1659-2775

When there is more than 1 patient, the numbers represent the range of values.

SCNN1A, *SCNN1B*, and *SCNN1G* tend to be deletions, splice-site, or nonsense mutations [7].

Notably, there appeared to be clustering of certain mutations in some regions of the country. For example, the *SCNN1A* mutation p.I68T fs*76 occurred in 5 families from the Eastern Region of Saudi Arabia. In addition, the entire *SCNN1A* deletion occurred in 2 unrelated families from AlMadina AlMunawarah in the West North side of Saudi Arabia. Although no relationship is known between these families at present, it is possible that these mutations are founder mutations or that there exists an unknown relationship between the ancestries of some of these families. Mutations in *SCNN1B* and *SCNN1G* were not found in Saudi Arabia but found in the 2 Omani families included in this study.

Furthermore, most of the mutations in this series were novel. This reflects a unique genotype of PHA 1b in this region, which might be related to the population's homogenous structure and its high rate of consanguinity [16]. Beyond this, it likely reflects a high rate of undiscovered mutations due to the rare nature of PHA 1b, since most previously reported studies were either single-case reports or limited case series due to the rarity of PHA. There have been only a couple of reports of PHA from Arab world: Attia and Marzouki reported a young female neonate who presented with severe hyperkalemia and hyponatremia, developed cardiac arrest, and was found to have PHA [21]. She was initially thought to have congenital adrenal hyperplasia but did not respond to glucocorticoids and mineralocorticoids and was later diagnosed as PHA. This diagnosis was based on clinical and biochemical findings, but no genetic testing was conducted [21]. Alshaikh described 2 Omani siblings who had PHA 1b and presented in the first week of life with nausea, vomiting, hypotension, and hyperkalemic metabolic acidosis. They also had respiratory and cutaneous symptoms and were found to have a novel missense *SCNN1A* mutation (c.385G>A, p.Gly129Ser) [22].

Moreover, the genotype-phenotype correlation in PHA 1b has not been well described. Although there were some variations in this study, the affected patients tended to have similar clinical and biochemical features, and their diseases were severe to very severe in the first few years after their diagnoses, followed by variable but noticeable improvements. However, only 1 patient was able to discontinue his treatments at the age of 6 years (patient H1). Although the data do not support definite genotype-phenotype correlation, single-point mutations were generally more likely than others to present with less-severe disease and to show improvement over time. Patients with gene structure–disrupting mutations, including deletion with frameshift and truncation, gross deletions, and nonsense mutations, tended to have a severe disease at the time of diagnosis and exhibited less improvement over time but continued to suffer from significant illness (Table 3). In some mutations, there was also some dichotomy between the clinical manifestations and electrolyte and hormonal changes with severe symptoms but only moderate electrolyte and hormonal changes in some mutations and vice versa (Table 3). In a recent study from the UK, 12 patients with genetically confirmed PHA 1 were studied [9]. Four of these patients had PHA 1a with mutations in *NR3C2*, and 8 had PHA 1b with *SCNN1A* and *SCNN1B* mutations. The clinical and biochemical features overlapped with each other, and the phenotypes could not predict the genotypes. However, patients with missense mutations of *SCNN1A* and *SCNN1B* experienced less marked rises in serum aldosterone [9]. Cayira et al recently reported 3 cases of PHA 1b with 4 novel variants in *SCNNA1* and *SCNN1B* in Turkey, a country not far from the Arab Gulf region [12]. These mutations included 2 novel pathogenic variants (c.87C>A[p.Tyr29*]/IVS9 + 1G>A [c.1346 + 1G>A]) in *SCNN1B* in one case; a novel homozygous pathogenic variant (p. His69Arg[c.206A>G]) in *SCNN1A* in another case with milder illness; and a homozygous, 1-base duplication, p.A200Gfs*6 (c.598dupG), in *SCNN1A* in a third case [12]. They concluded that although missense mutations have been reported in only a few cases, they seem to result in milder disease phenotypes [12]. In 2005, Edelheit et al reported 4 patients with PHA 1b and reviewed the literature on the subject [13]. The total number of ENaC mutations reported by that time (2005) was 22, including mutations in their 4 patients. Nineteen of these 22 mutations were mutations that interfered with the mRNA/protein length and included deletions, insertions, and splice-site mutations [13]. The patients with these mutations consistently presented with a severe form of the systemic PHA 1b. The other 3 patients had missense mutations associated with milder diseases than the other 19 patients [13]. Elsewhere, Hanokugolu et al studied 4 patients with PHA 1b over many years [8]. One had a compound heterozygous mutation with a monoallelic missense mutation (Gly327Cys), combined with another nonsense mutation. The other 3 patients had gene length–changing mutations (nonsense and splice-site mutations). The patient with a missense mutation had a milder disease, and his plasma renin and aldosterone values decreased over time, whereas the other patients continued to experience severe diseases, with increasing plasma renin and aldosterone values over time [8]. Thus, the literature and our study do not show clear genotype-phenotype correlation and there is an overlap in the severity of the clinical features, electrolyte disturbances, and aldosterone and renin levels between different types of mutations.

This study had strengths and weaknesses. To this research team's knowledge, this is the largest series of PHA 1b that has been reported in the literature. Thus, it provides a comprehensive characterization of PHA 1b in a substantial number of patients/families from a homogeneous and highly consanguineous population and adds significantly to the repertoire of ENaC mutations. It revealed a unique genotype characterized by many pathogenic gene structure–disrupting novel mutations (Table 2). Although this study did not conduct functional studies of the novel mutations found here, the mutations were mostly gene structure–altering mutations, including small and large frameshift deletions, splice-site mutations, and nonsense truncating mutations. By significantly altering their gene structures, these mutations are likely to disrupt the normal function of these genes, causing diseases. Only one mutation was a missense mutation and it was predicted by in silico analysis to be highly pathogenic. In addition, it changes the last codon of exon 9 and is likely also to interfere with splicing of exons 9 and 10, disrupting the structure of the gene.

In summary, this study examined the largest series of patients with PHA 1b from 2 Arab Gulf countries, along with their clinical and biochemical profiles. These genetic profiles were characterized by many novel genetic alterations, mostly deletion mutations but including splice-site, whole gene deletion, and nonsense mutations as well. The genotype-phenotype correlation is generally nonconclusive but suggests milder disease severity and a better prognosis in missense mutations and more severe disease and lower degree of improvement in gene structure–disrupting mutations.

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Additional Information

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Disclosures: None of the authors have any conflict of interest to declare.

Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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