Functional polymorphisms affecting the clinically important arginine-137 residue of AVPR2 do not influence serum sodium concentration at the population level

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Functional polymorphisms affecting the clinically important arginine-137 residue of AVPR2 do not influence serum sodium concentration at the population level. Physiol Genomics 45: 210–216, 2013. First published January 29, 2013; doi:10.1152/physiolgenomics.00161.2012.—The protein product of the AVPR2 gene, coding for the arginine vasopressin receptor type 2, is essential for vasopressin-dependent concentration of the urine. The arginine residue at position 137 in the protein product of this gene is uniquely pivotal for function. The R137H mutant inactivates the receptor conferring congenital nephrogenic diabetes insipidus, whereas activating mutations at this same residue (i.e., R137C and R137L) confer pathological water retention in the nephrogenic syndrome of inappropriate antidiuresis. These mutations were discovered in human subjects with conspicuous phenotypes in clinical water balance. Prevalence of these polymorphisms among asymptomatic individuals has not been assessed, nor has their contribution to broad interindividual variation in serum sodium concentration; no data addressing minor allele frequency are available. We genotyped two large cohorts using a validated high-throughput Pyrosequencing-based assay that we designed to capture the totality of genetic variation. Even at the population level, the AVPR2 gene on the X-chromosome, and participants were oversampled at the extremes of the population distribution for serum sodium concentration. In the Offspring Cohort of the Framingham Heart Study, male and female participants were genotyped. No pathological variants affecting R137 were detected among the 5,142 individuals has not been assessed nor has its contribution to interindividual variation in serum sodium concentration or to its heritability.

osmoregulation; arginine vasopressin; hyponatremia; hypernatremia

DISORDERED WATER BALANCE is reflected in the serum sodium concentration; a water excess (relative to extracellular electrolyte concentration) confers hyponatremia, whereas relative water deficit gives rise to hypernatremia. These dysnatremias are frequently encountered in clinical practice. Hyponatremia, for example, is the most common electrolyte abnormality affecting hospitalized patients (1). Severe dysnatremias can be lethal; however, even modest changes in serum sodium concentration cause reversible defects in cognition and coordination (25, 31). Water balance is regulated by water intake and water excretion. The former is dictated by thirst, whereas the latter is governed by arginine vasopressin (AVP) release from the posterior pituitary, and AVP-dependent function of the aquaporin-2 apical water channel in the kidney collecting duct (reviewed in Ref. 22). Both phenomena respond to the central osmosensing nuclei of the hypothalamus.

The protein product of the AVPR2 gene, the vasopressin receptor type 2, is essential for vasopressin-dependent concentration of the urine when water conservation is physiologically required. The arginine residue at position 137 in the protein product of this gene is uniquely pivotal for function. Mutation to histidine inactivates the receptor, conferring congenital nephrogenic diabetes insipidus and the attendant inability to concentrate the urine (4). The high output of dilute urine necessitates very high oral intake of hypertonic fluids to prevent lethal hypernatremia (relative water deficit). In contrast, activating mutations at this same residue (i.e., to either cysteine or leucine) cause an inability to shut off water conservation. The resultant pathological water retention (and accompanying hyponatremia) constitutes the nephrogenic syndrome of inappropriate antidiuresis (11). These mutations were discovered in human subjects with conspicuous phenotypes in clinical water balance, either hyponatremia or polyuria/hypernatremia. Prevalence of these polymorphisms among asymptomatic individuals has not been assessed nor has its contribution to interindividual variation in serum sodium concentration. Specifically, there are no allelic frequency data available in the public domain for any of these variants (dbSNP; e.g., http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=104894756; accessed 7/2/12).

The R137H inactivating mutation of AVPR2 (read as “arginine-to-histidine mutation at residue 137”) in this naming convention, and as used throughout this manuscript) was first described by Bichet et al. (4) in members of the Utah-based Q2 kindred affected with diabetes insipidus (5). In a heterologous expression model, the R137H-mutant receptor bound vasopres-
sin with high affinity but failed to activate downstream adenylyl cyclase (29). Although a subsequent study showed near-total retention of the mutant receptor in the cell interior (in contrast to expression at the plasma membrane), reduced agonist-inducible activation of G\textsubscript{i} adenyl cyclase system was confirmed (30). Barak et al. (2) attributed the constitutive intracellular localization to upregulation of the native arrestin-dependent receptor desensitization pathway; mutation of residues essential for the receptor-arrestin interaction restored plasma membrane localization. Therefore, multiple molecular mechanisms are potentially operative in R137H-mutant AVPR2 protein loss of function.

Activating mutations of AVPR2 affect this same arginine residue. Feldman et al. (11) reported two unrelated male infants presenting with central nervous system findings (irritability or seizures) and hyponatremia (serum sodium concentration of 118 and 123 meq/l). Each was hemizygous for a different mutation affecting R137 of AVPR2 (R137C and R137L). (AVPR2 resides on the X-chromosome; hemizygosity in males is conceptually akin to the homozygous mutant state in females.) Clinically, these mutations give rise to the syndrome of inappropriate antidiuresis Type D (8, 11, 12), wherein AVP levels are very low or undetectable (9), and individuals with these variants may be insensitive to the effects of vasopressin receptor antagonists (8). When transiently transfected into COS7 cells, the pathogenic variants conferred markedly elevated basal levels of adenylyl cyclase activity relative to the wild-type receptor, as measured via a cAMP-responsive reporter gene driven by tandem CRE (cAMP-response) elements. The effect of vasopressin stimulation was not tested, nor was receptor subcellular localization. Both the clinical phenotype(s) and the available biochemical data supported activating mutations. From the limited data available, it appears that female carriers may be either normonatremic or hyponatremic at baseline; they may also be predisposed to hyponatremia after water-loading (8, 11, 12).

An additional nonsynonymous polymorphism affects this arginine residue, the R137G variant of AVPR2. In contrast to the above, there are neither clinical nor biochemical/functional data assessing the impact of this mutation/polymorphism in any context. It appears in public databases (e.g., dbSNP); however, there is no source cited for these data.

Therefore, the R137 residue in the AVPR2 gene product is one of the most pivotal in the regulation of systemic water balance in humans, with variants conferring both hypernatremic and hyponatremic clinical phenotypes. In addition, treatment strategies are being conceived based upon the unique mechanisms that confer altered water homeostasis (35). However, virtually nothing is known about the prevalence (i.e., allelic frequency) of these variants at the population level and at the population extremes of serum sodium concentration. We sought to establish whether any of the four known nonsynonymous polymorphisms affecting the R137 residue of the AVPR2 protein influence serum sodium concentration at the population level and whether they are likely to contribute to disordered water balance in asymptomatic individuals with relatively high or low serum sodium concentration.

**METHODS**

**Genotyping Approach**

AVPR2 polymorphisms affecting R137. For single nucleotide polymorphism (SNP) rs104894756 (encompassing the R137H and R137L variants), there are individual occurrences reported in the National Center for Biotechnology Information Variation Viewer for the AVPR2 gene (http://www.ncbi.nlm.nih.gov/sites/variant?gene=554) referencing OMIM (Online Mendelian Inheritance in Man) and Omi- cia. The R137H variant is referenced under OMIM 304800 and was first described by Bichet et al. (4); the R137L variant was described by Feldman et al. (11). For rs104894761 (encompassing R137C and R137G), there are individual occurrences referring OMIM, Omicia, and Correlagen. The R137C variant was first described by Feldman et al. (11). There are no estimates of minor allele frequency for any. For the unpublished R137G variant of rs104894761, there are three independent observations listed in the online data set at dbSNP; however, the source is not described or referenced.

**Pyrosequencing assay design.** We devised an assay to capture the major genetic variants at R137 (PyroMark Assay Design (v 2.0; Qiagen), see Table 1]. These include the congenital nephrogenic diabetes insipidus-associated variant (R137H), the recently described variants associated with the nephrogenic syndrome of inappropriate antidiuresis (R137C and R137L, Ref. 11), and the R137G of unknown significance. We designed the assay to capture the totality of heterogeneity reported to affect the codon representing amino acid R137. This includes C/G/T (”B” in IUPAC code) at position 1 and A/G/T (“D” in IUPAC code) at position 2 of the codon corresponding to R137. Because there are no data available with respect to the haplotype structure in the vicinity of the R137 sequence variants, degeneracy was engineered into the amplification and Pyrosequencing steps so that the presence of a known SNP in close proximity to R137 would not preclude amplification and/or analysis. Primers were designed to minimize overlap with known AVPR2 SNPs. Primers were as follows: 1) forward primer (AVPR2_R137_F2_mix) TGTGTGGGCGTGATGAGTG, 2) reverse primer (AVPR2_R137_R3B) GGTACGCCAGCATGGGAC, and 3) sequencing primer (AVPR2_R137_S1_mix) GMCATGACGCTGGAC. Primer AVPR2_R137_R3B was 3’-biotinylated and PAGE-purified.

<table>
<thead>
<tr>
<th>Variant</th>
<th>SNP ID</th>
<th>Phenotype</th>
<th>Ref.</th>
<th>mAF</th>
<th>Codon</th>
<th>Residue at position 137</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R137C</td>
<td>rs104894761</td>
<td>nephrogenic syndrome of inappropriate antidiuresis</td>
<td>Feldman (2005)</td>
<td>unknown</td>
<td>TGC</td>
<td>Arg</td>
</tr>
<tr>
<td>R137G</td>
<td>rs104894761</td>
<td>unknown</td>
<td></td>
<td></td>
<td>GGC</td>
<td>Cys</td>
</tr>
<tr>
<td>R137L</td>
<td>rs104894756</td>
<td>nephrogenic syndrome of inappropriate antidiuresis</td>
<td>Feldman (2005)</td>
<td>unknown</td>
<td>CTC</td>
<td>Leu</td>
</tr>
<tr>
<td>R137H</td>
<td>rs104894756</td>
<td>congenital X-linked nephrogenic diabetes insipidus</td>
<td>Bichet (1993)</td>
<td>unknown</td>
<td>CAC</td>
<td>His</td>
</tr>
</tbody>
</table>

Single nucleotide polymorphism (SNP) ID (”rs” number) is shown, as is minor allele frequency (mAF). Codon represents the genotype for the codon corresponding to R137 in the wild-type allele; the new amino acid introduced by this variant is shown (residue at position 137).
To validate the assay, several complementary approaches were used. First, the assay was tested with anonymized human genomic DNA to confirm that the major human allele (R137) could be detected. The variant was readily detected in both the heterozygous (female) and hemizygous (male) state (Fig. 1). Then, site-directed mutagenesis was used to engineer the variants into anonymous (female) and hemizygous (male) state. The variant was readily detected in both the heterozygous and hemizygous state. The 3rd tracing (R137C/-) is anonymized DNA from a male participant hemizygous for the R137C mutation. The genotype is correctly called as T/T G/G (i.e., Y in the 1st position of the codon, in IUPAC code), confirming detection of the variant and correct assignment of the hemizygous state. The 2nd tracing is from an anonymized female donor), there are 2 copies of the wild-type allele (R137/R137), and the genotype is C/C G/G (i.e., homozygous wild-type at the 1st and 2nd positions of the R137 CGC codon). The 2nd tracing of the genomic DNA with a small amount of wild-type DNA. The 4th tracing (Water) is a control using water instead of genomic DNA; no amplicon is generated, and, because there is no product, there is no resultant sequence or called genotype.

**Populations for Genotyping**

Osteoporotic Fractures in Men (MrOS) study. MrOS was designed to assess the determinants of fracture in 5,994 healthy community-dwelling U.S. male subjects over 65 yr of age (23). Subjects were recruited from six geographically diverse centers (6). One center was excluded from this analysis because participants had provided only limited informed consent. Serum sodium and creatinine were measured on a single instrument using thawed previously frozen serum (Clinical Laboratory, Portland V.A. Medical Center); values were obtained for 5,527 and 5,533 subjects, respectively (32). AVP was not measured, and no water balance testing was performed. All participants were male. Genomic DNA was subjected to phi29-based whole genome amplification (Repli-G, Qiagen) to provide sufficient material for analysis. There were 398, 372, and 308 samples (and corresponding phenotypic information) available for analysis in the hyponatremic, normonatremic (“mean”), and hypernatremic strata, respectively (Table 2). The Hypo (hyponatremic) sodium group consisted of participants with serum sodium concentration corresponding to the lowest decile of the sample population following stratification on sex; serum sodium concentration ranged from 116 to 138 meq/l. Correspondingly, the Hyper (hypernatremic) sodium group consisted of participants with serum sodium concentration in the highest decile, with
RARE POLYMORPHISMS AFFECTING ARGININE-137 OF AVPR2

Values ranging from 145 to 153 meq/l. These deciles at the population extremes for serum sodium concentration corresponded to values $>-1.5$ SD units below or above the population mean for the MrOS population. Mean serum sodium concentration for the MrOS population was $141.38 \pm 2.66$ meq/l (mean $\pm$ SD); for the Mean group, we selected every third participant when participants with serum sodium concentration of either 141 or 142 meq/l were ordered by serum sodium concentration, and then by coded alphanumeric identifier (sodium concentrations were “binned” as integers at the time of reporting by the clinical laboratory). All three groups (Hypo, Hyper, and Mean) were included for analysis because an R137 polymorphism needn’t be synonymous with dysnatremia; patients with diabetes insipidus can maintain normal or near-normal serum sodium concentration provided thirst and access to water are intact, and patients with impaired water excretion may manifest varying or even subclinical degrees of water excess. Subjects were excluded as follows: 1) informed consent on file with parent study not compatible with investigation of this phenotype; 2) non-Caucasian race; 3) glucose $\geq 150$ mg/dl; or 4) estimated glomerular filtration rate $>2$ SD below the population mean (i.e., Cr $>1.3$). Both of the latter conditions associate with reduced serum sodium concentration and/or aberrant water balance independent of central osmoregulation (16, 36).

Genotyping: the Offspring Cohort of the Framingham Heart Study.

The Offspring Cohort of the Framingham Heart Study included men and women who were either offspring of the Original Cohort of the Study or were spouses of these offspring (10, 15). Genomic DNA was provided by the parent study. The Original Cohort included 5,209 respondents to a random sample of 2/3 of the adult population of Framingham, Massachusetts, 30–62 yr of age (by household) in 1948 (7). Offspring Cohort genomic DNA selected from plates 1 through 20 for each of the Gen2A/unr and Gen2B/unr genomic DNA plate sets was genotyped for the variants (see below). Based upon corrected serum sodium concentration (34), participant DNA was stratified by phenotype. Hypo represents participants with serum sodium concentration falling within the lowest 15% for sex (130.8–135.8 meq/l for F and 129.8–135.9 meq/l for M), whereas Hyper corresponds to a serum sodium concentration in the highest 15% (142.8–159.1 meq/l for F and 142.8–150.9 meq/l for M); Mean were participants with serum sodium concentration falling between these extremes. Subjects were excluded as follows: 1) glucose $\geq 150$ mg/dl or 2) estimated glomerular filtration rate $>2$ SD below the population mean (i.e., Cr $>1.3$). All participants were inferred to be Caucasian of European descent (e.g., Ref. 17).

Table 2. Results of R137 genotyping of MrOS and the Offspring Cohort of the FHS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Sex</th>
<th>Tested, n</th>
<th>Failed, n</th>
<th>Failed, % Successful, n</th>
<th>Alleles, n</th>
<th>Variant, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MrOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean(^1)</td>
<td>M</td>
<td>372</td>
<td>0</td>
<td>0.0</td>
<td>372</td>
<td>372</td>
</tr>
<tr>
<td>Hypo(^1)</td>
<td>M</td>
<td>398</td>
<td>2</td>
<td>0.5</td>
<td>396</td>
<td>396</td>
</tr>
<tr>
<td>Hyper(^1)</td>
<td>M</td>
<td>308</td>
<td>1</td>
<td>0.3</td>
<td>307</td>
<td>307</td>
</tr>
<tr>
<td>All(^1)</td>
<td>M</td>
<td>1,078</td>
<td>3</td>
<td>0.3</td>
<td>1,075</td>
<td>1,075</td>
</tr>
<tr>
<td>FHS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean(^2)</td>
<td>M</td>
<td>989</td>
<td>3</td>
<td>0.3</td>
<td>986</td>
<td>986</td>
</tr>
<tr>
<td>Mean(^2)</td>
<td>F</td>
<td>1,097</td>
<td>3</td>
<td>0.3</td>
<td>1,094</td>
<td>2,188</td>
</tr>
<tr>
<td>Hypo(^2)</td>
<td>M</td>
<td>139</td>
<td>2</td>
<td>2.2</td>
<td>136</td>
<td>136</td>
</tr>
<tr>
<td>Hypo(^2)</td>
<td>F</td>
<td>156</td>
<td>1</td>
<td>0.6</td>
<td>155</td>
<td>310</td>
</tr>
<tr>
<td>Hyper(^2)</td>
<td>M</td>
<td>141</td>
<td>0</td>
<td>0.0</td>
<td>141</td>
<td>141</td>
</tr>
<tr>
<td>Hyper(^2)</td>
<td>F</td>
<td>153</td>
<td>0</td>
<td>0.0</td>
<td>153</td>
<td>306</td>
</tr>
<tr>
<td>All(^2)</td>
<td>All</td>
<td>2,675</td>
<td>10</td>
<td>0.4</td>
<td>2,665</td>
<td>4,067</td>
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</table>

\(^{1}\) For Osteoporotic Fractures in Men (MrOS) Study, Hypo and Hyper correspond to the lowest and highest decile of serum sodium concentration, respectively. Mean approximates the decile centered on the population mean for serum sodium concentration, after stratifying on sex (see METHODS). \(^{2}\) For the Framingham Heart Study (FHS), Hypo and Hyper correspond to the lowermost and uppermost 15% of the population, respectively, when stratified on sex and ranked by serum sodium concentration. Mean refers to central 70% of the distribution (i.e., all other participants).
RESULTS

A Pyrosequencing-based assay was designed to capture all known nonsynonymous allelic variation affecting residue R137 of the AVPR2 protein (Table 1). The assay was successfully validated with human genomic DNA from individuals known to be heterozygous or hemizygous for the R137C allele (Ref. 12) (Fig. 1). The assay was also validated for all other pathogenic nonsynonymous allelic variants using site-directed mutagenesis of a plasmid template corresponding to the wild-type allele (Fig. 2). Therefore, the ability of this novel assay to detect known pathogenic R137 variants of interest was established. The advantage of the Pyrosequencing-based approach, in contrast to PCR-based amplification of the affected exon followed by direct sequencing, is that it can be performed on large numbers of samples in parallel in microplate format. With more common alleles (i.e., minor allele frequency >~3%), it can be applied to pooled genomic DNA reflecting an entire population; however, for present purposes, individual-level genotyping was performed because the possibility of low minor allele frequency could not be excluded.

We next sought to determine the frequency of the R137 allelic variants in large populations. The MrOS Study population was tested first because it was entirely male, and we inferred that the presence of a variant allele in the hemizygous state would have the greatest impact upon serum sodium concentration. Genomic DNA from 1,078 of the 5,840 participants with available banked genomic DNA and phenotypic concentration. Genomic DNA from 1,078 of the 5,840 participants with available banked genomic DNA and phenotypic information was genotyped, with heavy oversampling of the population extremes for serum sodium concentration; 398 were relatively hyponatremic (serum sodium concentration in the lowest decile of the population; see METHODS), 308 were relatively hypertonatremic (serum sodium concentration in the highest decile of the population; see METHODS), 308 were relatively hypernatremic (serum sodium concentration in the highest decile), and 372 had serum sodium concentration approximating the population mean. Of the 1,075 successful genotypes, no polymorphisms affecting R137 were observed. Even among the 703 participants with serum sodium concentration in the lowest and highest deciles of the population, selected for analysis to maximize the likelihood of detecting variants, no nonsynonymous variants were detected (Table 2).

We next sought to test a mixed-sex population, with the expectation that the heterozygous state for R137-affecting variants may give rise to a less extreme (i.e., asymptomatic) water balance phenotype. Genomic DNA from participants in the Offspring Cohort of the Framingham Heart Study were tested. This is the only group within the larger Framingham Heart Study in which serum sodium concentration has been ascertained. Among the 2,665 successful genotypes representing 4,067 AVPR2 alleles, there were no R137 polymorphisms detected. Note that there were fewer participants representing the extremes of the serum sodium concentration distribution in this cohort (i.e., ~150 per sex for either hypo- or hypernatremia) as these subphenotypes were not oversampled.

In summary, we were unable to detect any individuals with nonsynonymous allelic variants affecting the key residue R137 of the AVPR2 protein despite screening >5,000 alleles across two large cohorts. In addition, we obtained this result even after focusing upon those individuals most likely to be affected by such a variant in one large population (i.e., individuals with serum sodium concentration falling at the extremes of the population distribution). Whereas others have shown that variants at R137 are highly likely to be pathological, we conclude that they are unlikely to contribute significantly to interindividual variation of the serum sodium concentration at the population level. In addition, they are unlikely to contribute to the heritability observed in serum sodium concentration (34).

DISCUSSION

We have developed and validated a comprehensive assay to test for the presence of AVPR2 gene variants affecting the R137 codon. This codon is unique in AVPR2 in that variants confer phenotypes that are polar opposites: hypernatremia in the setting of X-linked nephrogenic diabetes insipidus (4), and hyponatremia in the nephrogenic syndrome of inappropriate antidiuresis (11). In addition, a nonsynonymous variant (R137G) has been described to which a phenotype has not been assigned. Because of the pivotal nature of this residue, it’s an attractive candidate for influencing systemic water balance at the population level. In addition, there are no data addressing the allelic frequencies of R137-relevant SNPs in unselected populations and in asymptomatic human subjects with serum sodium concentration at the extremes of the normal distribution.

We confirm through multiple control experiments that our Pyrosequencing-based assay is sensitive and specific and suitable for this investigation. The assay was designed to be compatible with a high-throughput approach (i.e., microplate format), to be independent of amplification and/or sequencing-induced artifact, to genotype both alleles (where present), and to capture the totality of known genetic variation at this codon in a single assay. There was a 99.7% assay success rate; failures invariably resulted from a failure of template amplification, as detected via agarose gel electrophoresis of the PCR reaction product, and not from a failure of Pyrosequencing (data not shown). These amplification failures were uniformly traceable to lower-than-expected concentrations of genomic DNA template in the original sample (data not shown). We do not believe that important R137 alleles are concealed in the failed assays for that reason, as well the following: 1) genomic DNA from positive controls (for R137C from the hetero- and hemizygous state) was successfully genotyped (Fig. 1); 2) variant SNPs were successfully amplified and Pyrosequenced from mutagenized template (Fig. 2); and 3) further amplification and direct (Sanger) sequencing of templates from representative “failed” Pyrosequencing assays did not demonstrate the presence of other variants.

Of the 5,142 AVPR2 alleles successfully genotyped (1,075 from MrOS and 4,067 from FHS, including 2,590 from male and 2,804 from female participants), there were no R137-affecting variants detected. Therefore, we estimate that the aggregate minor allele frequency (mAF) for the known nonsynonymous SNPs affecting R137 is <0.019%. In addition, we show that the mAF is necessarily low (<0.06% in aggregate) even among participants at the extremes of the population distribution for serum sodium concentration (i.e., those we infer are most likely to harbor a dysnatremia-associated AVPR2 variant). For example, among relatively hyponatremic males (the lowest decile or the lowest 15% of the population, respectively, for MrOS and FHS; n = 532), the mAF for each SNP is estimated to be <0.19%, whereas for hyponatremic females (n = 310), it is predicted to be <0.32%. Similarly for relatively...
hypernatremic males ($n = 448$), the mAF < 0.22% and for females ($n = 306$), mAF < 0.33%. Therefore, for any given patient with moderately aberrant serum sodium concentration, the likelihood that this phenotype is attributable to an R137-based AVPR2 variant is small. Notably, we have not been able to screen a large number of patients presenting with marked and/or symptomatic hypo- or hypernatremia to establish the rarity of these variants in this highly selected clinical subset.

Nonetheless, in our two large populations there were a substantial number of participants with serum sodium concentration that is clinically abnormal (i.e., < 135 or > 145 meq/l). Although we selected relatively healthy populations for investigation, to minimize the impact of nongenetic factors upon systemic water balance, a broad range of serum sodium concentrations was represented. By our design, the MrOS participants whom we genotyped were heavily weighted toward the extremes of the serum sodium distribution. Whereas we did not genotype all 5,527 participants from whom serum sodium concentration was available, we did genotype all participants with serum sodium concentration falling within the lowest or highest deciles (and not subject to exclusion). Among these, for example, there were 85 participants with sodium concentration < 136 meq/l (> 2 SD below the population mean, including 12 that were ≤ 130 meq/l) and 167 participants with sodium concentration > 145 meq/l (including 26 that were > 147 meq/l). Among the genotyped FHS participants, there were 74 with serum sodium concentration < 134 meq/l and 37 with serum sodium concentration > 145 meq/l (> 2 SD below or above the population mean, respectively). Therefore, a substantial number of clinically hypo- and hypernatremic participants were genotyped.

The present analysis was restricted to Caucasian participants in MrOS and FHS; in both cohorts, this represented the predominant race (data not shown). Importantly, the reported R137 variants have thus far only been observed in Caucasians (e.g., Refs. 8, 12, 33). Although there may be additional variants in other races/ethnicities, the present study was undertaken to determine allelic frequency of these specific variants.

Although the R137 codon of AVPR2 is an attractive candidate for a water balance quantitative trait locus, variants in other genes pivotal to central osmosensing and renal water handling could conceivably influence water balance at the population level including AQP2, AVP, SCN7A (13), TRPV1 (31a), and others. The protein product of the transient receptor potential vanilloid-4 (TRPV4) gene has been implicated in systemic osmoregulation (18–20). A nonsynonymous TRPV4 polymorphism was associated with serum sodium concentration in two elderly male Caucasian populations, and the variant channel exhibited loss-of-function in vitro with respect to osmotic but not pharmacological stimuli (32).

In summary, these are the first data to address the allelic frequency of the clinically important nonsynonymous SNPs affecting the R137 residue of the AVPR2 gene. In addition to screening unselected populations, we focused upon the extremes of the distribution of serum sodium concentration and conclude that genetic variants affecting this residue are exceedingly rare among the Caucasian populations investigated. We further conclude that variants at this residue are unlikely to broadly contribute to interindividual variability in serum sodium concentration or to the heritability of serum sodium concentration previously shown at the population level (34).

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author Contributions

Author contributions: Y.F. and T.C. performed experiments; Y.F., D.B., E.O., and D.M.C. analyzed data; Y.F., T.C., D.B., E.O., and D.M.C. edited and revised manuscript; Y.F., T.C., D.B., E.O., and D.M.C. approved final version of manuscript; T.C., D.B., E.O., and D.M.C. interpreted results of experiments; D.M.C. conception and design of research; D.M.C. prepared figures; D.M.C. wrote manuscript; T.C., D.B., E.O., and D.M.C. interpreted results of experiments; Y.F., T.C., D.B., E.O., and D.M.C. analyzed data; Y.F., T.C., D.B., E.O., and D.M.C. edited and approved final version of manuscript; T.C., D.B., E.O., and D.M.C. revised manuscript; Y.F., T.C., D.B., E.O., and D.M.C. acquired funding and wrote manuscript.

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