Clinical Research

Association of ATP1A1 and Dear Single-Nucleotide Polymorphism Haplotypes With Essential Hypertension
Sex-Specific and Haplotype-Specific Effects

Nicola Glorioso, Victoria L.M. Herrera, Pia Bagamasbad, Fabiana Filigheddu, Chiara Troffa, Giuseppe Argiolas, Emanuela Bulla, Julius L. Decano, Nelson Ruiz-Opazo

Abstract—Essential hypertension remains a major risk factor for cardiovascular and cerebrovascular diseases. As a complex multifactorial disease, elucidation of susceptibility loci remains elusive. ATP1A1 and Dear are candidate genes for 2 closely linked rat chromosome-2 blood pressure quantitative trait loci. Because corresponding human syntenic regions are on different chromosomes, investigation of ATP1A1 (chromosome [chr]-1p21) and Dear (chr-4q31.3) facilitates genetic analyses of each blood pressure quantitative trait locus in human hypertension. Here we report the association of human ATP1A1 (P<0.000005) and Dear (P<0.03) with hypertension in a relatively isolated, case/control hypertension cohort from northern Sardinia by single-nucleotide polymorphism haplotype analysis. Sex-specific haplotype analyses detected stronger association of both loci with hypertension in males than in females. Haplotype trend-regression analyses support ATP1A1 and Dear as independent susceptibility loci and reveal haplotype-specific association with hypertension and normotension, thus delineating haplotype-specific subsets of hypertension. Although investigation in other cohorts needs to be performed to determine genetic effects in other populations, haplotype subtyping already allows systematic stratification of susceptibility and, hence, clinical heterogeneity, a prerequisite for unraveling the polygenic etiology and polygene–environment interactions in essential hypertension. As hypertension susceptibility genes, coexpression of ATP1A1 and Dear in both renal tubular cells and vascular endothelium suggest a cellular pathogenic scaffold for polygenic mechanisms of hypertension, as well as the hypothesis that ATP1A1 and/or Dear could contribute to the known renal and vascular endothelial dysfunction associated with essential (polygenic) hypertension. (Circ Res. 2007;100:1522-1529.)

Key Words: α,Na,K-ATPase ■ Dear ■ hypertension ■ genetics ■ risk factor

Essential hypertension is a major public health concern because of its high prevalence and its role as a leading risk factor for leading causes of death and morbidity in the developed world: coronary artery disease, stroke, chronic renal disease, and peripheral vascular disease. As a multifactorial disorder in which the onset and severity of the condition are influenced by both genetic and environmental factors, elucidation of underlying genetic mechanisms of hypertension is critical but remains elusive because of the polygenic nature and the complexity that is brought on by environmental and the intrinsic genetic heterogeneity of human populations. For complex multifactorial diseases with clinical heterogeneity, such as hypertension, genetic studies of inbred rat models of polygenic (essential) hypertension are instrumental in identifying blood pressure quantitative trait loci (BP-QTLs) and candidate susceptibility genes for subsequent testing in human essential hypertension.

We have recently detected 2 closely linked, sex-specific BP-QTLs on chromosome-2 (chr2) affecting salt-sensitive hypertension in a total genome scan of (Dahl salt–resistant [S] × Dahl salt–resistant [R])F2 intercross male and female rat hybrids, respectively. These 2 chr2 BP-QTLs correspond to 2 candidate genes supported by cumulative experimental evidence. Briefly, molecular genetic and transgenic analyses demonstrate that a functionally significant Q276L variant of the α,Na,K-ATPase (ATP1A1), which exhibits abnormal K transport and K affinities, most likely underlies the chr2–196.7 Mb qtl of salt-sensitive hypertension in F2[Dahl R×S] intercross male rats. In parallel, molecular genetic studies show that a functionally significant Dear (dual endothelin [ET]-1/angiotensin II receptor) S44/M74 variant, located 20 Mb from ATP1A1, is the likely candidate gene variant for the second chr2–181.7 Mb qtl, exhibiting cosegregation with salt-sensitive hypertension in F2 intercross female rats. Female-specific effects are corroborated in heterozygous Dear−/−-deficient mice, which exhibit lower BP in adult females.
Although closely linked on rat chr2, analysis of corresponding syntenic regions in humans localize ATP1A1 and Dear to different syntenic regions: ATP1A1 on chr1p21, spanning 30 Kb of genomic DNA with 22 exons; and Dear on chr4q31.3, encompassing ~5 Kb of genomic DNA with 2 exons. The distinct chromosomal location of ATP1A1 and Dear in humans serendipitously allows the independent association analysis of ATP1A1 and Dear loci with human essential hypertension, without the need for congeneric rat experimentation and, at the same time, directly addresses translational relevance to humans.

We, therefore, investigated the putative role of ATP1A1 and Dear genes in human essential hypertension via association analysis of common single-nucleotide polymorphisms (SNPs) in ATP1A1 and Dear genes, respectively, with hypertension susceptibility in a northern Sardinian hypertension case/control cohort.

Materials and Methods

Study Population

The study cohort has been previously described. Briefly, it consists of 712 subjects, with 433 patients with essential hypertension and 279 normotensives, all enrolled at the Hypertension and Cardiovascular Prevention Center of the University of Sassari Medical School, Sassari, Sardinia, Italy. The study protocols were approved by the local ethics committee of the Local Health Unit, University of Sassari, Sardinia, Italy. All subjects were white, unrelated, born in local ethics committee of the Local Health Unit, University of Sassari Medical School, Sassari, Sardinia, Italy. The study protocols were approved by the local ethics committee. All subjects were white, unrelated, born in different domains of northern Sardinia that have been shown to have a high degree of genetic homogeneity, and ascertained to be Sardinian for at least 6 generations. Hypertensive subjects with BP of >160/95 mm Hg (n=433) with no secondary hypertension etiology were considered for the study. BP measurements used for phenotype characterization were those obtained before any medications. Normotensive controls (n=279) were limited to those older than 50 years who had not been previously diagnosed or treated as hypertensive, had no family history of hypertension, cardiovascular or cerebrovascular disease, and had BP of <138/85 mm Hg on at least 4 occasions.

Characterization of Human Dear cDNA and Expression Studies

Human Dear cDNA (444-bp long) was obtained by RT-PCR from human kidney PolyA+ RNA (forward primer, 5’-CAATTTACAGCCCTCTTACC-3’; reverse primer, 5’-CCTCTTCAATGCCTTGTCG-3’), subcloned into the PT Vector System (Clontech, Palo Alto, Calif) and transiently expressed in Cos1 cells (American Type Culture Collection) as described. Cos1 cells were transfected with the expression vector via lipofection-mediated gene transfer, and cell membranes were isolated 72 hours postincubation for hormone binding. Binding of [125I]Tyr4–angiotensin II and [125I]Tyr13–ET-1 to COS-1 membranes was performed by a rapid filtration method as described. Briefly, [125I]Tyr4–angiotensin II (2.5 to 10 nmol/L) or [125I]Tyr13–ET-1 (0.045 to 10 nmol/L) was incubated with membranes (100 μg) for 20 minutes at 37°C in 100 μL of buffer A (5 mol/L MgCl2, 0.2 mmol/L EDTA, 10 mg/mL BSA, 10 mmol/L Hepes, pH 7.4). Binding reactions were terminated by the addition of 1 mL of iced-cold buffer A and immediately filtered through a Whatman GF/C filter (presoaked overnight at 4°C in 10 mg/mL BSA) and subsequently washed with 15 mL of iced-cold buffer A. Specific binding was determined as the difference between the total radioactivity bound to membranes and the radioactivity bound to blanks containing 1 μmol/L angiotensin II or 1 μmol/L ET-1. Results are expressed as the mean±SD from 3 to 5 independent experiments. Displacement of [125I]ET-1 binding was used to determine relative binding affinities for ET-1 and vascular endothelial growth factor-signal peptide (VEGFsp) to human Dear expressed in transfected Cos-1 cells essentially as described previously. VEGFsp was synthesized from the following peptide sequence: NH2-MNFLLSWVHTALLYLHHAWSQA-COOH.

Western Blot Analysis

Western blot analysis was done essentially as described using equal amounts of membrane protein (50 μg) from control mock-transfected Cos1 cells and Cos1 cell transfecants stably expressing...
human Dear and rabbit IgG anti–human Dear anti-peptide–specific antibody (1:200 dilution, 16 hours at 4°C) developed against human Dear–specific synthetic peptide M1TMFKGSNE9, to detect human Dear polypeptide. Immunoreactive polypeptides were detected by chemiluminescence using the ECL Western Detection Kit (GE Healthcare).

**Immunohistochemistry**

We analyzed human kidney sections (n = 5 individuals) contained in validated human normal tissue arrays (Pantomics Inc). Immunohistochemistry was done as described6 using a rabbit IgG anti–human Dear anti-peptide–specific antibody (40 μg/mL, 16 hours at 4°C) raised against human Dear–specific synthetic peptide: M1TMFKGSNE9. Specificity of the anti–human Dear anti-peptide antibody was ascertained by addition of 100× molar excess of antigenic peptide (M1TMFKGSNE9) and addition of 100× molar excess of an unrelated synthetic peptide (GNHFGCVVPGR) and corroborated by detection of the expected-size polypeptide of approximately 10-kDa on Western blot analysis of human Dear Cos-1–transfectant cell membranes.

**Genotyping**

SNP genotyping was performed by the Molecular Genetics Core Facility at the Boston University School of Medicine on an Applied Biosystems 7900 Real-Time PCR System. ATP1A1 and Dear SNPs were selected from the Applied Biosystems SNP database, and the SNP assays (TaqMan assays) were procured from Applied Biosystems. On average, genotyping completeness rate was 89%. We tested 16 ATP1A1 SNPs within the 5’ end of the ATP1A1 transcription unit. The genotyping assays for 7 ATP1A1 SNPs failed, and, from the remaining 9 SNPs, 1 failed Hardy–Weinberg equilibrium. Therefore, a total of 8 ATP1A1 SNPs were included in the analysis. For the Dear locus, 12 SNP assays were attempted, 6 were successfully assayed, 1 failed Hardy–Weinberg equilibrium; thus 5 Dear SNPs were considered for analysis.

**Statistical Analysis**

Departure from Hardy–Weinberg equilibrium, investigation of linkage disequilibrium patterns, and single-point association analysis comparing unrelated affected individuals and normotensive controls were done through the use of HelixTree genetic analysis software (version 4.4.1; Golden Helix Inc, Bozeman, Mont). The missing genotypes were not included (imputed) in the association analysis. A multiplicity-adjusted F test was used to derive the adjusted probability value (split significance) reported. Haplotype associations of the SNPs were investigated in the case/control cohort through the use of haplotype trend regression,13,16 as implemented in HelixTree Genetic Analysis Software. We analyzed variable windows from 2 to 8 marker haplotypes for ATP1A1 and from 2 to 5 marker haplotypes for Dear. The combinations of SNPs with the most significant probability values are reported.

**Results**

**Study Population**

We selected the northern Sardinian cohort because it is a relatively isolated genetic population,11,12 thus reducing putative confounders from genetic background heterogeneity and environmental factor variability. To ascertain phenotype accuracy, we used stringent, clinically pertinent criteria to distinguish hypertensive cases and normotensive controls, thus ascertaining a robust case/control cohort.10 Briefly, phenotypic characteristics of the study population (Table 1) were as follows: 433 hypertensives with group mean systolic BP (SD) of 174.4 (14.7) mm Hg and mean diastolic BP (SD) of 110.5 (9.9) mm Hg; and 279 normotensives with group mean systolic BP (SD) of 127.6 (11.3) mm Hg, and mean

**TABLE 1. Characteristics of the Study Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>NT* (Total)</th>
<th>HT (Total)</th>
<th>Male NT</th>
<th>Female NT</th>
<th>Male HT</th>
<th>Female HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>279</td>
<td>433</td>
<td>131</td>
<td>148</td>
<td>237</td>
<td>196</td>
</tr>
<tr>
<td>Age, mean (SD), yr</td>
<td>65.4 (10.6)</td>
<td>51.0 (10.2)</td>
<td>66.1 (8.9)</td>
<td>64.8 (11.9)</td>
<td>51.8 (10.6)</td>
<td>50.0 (9.6)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>26.2 (3.9)</td>
<td>27.7 (4.0)</td>
<td>26.3 (3.0)</td>
<td>26.2 (4.6)</td>
<td>28.0 (3.8)</td>
<td>27.4 (4.3)</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>127.6 (11.3)</td>
<td>174.4 (14.7)</td>
<td>127.9 (10.7)</td>
<td>127.4 (11.9)</td>
<td>173.2 (14.6)</td>
<td>175.9 (14.8)</td>
</tr>
<tr>
<td>DBP, mean (SD), mm Hg</td>
<td>77.6 (7.2)</td>
<td>110.5 (9.9)</td>
<td>77.2 (6.8)</td>
<td>78.0 (7.4)</td>
<td>111.9 (10.4)</td>
<td>108.8 (9.0)</td>
</tr>
</tbody>
</table>

NT indicates normotensives; HT, hypertensives; total, males + female subjects; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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1. [At least 5 references needed]
diastolic BP (SD) of 77.6 (7.2) mm Hg. Stratification by sex did not significantly change the mean BP values when comparing hypertensives versus normotensives (Table 1). SNP association and single and combinatorial SNP haplotype association tests were performed for the total case/control cohort, as well as for sex-specific subsets, to address cumulative evidence for sex-specific regulation of cardiovascular functions.17–20

Characterization of the Dear Gene

Because human Dear has not been characterized, we first isolated human Dear cDNA from a human kidney cDNA library and showed that it is composed of 85 amino acids (with a predicted molecular mass of 9677.2 kDa, Figure 1A) with dual high-affinity binding to ET-1 and VEGF, but not to angiotensin II (Figure 1B and 1C), similar to mouse Dear.9 Immunosstaining of normal human kidney sections detects differential Dear expression in renal tubular epithelium: proximal convoluted tubules, thin loops of Henle > distal convoluted tubules, collecting duct and thick loops of Henle (Figure 2A through 2C). Dear expression is also detected in glomerular capillary endothelium and renal micro- and macro-vascular endothelium (Figure 2A and 2B). Interestingly, nuclear immunostaining is detected in the different renal tubules (Figure 2A through 2C).

Dear is localized to chromosome 4 (153,612,479 to 153,617,275 bp) and comprises 2 exons (Figure 3): exon 1, containing the 5′ untranslated region (5′-UTR) plus amino acids 1 to 5; and exon 2, encoding amino acids 6 to 85 plus the 3′-UTR.

Allele Frequencies and Linkage Disequilibrium

We studied 8 SNPs in the 5′ half of the ATP1A1 gene, as well as 5 SNPs within and around the Dear locus that were informative and passed the Hardy–Weinberg test in our Sardinian cohort. As seen in Figure 3, 4 ATP1A1 SNPs are in the 5′-flanking intergenic region (SNP1-SNP4), 2 in intron 1 (SNP5 and SNP6), 1 in intron 8 (SNP7), and 1 in intron 10 (SNP10). On the other hand, 2 Dear SNPs are in the 5′-flanking intergenic region (SNP9 and SNP11), 1 in the 5′-UTR (SNP11), and 2 in the 3′-flanking intergenic region (SNP12 and SNP13). Genomic location, allele frequency and Hardy–Weinberg test results for the 8 ATP1A1 and 5 Dear informative SNPs studied are presented in Table 2. All SNPs analyzed did not deviate from Hardy–Weinberg equilibrium in both normotensive and hypertensive cohorts. Robust, pairwise linkage disequilibrium was detected between the

![Figure 3. Structure of the human ATP1A1 (5′ end) and Dear genes and location of the SNPs analyzed. Exons (shown as boxes) 1 to 13 for ATP1A1 and exons 1 (5′-UTR) amino acids 1 to 5) and 2 (amino acids 6 to 85 plus 3′-UTR) for Dear are shown. Dear UTRs (5′-UTR in exon 1 and 3′-UTR in exon 2) are unfilled. The locations of the SNPs genotyped are indicated by vertical lines.](https://www.ahajournals.org/content/journals/10.1161/HCJ.0000000000000010)
Single-Point Association Analyses

We first examined single-point associations between *ATP1A1* and *Dear* SNPs with hypertension susceptibility. None of the *ATP1A1* SNPs demonstrated significant association with hypertension in the total cohort (Table 3). After sex stratification, 1 *ATP1A1* SNP (SNP1; Table 3) was associated with hypertension (P = 0.0422) albeit only in the male population. On the other hand, 3 *Dear* SNPs (SNP10, SNP11, and SNP12) showed significant association (P < 0.05; Table 3) with hypertension in the total cohort. Sex-specific analyses detected significant associations of 3 *Dear* SNPs (SNP11, SNP12, and SNP13; Table 3) with hypertension only in the male cohort. We note that SNP11, showing the strongest association (P = 0.0172), is located within the *Dear* 5'-UTR (Figure 3), thus supporting the likelihood of *Dear*-specific involvement in hypertension susceptibility.

Haplotype Trend Regression Analyses

We next performed haplotype analyses on these 2 loci to assess possible stronger associations with disease assessing a global test of association across all haplotypes, as well as haplotype-specific association by haplotype trend regression analysis. Analysis of *ATP1A1* across all haplotypes (a more conservative global test of association) detected highly significant evidence (global, P = 0.0000045; Table 4) of an association with hypertension susceptibility in the combined cohort. Subsequent analysis of individual *ATP1A1* haplotype effects detected strong association of 1 haplotype, h1 T-G-T-C, with hypertension (P = 0.000015; Table 4). Independent investigation in male and female cohort subsets showed stronger haplotype association of *ATP1A1* with hypertension in males (global, P = 0.0027; Table 4) compared with that observed in females (global, P = 0.0196; Table 4), respectively. One haplotype, h6 T-G (Table 4), was significantly associated with hypertension in both female (P = 0.0028) and male (P = 0.0096) cohorts. Notably, an *ATP1A1* haplotype (h9 T-T; Table 4) was uniquely associated with normotension (P = 0.0064) in the male cohort. Similarly, analysis of *Dear* haplotypes in the combined cohort detected association of h12 haplotype (G-G; Table 4) with normotension (P = 0.0121). In contrast, independent investigation of males and females revealed a h13 haplotype (C-C; Table 4) that was associated with hypertension in the male population (P = 0.0328).

Discussion

The investigation of potential association of *ATP1A1* and *Dear* with hypertension susceptibility was based on cumulative evidence obtained in animal models of polygenic hypertension, in particular, our studies in the Dahl rat model linking *ATP1A1* 3–7 and *Dear* 3,8 to salt-sensitive hypertension. Altogether, concordance of results validates findings in the...
Dahl rat model as paradigms for investigation in humans. For *ATP1A1*, a stronger association of the *ATP1A1* locus with salt-sensitive hypertension was detected in males in the Dahl rat model compared with females. Similarly, *ATP1A1* showed stronger haplotype association with hypertension susceptibility in the male Sardinian population (Table 4). For *Dear*, observations are concordant as well. In the Dahl rat model, *Dear* showed sex-specific genetic linkage to hypertension affecting primarily the female population. Concordantly, *Dear* single-point and haplotype associations demonstrate sex specificity, albeit with a different sex, with associations detected exclusively in the male Sardinian population.

Our results corroborate earlier reports linking the *ATP1A1* locus with hypertension susceptibility in a Quebec family study using microsatellite markers in close proximity to the *ATP1A1* locus. This is the first report associating *Dear* with hypertension susceptibility in humans. Furthermore, the association of three haplotypes (h1, h6, h9) defined by SNPs located in the 5′-flanking regulatory region delineate the *ATP1A1* 5′-regulatory region as most likely harboring causal molecular variants contributing to modulation of hypertension susceptibility in this northern Sardinian case/control cohort. Likewise, *Dear* haplotype analyses also suggest the existence of regulatory variants within the 5′-flanking region (h12 associated with normotension), as well as potential structural variants within the 3′-half of the *Dear* transcription unit (h13 associated with hypertension). We note that elucidation of specific causative SNPs will require further experimentation.

Interestingly, the detection of variants (or haplotypes) for the same gene modulating susceptibility to hypertension in an antithetic fashion, with some increasing risk for hypertension (*ATP1A1*-h1, *ATP1A1*-h6, and *Dear*-h13) and others showing association with normotension (*ATP1A1*-h9 and *Dear*-h12), demonstrates the complexity of polygenic traits because multiple alleles underlie the queried phenotype and allelespecific effects contribute to both ends of the phenotype. These observations, in conjunction with sex-specific haplotype effects, highlight the challenge of elucidating the genetic basis of polygenic (essential) hypertension.

It is important to note that both *ATP1A1* and *Dear* are expressed in renal tubular epithelium and vascular endothelium (Figure 2A and 2B). Coexpression of both hypertension susceptibility variants in cell types implicated in BP regulation, such as endothelium and renal tubular epithelium, suggest a unifying hypothesis that polygenic expression convergence of multiple hypertension susceptibility gene variants on the same cellular target acts as a pathogenic scaffold for polygenic hypertension mechanisms. Intuitively, altered *ATP1A1* and *Dear* functions in endothelium could contribute, in combination, to endothelial dysfunction through a putative imbalance of endothelial repair to turnover, because *ATP1A1* is implicated in cell proliferation and *Dear* in angiogenesis, the latter involving endothelial cell proliferation, migration, and survival. Likewise, *ATP1A1* and *Dear* in renal tubular epithelial cells could affect sodium homeostasis, because ET-1 decreases renal Na,K-ATPase activity. Based on this observation, a net decrease in ET-1/*Dear* activation could result in greater renal Na,K-ATPase activity and increased Na reabsorption given the same sodium load, hence salt sensitivity. Further analyses into *ATP1A1* and *Dear* causal SNPs and their respective, as well as combinatorial functional, consequences are necessary.

### Table 3. Single-Point Association Analysis of SNPs in *ATP1A1* and *Dear* With Hypertension Susceptibility

<table>
<thead>
<tr>
<th>Gene (chr)</th>
<th>Position</th>
<th>Males + Females</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ATP1A1</em> (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNP1</td>
<td>116,618,393</td>
<td>0.3410</td>
<td><strong>0.0422</strong></td>
<td>0.7430</td>
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<tr>
<td>SNP2</td>
<td>116,620,772</td>
<td>0.0686</td>
<td>0.0884</td>
<td>0.5730</td>
</tr>
<tr>
<td>SNP3</td>
<td>116,623,468</td>
<td>0.1100</td>
<td>0.2030</td>
<td>0.5300</td>
</tr>
<tr>
<td>SNP4</td>
<td>116,625,520</td>
<td>0.2400</td>
<td>0.1370</td>
<td>0.8310</td>
</tr>
<tr>
<td>SNP5</td>
<td>116,630,427</td>
<td>0.2310</td>
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</tr>
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<td>SNP6</td>
<td>116,636,357</td>
<td>0.1080</td>
<td>0.1570</td>
<td>0.1390</td>
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<tr>
<td>SNP7</td>
<td>116,644,605</td>
<td>0.1840</td>
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</tr>
<tr>
<td>SNP8</td>
<td>116,647,110</td>
<td>0.1720</td>
<td>0.1290</td>
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</tr>
<tr>
<td>SNP9</td>
<td>153,606,550</td>
<td>0.0851</td>
<td>0.1920</td>
<td>0.1970</td>
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<tr>
<td>SNP10</td>
<td>153,609,666</td>
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<tr>
<td>SNP11</td>
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<td><strong>0.0172</strong></td>
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<td>SNP12</td>
<td>153,623,112</td>
<td>0.0338</td>
<td><strong>0.0334</strong></td>
<td>0.3740</td>
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<td>SNP13</td>
<td>153,628,569</td>
<td>0.1320</td>
<td><strong>0.0447</strong></td>
<td>0.1020</td>
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</tbody>
</table>

Association analysis was performed with the HelixTree genetic analysis software. *P* indicates adjusted *P* value derived from a multiplicity-adjusted *F* test performed on raw *P* values. Significant results (*P*<0.05) are shown in bold.
including investigation of vascular endothelial versus renal epithelial contributions to hypertension.

In summary, our results show that (1) animal models of essential hypertension can be highly informative in deciphering candidate susceptibility loci for human hypertension, (2) variants can affect hypertension susceptibility in a sex-specific manner, (3) variants within a locus can be associated with both hypertension or normotension, and that (4) multiple variants within a locus can modulate risk for essential hypertension. These findings provide insight into the genetic basis of clinical heterogeneity of essential hypertension and corroborate the mandate for sex-specific investigative and treatment approaches. Although testing in other cohorts is necessary, our studies support the hypothesis that $ATP1A1$ and Dear genes are hypertension susceptibility genes in this northern Sardinian cohort. More importantly, along with other gene haplotypes associated with hypertension, our data forward the feasibility of stratification of hypertension patients by haplotype subtyping. This would then help unravel the clinical heterogeneity of essential hypertension, as well as facilitate the elucidation of its elusive polygenic basis and polygene–environment interactions, both of which are central to mechanism-based intervention and prevention strategies.

Acknowledgments
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Disclosures
None.

References

### TABLE 4. Association Analysis of Haplotypes in $ATP1A1$ and Dear With Hypertension Susceptibility

<table>
<thead>
<tr>
<th>Combination of SNPs Using Sliding Window With Significant $P$ Values (Global $P$)</th>
<th>Haplotype</th>
<th>Alleles (name)</th>
<th>Frequency</th>
<th>Regression $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male + female cohort</td>
<td>$T-G-T-C$ (h1)</td>
<td>0.018</td>
<td>$0.000015$ (HT$^*$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T-T-T-T$ (h2)</td>
<td>0.016</td>
<td>0.1651</td>
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<tr>
<td></td>
<td>$T-T-C-T$ (h3)</td>
<td>0.039</td>
<td>0.0756</td>
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<tr>
<td></td>
<td>$C-G-T-C$ (h4)</td>
<td>0.904</td>
<td>0.5151</td>
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<tr>
<td></td>
<td>$C-T-C-T$ (h5)</td>
<td>0.014</td>
<td>0.7255</td>
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<tr>
<td>Female cohort</td>
<td>$T-G$ (h6)</td>
<td>0.016</td>
<td>$0.0028$ (HT)</td>
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<tr>
<td></td>
<td>$C-T$ (h7)</td>
<td>0.019</td>
<td>0.6702</td>
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<tr>
<td></td>
<td>$C-G$ (h8)</td>
<td>0.897</td>
<td>0.6620</td>
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<td></td>
<td>$T-T$ (h9)</td>
<td>0.068</td>
<td>0.3416</td>
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<tr>
<td>Male cohort</td>
<td>$T-G$ (h6)</td>
<td>0.024</td>
<td>$0.0096$ (HT)</td>
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</tr>
<tr>
<td></td>
<td>$C-T$ (h7)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>$C-G$ (h8)</td>
<td>0.903</td>
<td>0.6213</td>
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<tr>
<td></td>
<td>$T-T$ (h9)</td>
<td>0.047</td>
<td>$0.0064$ (NT$^†$)</td>
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</tr>
<tr>
<td>Male + female cohort</td>
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<td>0.045</td>
<td>0.1353</td>
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<td>$G-A$ (h11)</td>
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<td>0.0631</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$G-G$ (h12)</td>
<td>0.248</td>
<td>$0.0121$ (NT)</td>
<td></td>
</tr>
<tr>
<td>Male cohort</td>
<td>$C-C$ (h13)</td>
<td>0.689</td>
<td>$0.0328$ (HT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T-T$ (h14)</td>
<td>0.300</td>
<td>0.0808</td>
<td></td>
</tr>
</tbody>
</table>

Haplotype trend regression analysis was used for the association test, implemented with the HelixTree Genetic Analysis Software. Global $P$ values refer to the global test of association across all detected haplotypes. $^*$Haplotype associated with hypertension; $^†$haplotype associated with normotension. Significant results ($P$/$0.05$) are shown in bold.


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