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A New Locus on Chromosome 12p13.3 for Pseudohypoaldosteronism Type II, an Autosomal Dominant Form of Hypertension

Sandra Disse-Nicodème,[1] Jean-Michel Achard,[3] Isabelle Desitter,[1] Anne-Marie Houot,[1] Albert Fournier,[3] Pierre Corvol,[1,2] and Xavier Jeunemaitre[1,2]

[1]INSERM U36, Collège de France, and [2]Laboratoire de Génétique Moléculaire, Hôpital Broussais, Assistance Publique-Hôpitaux de Paris, Paris; and [3]Service de Néphrologie, Hôpital d'Amiens-Sud, Amiens, France

Pseudohypoaldosteronism type II (PHA2) is a rare autosomal dominant form of volume-dependent low-renin hypertension characterized by hyperkalemia and hyperchloremic acidosis but also by a normal glomerular filtration rate. These features, together with the correction of blood pressure and metabolic abnormalities by small doses of thiazide diuretics, suggest a primary renal tubular defect. Two loci have previously been mapped at low resolution to chromosome 1q31-42 (*PHA2A*) and 17p11-q21 (*PHA2B*). We have now analyzed a new, large French pedigree, in which 12 affected members over three generations confirmed the autosomal dominant inheritance. Affected subjects had hypertension together with long-term hyperkalemia (range 5.2–6.2 mmol/liter), hyperchloremia (range: 100-109 mmol/liter), normal plasma creatinine (range: 63–129 mmol/liter) and low renin levels. Genetic linkage was excluded for both *PHA2A* and *PHA2B* loci (all LOD scores $Z < -3.2$ at recombination fraction [θ] 0), as well as for the thiazide-sensitive sodium-chloride cotransporter gene. A genome-wide scan using 383 microsatellite markers showed a strong linkage with the chromosome 12p13 region (maximum LOD score $Z = 6.18$, $\theta = 0$, at D12S99). Haplotype analysis using 10 additional polymorphic markers led to a minimum 13-cM interval flanked by D12S1652 and D12S336, thus defining a new *PHA2C* locus. Analysis of two obvious candidate genes (*SCNN1A* and *GNb3*) located within the interval showed no deleterious mutation. In conclusion, we hereby demonstrate further genetic heterogeneity of this Mendelian form of hypertension and identify a new *PHA2C* locus, the most compelling and precise linkage interval described to date.

Introduction

The complex interplay between the many inherited and environmental factors that regulate arterial blood pressure makes it very difficult to identify the susceptibility genes responsible for essential hypertension. Genetic analysis of rare, severe Mendelian forms of hypertension may facilitate this identification and open up new avenues toward a better understanding of blood pressure regulation (Lifton 1996).

Pseudohypoaldosteronism type II (PHA2), also known as Gordon's syndrome, is a volume-dependent form of hypertension characterized by hyperkalemia, hyperchloremic acidosis, and a normal filtration rate (Arnold and Healy 1969; Gordon et al. 1970). The low renin levels are thought to be due to volume repletion, whereas plasma aldosterone is thought to vary according to the contradictory influences of the plasma renin and potassium concentrations (Gordon et al. 1995). The etiology of this disease is still unknown, but there is some evidence for a primary renal defect along the distal tubule. Affected patients are very sensitive to small doses of thiazide diuretics, and the features of PHA2 are the mirror image of the Gitelman syndrome, in which there are inactivating mutations in the thiazide-sensitive sodium-chloride cotransporter gene (Simon et al. 1996). The autosomal dominant inheritance of the trait is suggested by the apparent transmission of PHA2 features from one parent to offspring in >10 families (Gordon et al. 1995). Mansfield et al. (1997) studied eight families, in which at least two subjects over two generations were affected, and identified a 20–33-cM *PHA2A* locus on chromosome 1 and a 21–43-cM *PHA2B* locus on chromosome 17, assuming autosomal dominant inheritance. The locus on chromosome 17 was confirmed in another Australian kindred (O'Shaughnessy et al. 1998) and is particularly interesting, since it overlaps a region suggested to be linked to human and rat essential hypertension (Julier et al. 1997).

We have now identified a new, large French family with 12 individuals affected by PHA2 over three generations. We have used genetic markers mapped on *PHA2A* and *PHA2B* loci to exclude these two loci and

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Address for correspondence and reprints: Dr. Xavier Jeunemaitre, INSERM U36, Collège de France, 3 rue d'Ulm, 75005 Paris, France. E-mail: xavier.jeunemaitre@college-de-france.fr

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the involvement of the thiazide-sensitive sodium chloride cotransporter *SLC12A3* gene. A genomewide scan identified a new 13-cM *PHA2C* locus located on chromosome 12.

Subjects and Methods

Pedigree

A large family from the north of France was identified through a proband who had the typical features of PHA2. We obtained informed consent from each participant, or from the parents of the younger children, and carried out a standard phenotypic analysis. It included each subject's medical history, a clinical examination with at least three manual blood pressure measurements in sitting position, and basal biochemical data. Following this examination, each individual found to have high blood pressure level or hyperkalemia underwent a second complete determination of the ionogram $\geq$1 wk later. Subjects were considered affected if they were currently being treated for hypertension or had persistent hyperkalemia ($K^+ > 5.2$ mmol/liter) with normal plasma creatinine, high blood pressure (systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg for adults, values above the 95th percentile of those observed in childhood and adolescence in a French population for offspring [Andre et al. 1982]). Subjects were considered unaffected if they had normal blood pressure and normokalemia. Others were considered undetermined.

Analysis of Candidate Loci

DNA was extracted from blood samples by conventional techniques. All individuals with an unambiguous phenotype (34/39) were genotyped using eight microsatellite markers spanning the *PHA2A* locus on chromosome 1, six markers spanning the *PHA2B* locus on chromosome 17, and three markers spanning the *SLC12A3* locus on chromosome 16, from the kit ABI PRISM Linkage Mapping Set version 2 (PE Applied Biosystems). Custom primers were designed to genotype additional markers D16S408 and D16S494, which were selected upon their map location in the vicinity of the *SLC12A3* gene and their heterozygosity. PCR reactions were carried out in 7.5-μl volumes, containing 50 ng genomic DNA, 1 × GeneAmp PCR buffer II (Roche Molecular System), 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.33 μM each of both primers, and 0.3 U Ampli*Taq* Gold DNA polymerase (Roche Molecular System). Allele sizes were determined by separation on acrylamide gels on an ABI 377 sequencer, and normalized to a control DNA sample CEPH 1347-02. Signal analysis and genotype determination were automated using GENESCAN 2.1 and GENOTYPER 1.1 software.

Genomewide Scan

Genotyping was performed by the Australian Genome Research Facility using 383 autosomal microsatellite markers of the kit ABI PRISM Linkage Mapping Set version 2 (PE Applied Biosystems).

High-Resolution Mapping

Ten markers mapping to chromosome 12p13.3, between D12S352 and D12S336, and evenly distributed through this linkage interval (D12S94, D12S91, D12S100, D12S1626, D12S1652, D12S1725, D12S1624, D12S314, D12S356, and D12S1623) were analyzed in the 39 individuals of the family, using custom fluorescent dye-labeled primers and the protocol described above. In order to avoid false-positive results caused by genotyping errors, these markers were genotyped twice by two independent investigators (S.D.-N. and I.D.).

Simulations, Linkage, and Haplotype Analyses

Transmission of the trait was assumed to be autosomal dominant, with a disease-allele frequency of 0.005. Conservative and stringent models of inheritance were used, as described by Mansfield et al. (1997). In the conservative model, the phenocopy rate was set at 1% and penetrance at 95%, whereas, in the stringent model, penetrance was maximum with no phenocopies. The maximum expected LOD score was estimated by genotype simulation: 900 replicates were generated using the SLINK program (Weeks et al. 1990; Terwilliger and Ott 1994). Two-point LOD scores were then simulated using the MSIM program. Simulated allele frequencies were set at 0.3, 0.2, 0.2, 0.1, 0.1, and 0.1 (heterozygosity 0.8). Error checking on genotypic data was performed using UNKNOWN program, and two-point LOD scores were computed for the 383 markers by MLINK from version 5.2 of the LINKAGE package (Terwilliger and Ott 1994; Lathrop et al. 1985). Allele frequencies for each marker were obtained online from the Genome Database and were similar to those observed in our white family. Recombination fractions were set to be identical in males and females. Haplotype analysis and pedigree drawing were performed with Cyrillic version 2.1 (Cherwell Scientific).

Mutation Screening

All exons of *SCNN1A* and *GNb3* genes (GenBank accession numbers Z92978–Z92981 and Y12048–Y12058) were PCR amplified from the genomic DNA of affected subject III.1 versus unaffected subject III.5, by use of flanking intronic primers. Primer sequences are available from authors upon request. Double-strand sequencing was performed using the dideoxy termination

method on an ABI 377 instrument (Prism Big Dye Terminators Cycle Sequencing kit, PE Applied Biosystems). Southern blots were performed using genomic DNA from affected individual III.9 and unaffected individual III.7, digested with *Hin*dIII, *Eco*RI, *Pst*I and hybridized with ^{32}P-labeled cDNA probes (*SCNN1A* exon 3 and *GNb3* exon 11).

Results

Clinical and Biochemical Data

The family described here consists of 39 phenotyped individuals, 12 of whom are considered to be affected (table 1 and fig. 1). The proband III.1 was diagnosed as hypertensive at age 37 years and was treated with a combination of beta blockers and furosemide. Ten years later, after having stopped his treatment for an unknown period, he was hospitalized because of hypertension (180/110 mmHg) and hyperkalemia (6.2 mmol/liter), despite having a normal serum creatinine (120 mmol/liter). Repeated measurements confirmed the typical biological features of PHA2 (average values are indicated in table 1). Hydrochlorothiazide (50 mg) administered for 1 mo normalized both blood pressure and plasma potassium. A history of resistant hypertension and stroke in several of his relatives led us to carry out a systematic clinical and biochemical evaluation of the family (S. Disse-Nicodème, X. Jeunemaitre, A. Fournier, P. Corvol, and J.-M. Achard, unpublished data). As indicated in table 1, 10 subjects were considered to be clearly affected, since they had (1) either high blood pressure or a history of hypertension, (2) hyperkalemia in the absence of diuretics (range 5.2–6.2 mmol/liter), and (3) a tendency toward hyperchloremia and acidosis. Two young subjects (IV.6, age 28 years, and IV.10, age 18 years) were not hypertensive but had typical biochemical features and were also considered affected. The eight affected subjects whose plasma renin and aldosterone concentrations could be evaluated under satisfactory conditions, had low renin levels and abnormally low aldosterone levels with respect to the hyperkalemia (table 1). The disease transmission over three generations and the number of affected subjects in each sibship strongly suggested an autosomal dominant inheritance.

Exclusion of PHA2A and PHA2B Loci

The SLINK and MSIM programs were used to estimate the maximum power of linkage expected with 34/39 members of the family. A maximum expected LOD score $Z = 7.17$ was reached at recombination fraction (θ) 0 with the stringent model (no phenocopy and 100% penetrance), showing that the family could be informative. We used microsatellite markers of the 1q42-q44 and 17p21-q21 regions, corresponding to the *PHA2A* and *PHA2B* loci, to test their implication in our family. Linkage was significantly excluded with all pairwise LOD scores $Z < -3.2$ using both the conservative and the stringent model (table 2). We also tested five markers at the thiazide-sensitive sodium-chloride cotransporter *SLC12A3* locus (GenBank accession number NM_000339 or X91220). Calculation of two-point LOD scores (table 2), as well as multipoint LOD score (data not shown) demonstrated exclusion of the *SLC12A3* locus under both genetic models.

Genomewide Scan

In the linkage analysis, 383 markers, representing all autosomes, were used. Because they led to non-Mendelian allelic segregation, 24 incorrect genotypes (0.18%) were detected and were removed from the statistical analysis. With the stringent model, the LOD scores were <-2 for 374 markers (data not shown). Eight markers gave non-significant positive results (data available upon request). The maximum pairwise LOD score was $Z_{max} = 6.18$ at $\theta = 0$ from D12S99 (table 2). The results were similar using the conservative model with $Z_{max} = 5.90$ at $\theta = 0$ from D12S99. The interval of positive linkage surrounding D12S99 spanned 22 cM from pter to D12S336, for which linkage was clearly excluded.

High-Resolution Mapping

We refined the linkage region by genotyping 10 additional markers mapping within the linkage interval, and calculating pairwise LOD scores. When the stringent model was used, LOD scores were negative at $\theta = 0$ for the markers located in the region distal from D12S1725, but were positive for all the markers located in the proximal part of the locus, with a maximum value for D12S1725 and D12S1623 ($Z_{max} = 7.47$ at $\theta = 0$) (table 2). We could not refine the linkage interval using the conservative model. Haplotype analysis showed two recombination events in individuals III.23 and IV.12, which allowed us to map the disease locus between markers D12S1652 and D12S336 (fig. 1). This interval on chromosome 12p13.3 (fig. 2), represents 13 cM on the genetic map and was called *PHA2C*. The flanking markers D12S1652 and D12S336 are both anchored on the physical map constructed at the Albert Einstein College of Medicine Human Genome Research Center.

Mutation Screening on Two Candidate Genes

Searches of the Genome Database and Online Mendelian Inheritance in Man identified 32 genes of known function, including the α subunit of the epithelial sodium channel (*SCNN1A*) and the G protein beta-subunit type 3 (*GNb3*) (fig. 2). Southern blotting and direct sequencing of all the coding sequences of these two genes, were

Table 1

Clinical and Biochemical Features at Diagnosis of the Pedigree

Subject ID	Sex	Body-Mass Index (kg/m^2)	Age (years)	Affection Status[a]	Blood Pressure (mmHg)		History of Hypertension	Antihypertensive Treatment[b]	K^+ (mmol/liter)	Cl^- (mmol/liter)	HCO_3^- (mmol/liter)	Creatinine (μmol/liter)	Active Renin (pg/ml)	Aldosterone (pg/ml)
					Systolic	Diastolic								
II.2	F	30	69	A	180	110	Y	Y_T	4.4/6.2[c]	104	31	102	…	…
II.5	F	25	61	NA	150	85	N	N	4.3	105	28	69	…	…
II.6	F	19	64	A	180	100	Y[d]	Y	5.5	106	25	87	2	105
II.8	F	18	70	A	120	70	Y[d]	Y	5.6	107	27	91	…	…
III.1	M	30	48	A	165	95	Y	N	5.9	109	22	129	2	202
III.2	F	25	46	NA	135	80	N	N	4.5	104	29	65	…	…
III.3	M	28	50	A	130	70	Y	Y_T	4.5	100	28	…	…	…
III.4	F	27	43	NA	145	85	N	N	4.8	101	27	88	10	281
III.5	M	26	49	NA	120	80	N	N	4.9	105	33	117	…	…
III.7	M	26	43	NA	140	90	N	N	4.4	104	35	106	…	…
III.9	F	28	51	A	155	95	Y	Y_T	4.4/5.2[c]	104	29	118	5	202
III.10	F	24	42	U	120	80	N	N	4.7	105	29	87	…	…
III.11	M	26	34	NA	135	80	N	N	3.7	104	33	131	…	…
III.12	F	25	30	U	150	90	N	N	4	99	28	65	…	…
III.13	M	20	40	NA	140	90	N	N	4.4	105	25	79	…	…
III.14	M	25	30	U	160	100	Y	N	4.4	101	32	103	…	…
III.15	F	39	43	U	130	80	Y	Y	4.6	104	30	65	…	…
III.16	F	23	37	NA	110	70	N	N	4.5	104	29	73	…	…
III.17	F	20	24	NA	130	80	N	N	4.5	103	30	64	…	…
III.18	M	28	46	A	140	90	Y	Y	5.3	107	26	93	5.5	223
III.19	F	21	45	NA	120	75	N	N	3.7	104	31	69	…	…
III.20	M	24	42	NA	150	90	N	N	4.1	103	28	74	…	…
III.21	M	23	43	NA	135	75	N	N	4.2	103	27	106	…	…
III.22	M	19	44	NA	135	85	N	N	4.3	102	30	52	16	104
III.23	F	31	47	NA	120	80	N	N	4.7	103	29	82	…	…
III.25	F	18	41	A	155	100	Y	N	5.5	106	26	63	2	191
IV.1	M	22	20	NA	125	75	N	N	4.1	105	30	88	…	…
IV.2	M	28	23	U	145	85	N	N	4.2	103	28	…	…	…
IV.3	M	21	29	NA	130	80	N	N	3.6	103	28	93	…	…
IV.4	F	26	14	A	140	90	Y	N	5.8	108	22	64	6	132
IV.5	M	21	16	NA	130	70	N	N	4.4	101	26	98	…	…
IV.6	M	20	28	A	120	70	N	N	6.1	109	27	110	…	…
IV.7	F	28	33	A	150	95	Y	Y	5.2	106	27	72	…	…
IV.8	F	21	32	NA	120	70	N	N	4.2	99	33	106	…	…
IV.9	F	23	30	NA	130	80	N	N	4.4	102	26	85	…	…
IV.10	M	20	18	A	130	90	N	N	5.4	108	26	77	…	…
IV.11	F	27	25	NA	135	85	N	N	4.3	99	29	64	…	…
IV.12	M	18	16	NA	120	70	N	N	4.4	103	30	79	…	…
IV.13	F	22	18	NA	115	65	N	N	4.3	101	32	61	…	…

[a] A = affected; NA = not affected; U = undetermined.

[b] Y_T = thiazide diuretics treatment.

[c] Washout value.

[d] Including a history of stroke.

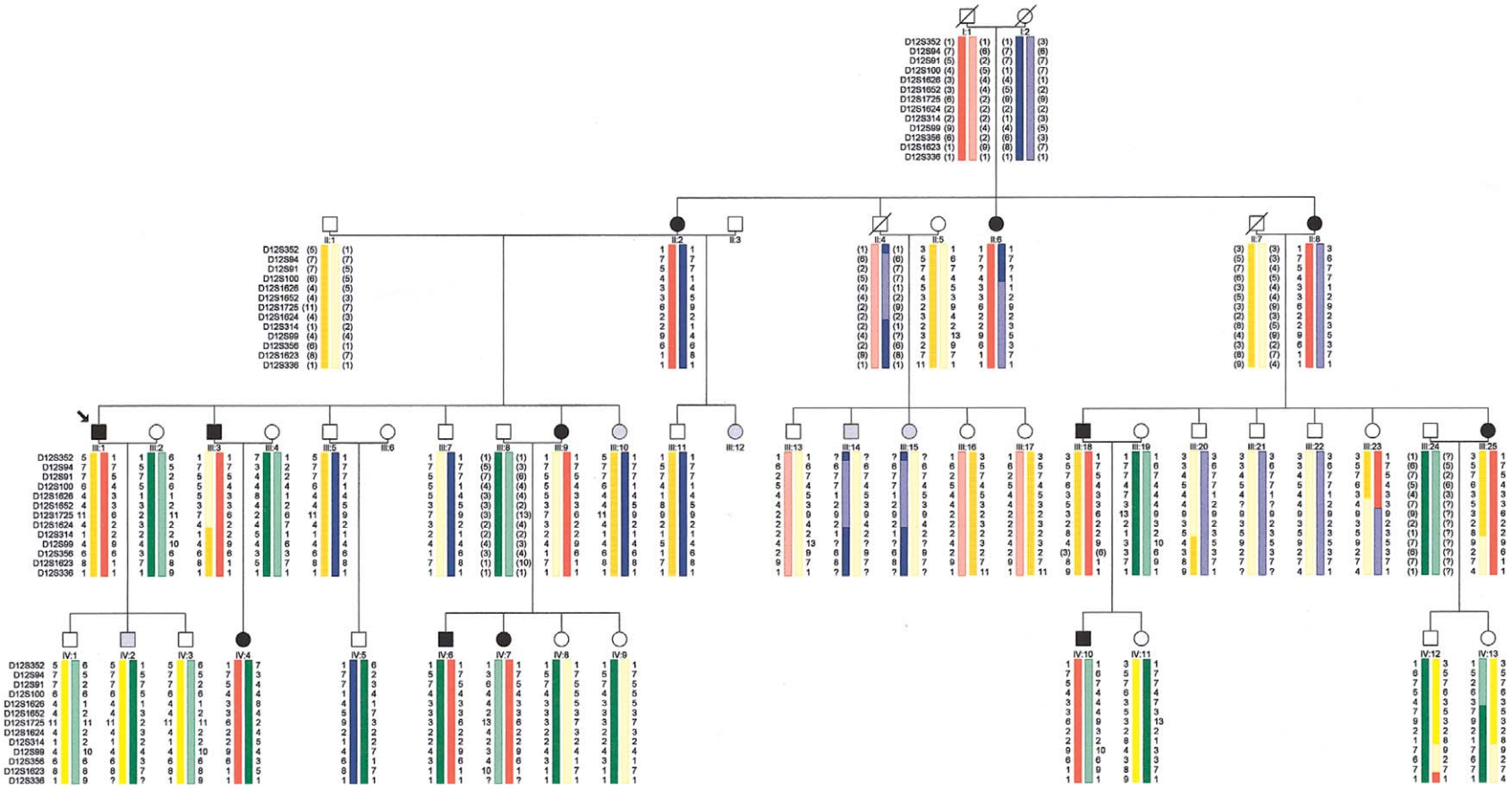

Figure 1 Pedigree structure and haplotypes for the PHA2-affected family. Females are designated by circles and males by squares. Affected status is indicated by blackened symbols, unaffected status by unblackened symbols, and undetermined status by grey symbols. Diagonal lines indicate deceased individuals. Genotypes for markers D12S352, D12S94, D12S91, D12S100, D12S1626, D12S1652, D12S1725, D12S1624, D12S314, D12S99, D12S356, D12S1623, and D12S336 (telomere to centromere) are shown; inferred genotypes are shown in parentheses. The disease-associated haplotype is indicated by red bars; a color change within a bar indicates the presence of a crossover event.

Table 2

Pairwise LOD Scores

Locus and Marker	Stringent LOD/Conservative LOD at θ=						
	0	.01	.05	.1	.2	.3	.4
PHA2A:							
D1S413	−∞/−8.55	−12.65/−8.15	−8.12/−6.83	−5.86/−5.04	−3.05/−2.70	−1.52/−1.37	−.59/−.54
D1S249	−∞/−9.61	−15.77/−9.09	−9.60/−6.94	−6.36/−4.95	−3.14/−2.62	−1.49/−1.29	−.53/−.47
D1S425	−∞/−9.78	−15.32/−9.03	−8.04/−6.09	−5.06/−4.10	−2.41/−2.06	−1.15/−1.01	−.44/−.39
D1S213	−∞/−6.00	−9.57/−4.97	−4.86/−3.23	−2.99/−2.18	−1.36/−1.07	−.62/−.51	−.23/−.19
D1S2800	−∞/−11.97	−12.44/−8.22	−6.37/−5.02	−3.91/−3.26	−1.74/−1.51	−.72/−.64	−.21/−.19
D1S2785	−∞/−6.37	−7.97/−5.19	−4.63/−3.41	−3.16/−2.39	−1.48/−1.19	−.59/−.48	−.14/−.11
D1S2842	−∞/−3.20	−7.21/−2.94	−4.51/−2.29	−2.83/−1.76	−1.24/−.94	−.48/−.39	−.11/−.09
D1S2836	−∞/−5.48	−4.34/−2.94	−2.26/−1.79	−1.39/−1.12	−.49/−.36	−.07/−.03	.02/.02
PHA2B:							
D17S799	−∞/−11.71	−14.96/−8.14	−7.77/−5.03	−4.77/−3.38	−2.07/−1.60	−.80/−.63	−.19/−.14
D17S921	−∞/−10.20	−12.60/−7.15	−7.39/−4.57	−4.61/−3.11	−2.03/−1.52	−.83/−.65	−.24/−.18
D17S1857	−∞/−7.25	−8.92/−6.00	−4.85/−3.90	−3.20/−2.69	−1.59/−1.36	−.68/−.59	−.18/−.15
D17S798	−∞/−11.71	−13.29/−6.92	−6.94/−4.24	−4.26/−2.88	−1.85/−1.40	−.72/−.56	−.17/−.12
D17S1868	−∞/−6.47	−7.35/−5.06	−3.82/−3.07	−2.36/−1.99	−1.06/−.93	−.47/−.42	−.18/−.16
D17S787	−∞/−8.00	−15.41/−7.12	−8.82/−5.21	−5.59/−3.83	−2.59/−2.06	−1.15/−.97	−.38/−.33
SLC12A3:							
D16S3068	−∞/−9.54	−14.71/−7.41	−7.43/−4.50	−4.45/−2.96	−1.83/−1.36	−.67/−.53	−.15/−.12
D16S3136	−∞/−7.73	−14.26/−7.51	−8.41/−5.73	−5.44/−4.05	−2.64/−2.19	−1.24/−1.09	−.46/−.41
D16S415	−∞/−7.26	−10.66/−5.80	−5.25/−3.65	−3.09/−2.34	−1.20/−.96	−.36/−.29	−.01/−.00
D16S408	−∞/−3.49	−1.40/−1.28	−.72/−.61	−.44/−.34	−.19/−.12	−.08/−.04	−.02/−.01
D16S494	−∞/−1.89	−4.57/−1.51	−2.67/−.98	−1.82/−.69	−.98/−.38	−.52/−.20	−.21/−.08
PHA2C:							
D12S352	1.42/1.45	1.43/1.44	1.43/1.38	1.35/1.28	1.06/.98	.70/.63	.32/.29
D12S94	−∞/2.04	1.46/2.06	1.93/2.04	1.92/1.92	1.57/1.51	1.04/.98	.46/.42
D12S91	−∞/3.20	2.68/3.20	3.04/3.08	2.90/2.82	2.24/2.12	1.38/1.28	.47/.42
D12S100	−∞/5.33	4.82/5.30	5.09/5.09	4.83/4.71	3.90/3.74	2.69/2.55	1.28/1.19
D12S1626	−∞/4.57	3.97/4.55	4.35/4.39	4.16/4.07	3.35/3.22	2.28/2.16	1.04/.97
D12S1652	−∞/4.10	3.58/4.09	3.92/3.95	3.75/3.67	3.03/2.90	2.07/1.94	.94/.87
D12S1725	7.47/7.13	7.36/7.01	6.87/6.54	6.24/5.93	4.86/4.60	3.32/3.11	1.59/1.48
D12S1624	2.12/2.01	2.09/1.99	1.98/1.88	1.82/1.72	1.46/1.37	1.04/.98	.56/.52
D12S314	4.25/3.98	4.19/3.92	3.92/3.67	3.55/3.31	2.71/2.51	1.76/1.62	.76/.69
D12S99	6.18/5.90	6.08/5.80	5.67/5.40	5.13/4.88	3.97/3.76	2.67/2.51	1.25/1.16
D12S356	4.43/4.20	4.35/4.13	4.03/3.82	3.62/3.42	2.73/2.57	1.76/1.64	.76/.70
D12S1623	7.47/7.13	7.36/7.01	6.87/6.54	6.24/5.93	4.86/4.59	3.32/3.11	1.59/1.47
D12S336	−∞/−.05	−.80/.00	−.10/.13	.10/.22	.24/.27	.22/.23	.14/.14

Note.—Pairwise LOD scores obtained at different recombination fractions. The genetic map in the vicinity of *SLC12A3* locus was estimated from the Location Database and the Genome Database: D16S3068-11 cM-D16S3136-8 cM-D16S415-.5 cM-*SLC12A3*-.5 cM-D16S408-3 cM-D16S494.

performed in the proband (subject III.1) and one unaffected subject (III.5). No deletion or deleterious mutation was found. A G→A substitution was found in the affected subject at position −92 from initiation codon, in the untranslated exon 1 of *SCNN1A*. This polymorphism was also present in 222 of 277 normotensive controls. We also detected a R181W missense mutation in exon 3 of *SCNN1A*, at the heterozygous state in the unaffected control subject. Sequence analysis of exon 3 in the family showed that this polymorphism cosegregated with the dark blue unaffected haplotype in figure 1. The only nucleotide variation found in the *GNb3* gene was the previously described C825T polymorphism. The T allele was present in all affected subjects of the family, but also in 13 unaffected subjects, demonstrating that this allele was not the primary cause of PHA2.

Discussion

Although they have not yet revealed any major gene implicated in essential hypertension, Mendelian forms of hypertension in humans have contributed to a better understanding of the molecular pathways underlying the regulation of blood pressure. They should also make it easier to identify novel candidate genes for more common forms of hypertension. Typical PHA2 is a rare disorder, and only a few sporadic cases and 13 families containing 64 affected individuals have been described

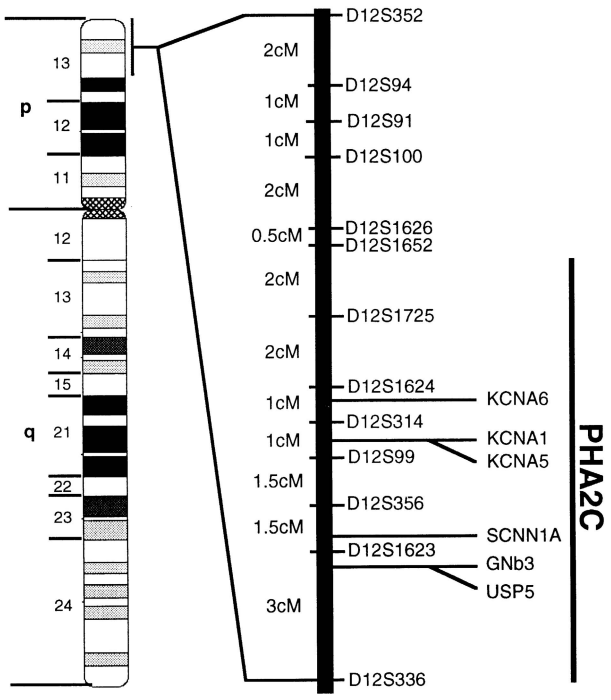

Figure 2 Ideogram of chromosome 12 (left). The linkage region on 12p13.3 is expanded (middle) to show the markers used for the haplotype analysis and their genetic distances (in cM) from each other. *PHA2C* locus spans the D12S1652–D12S336 interval. The positions of the main candidate genes belonging to the locus are shown (right). *KCNA1, KCNA5, KCNA6:* voltage-dependent potassium channel family members 1, 5, and 6; *SCNN1A:* amiloride-sensitive epithelial sodium channel alpha subunit; *GNb3:* guanine-nucleotide binding protein beta 3 subunit; *USP5:* ubiquitin specific protease 5.

so far (Gordon et al. 1995; Mansfield et al. 1997). The genetic dissection of PHA2 is particularly interesting because of the extreme sensitivity of affected patients to thiazide diuretics. This class of drug is widely used to treat essential hypertension and reduces both blood pressure and cardiovascular mortality and morbidity (World Health Organization–International Society of Hypertension 1999). This particular sensitivity and the clinical and biological characteristics of PHA2 suggest an overactivity of the thiazide-sensitive sodium-chloride cotransporter. However, the pathophysiology of PHA2 is likely to be more complex and several potential primary defects have been proposed. These include (1) a specific defect of the renal potassium secretory mechanism (Arnold and Healy 1969; Brautbar et al. 1978) or a more generalized cellular defect in transmembrane potassium transport rather than an isolated renal tubular defect (Farfel et al. 1978), (2) an abnormally increased rate of chloride reabsorption by the tubule and an altered sensitivity to mineralocorticoids (Schambelan et al. 1981), and (3) an excessive renal sodium reabsorption proximal to the site of aldosterone action (Gordon et al. 1970). The discovery of two independent genetic loci causing the disease (Mansfield et al. 1997) suggests that several proteins that may or may not belong to the same pathophysiological pathway, may be involved.

We have now demonstrated further genetic heterogeneity with the mapping of a third locus at chromosome 12p13.3 responsible for PHA2. The *PHA2C* locus is a 13-cM interval in which several genes may be potential candidates, particularly two of them, *SCNN1A* and *GNb3*. *SCNN1A* encodes the α subunit of the amiloride-sensitive epithelial sodium channel which is in the distal nephron. Mutations affecting the structure of this subunit, as well as those of the β and γ subunits, can cause pseudohypoaldosteronism type I (MIM 264350), a salt-wasting syndrome in infancy (Chang et al. 1996), whereas mutations affecting the proline-rich region of the C-terminal part of either the β or γ subunits cause Liddle syndrome (Lifton 1996). Although this syndrome is also an autosomal dominant form of hypertension in which there is volume expansion, its biological features are clearly different, with hypokalemia, alkalosis and completely suppressed renin and aldosterone levels (MIM 177200). *GNb3* encodes the guanine nucleotide-binding protein beta 3 subunit that may contribute to sodium transport through the membrane. Siffert et al. (1998) described a 825 C→T polymorphism in which the T allele was associated with a splice variant in which nucleotides 498-620 of exon 9 were deleted. This in-frame deletion could be responsible for the enhanced G-protein reactivity observed in the lymphoblasts and fibroblasts from selected patients with essential hypertension.

We found neither deletion nor deleterious point mutation in the coding and splicing consensus sequences of the *SCNN1A* and *GNb3* genes in our PHA2 family by Southern blotting and direct sequencing. This analysis does not exclude variants in the noncoding regions, especially in the 5′ regulatory sequences. Other genes in the region may also be interesting candidates (fig. 2). The *USP5* gene is a member of the ubiquitin C-terminal hydroxylase family which is involved in ubiquitin-dependent proteolysis. Nonsense mutations causing Liddle syndrome truncate the carboxyl terminus of *SCNN1B* and *SCNN1G* implicated in the ubiquitin-dependent degradation of the channel (Staub et al. 1996). A defect in this pathway thus could be responsible for the overactivity of a channel regulating ion transport in kidney. The *KCNA1, KCNA5,* and *KCNA6* genes belong to a cluster of potassium channels and are located at the *PHA2C* locus. The encoded proteins have the characteristic structure of voltage-gated ionic channels, with six potential membrane-spanning segments. Defects in one of these genes could be the primary cause of hyperkalemia in the affected subjects. However, the delayed rectifier function of KCNA potassium channels is

generally associated with the repolarization of nerve cells following an action potential, rather than with ion transport in kidney, and mutations in *KCNA1* have been found to cause episodic ataxia type 1 (Browne et al. 1994). Finally, another autosomal dominant form of hypertension has been mapped to chromosome 12p (Schuster et al. 1996). However, there are no particular biochemical features associated with this disease and the corresponding locus is more centromeric. Future determination of the molecular basis of PHA2 should lead to the identification of a possible new molecular pathway involved in blood pressure regulation and of new therapeutic targets in arterial hypertension.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Albert Einstein College of Medicine, http://sequence.aecom.yu.edu/chr12/12p13.3.1.html and http://sequence.aecom.yu.edu/chr12/12p13.3.2.html (for a physical map of the 12p13.3 region)

Australian Genome Research Facility, http://www.agrf.org.au/ (for the genotyping)

GenBank, http://www.ncbi.nlm.nih.gov/ (for the sequences of *SCNN1A*, *GNb3* and *SLC12A3*)

Genome Database, http://www.gdb.org/ (for the genes located in the 12p13.3 region)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim/ (for a review on PHA2)

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