Original articles

Angiotensin-related genes involved in essential hypertension: allelic distribution in an Italian population sample

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Background. **Blood pressure is a quantitative multifactorial trait influenced by environmental and genetic determinants. Although several candidate genes have been associated with the development of essential hypertension, the mechanisms of individual susceptibility still remain unclear. Knowledge on the distribution of genetic polymorphisms in different populations is fundamental for the assessment of the predictive value of genetic variation.**

Methods. **We genotyped 300 healthy normotensive subjects from the Italian population for three polymorphisms, at the angiotensinogen (AGT, M and T), angiotensin II type 1 receptor (AT1R, A and C) and angiotensin-converting enzyme (ACE, D and I) genes. Polymorphisms were analyzed by polymerase chain reaction and restriction enzyme digestion. Statistical analysis was performed to verify the agreement with the Hardy-Weinberg equilibrium.**

Results. **The observed allelic distribution was in accordance with estimates reported for Caucasian populations. Variant allelic frequencies were 0.36 for the T and C alleles at the AGT and AT1R locus and 0.47 for the I allele of the ACE gene. AT1R and ACE genotype frequencies were in Hardy-Weinberg equilibrium, while there was a deviation of the AGT genotypes from those predicted by the equation.**

Conclusions. **The studied polymorphisms are largely distributed in the Italian population sample, with a frequency of homozygous subjects for mutant alleles ranging from 9 to 22%. Epidemiology of mutations in the genes involved in blood pressure regulation provides tools to evaluate susceptibility to hypertension.**

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Introduction

In the general population blood pressure follows a normal distribution, and hypertension is defined on the basis of a threshold for therapeutic interventions. Family studies have shown a greater concordance of blood pressure levels in monozygotic than dizygotic twins, as well as in biological siblings than adoptive children[1].

Essential hypertension occurs as a consequence of a complex interplay between multiple environmental and genetic determinants, within a polygenic model[2]. During the last decades, advances in molecular biology have permitted clarification of the pathogenic mechanisms of hypertensive disorders. One of the main lines of investigation has included classic linkage analysis in families segregating for rare, Mendelian forms of hypertension. Genes responsible for glucocorticoid-remediable aldosteronism, Liddle's syndrome, and apparent mineralocorticoid excess have been mapped and cloned[3].

To study the predisposing genes involved in common essential hypertension, other strategies, such as analysis of affected sibling pairs or population association studies, have been used. The distribution of molecular variants of candidate genes has been compared in unrelated cases and controls, searching for marker genotypes with a possible causal role or located in close proximity to putative susceptibility loci[2]. In order to analyze co-segregation between the disease and several genotypes, animal studies that involve rodent models exhibiting spontaneous hypertension have also been applied[4].

By using the above-described methods, evidence has been obtained for a number of involved loci. Among the others, attention has focused on genes coding for α-adducin (on chromosome 4p16.3), insulin (19p13.2) and glucagon (17q25) receptors, enzymes

such as phospholipase A_2 (12q23-q24.1) and kallikrein (19q13), regulatory molecules such as endothelin-1 (6p23-p24), α_2-adrenergic (10q24-q26) and β_2-adrenergic (5q32-q34) receptors, as well as lipoprotein lipase (8p22) and glucocorticoid receptor (5q31) loci[2,5]. Molecular variants of genes encoding for components of the renin-angiotensin system have been associated with essential hypertension and ischemic heart disease, although with contrasting results[6-9].

In hypertensive sibling pairs linkage was observed for a point mutation leading to the substitution of threonin for methionin (M235T) at amino acid position 235 in the coding sequence of the angiotensinogen (AGT) gene (1q42-q43)[10].

Diallelic polymorphisms have been detected in the angiotensin II type 1 receptor (AT1R) gene at 3q21-q25: an A/C transversion located at position 1166 (A1166C polymorphism) has shown an increased frequency in hypertensive subjects, especially those with a more resistant form[11,12].

A polymorphic form of the angiotensin-converting enzyme (ACE) gene on chromosome 17q23 and involving the presence (insertion, I) or absence (deletion, D) of a 287-base pair Alu repeat sequence in intron 16 has been described[13]. In a large population-based study evidence has been obtained for the association and linkage of the ACE locus with hypertension as well as with the diastolic blood pressure in males[14].

In the present study we evaluated the distribution of AGT, AT1R and ACE gene polymorphisms in a representative sample of the Italian population.

Methods

A group of 300 unrelated normotensives (male/female ratio 5.5/1; geographical origin: northern Italy 6.4%, central and southern Italy 45% and 38.6% respectively, Sicily and Sardinia 10%) were recruited from a population-based sample of Italian subjects identified on a volunteer basis through local outpatient and university departments. Patients were selected from a population included in a larger genetic epidemiology study on the basis of the following criteria: 1) age 20-65 years, 2) no personal or family history of hypertension, 3) blood pressure levels < 140/90 mmHg (derived from repeated measurements performed in accordance with international guidelines[15]), 4) absence of antihypertensive treatment, and 5) exclusion of other cardiovascular disorders or clinically relevant diseases. Having obtained the patient's informed consent, a 4 ml volume of blood was collected in a heparinized tube. Genomic DNA was isolated following standard protocols[16].

Genotyping (Fig. 1) was performed by means of the polymerase chain reaction technique, with further restriction enzyme analysis when required. To study AGT gene polymorphism, an amplification reaction was performed involving a modified downstream primer that introduces a restriction half-site, as previously described[17]. After digestion with *Tth*111I enzyme, the expected products were separated on a 3% agarose (NuSieve) gel and visualized by ethidium bromide staining. AGT normal (M) and variant (T) alleles were detected as bands 165 and 141 base pairs in size respectively.

Genotypes for the A1166C polymorphism at the AT1R locus were determined by a previously reported mismatch-polymerase chain reaction/restriction strategy[18]. Digestion with the *Hae*III enzyme produced two fragments of 255 and 233 base pairs, corresponding to the A1166 and C1166 alleles.

The ACE gene I/D polymorphism was detected by polymerase chain reaction analysis, avoiding misclassification of heterozygous samples, as described elsewhere[9]. The expected I and D alleles, 319 and 597 base pairs in size, were visualized after electrophoresis on a 2.5% agarose gel and ethidium bromide staining.

Allele frequencies were determined by gene counting. To verify the Hardy-Weinberg equilibrium, χ^2 analysis was used to compare the observed and expected numbers calculated from gene frequencies.

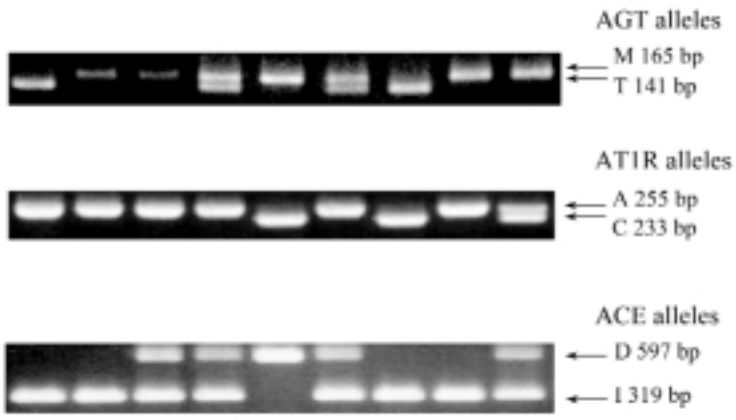

Figure 1. *Angiotensinogen (AGT), angiotensin II type 1 receptor (AT1R) and angiotensin-converting enzyme (ACE) gene polymorphisms. Representative agarose gels, stained with ethidium bromide, showing heterozygous (MT, AC, DI) and homozygous genotypes for mutant (TT, CC, II) and wild-type (MM, AA, DD) alleles. The molecular weights of the detected bands are shown.*

Results

The genotype and allele frequencies of the renin-angiotensin system genes in our population sample are shown in table I. There was an overall frequency of 0.64 for the M and of 0.36 for the T allele of the AGT gene. We found that 157 (52.3%) of 300 subjects were classified as heterozygous MT, 115 (38.4%) were homozygous MM, and 28 (9.3%) were homozygous for the AGT T variant allele. Among the study population, a 0.36 frequency was observed for the AT1R C mutant allele: 132 (44%) of 300 subjects were heterozygous AC and 42 (14%) were homozygous CC. The allelic frequencies of ACE I and of D polymorphism were 0.47 and 0.53 respectively. Eighty-three (27.7%) out of 300 subjects were homozygous DD, 67 (22.3%) were homozygous II, and 50% of the whole study population was heterozygous ID.

The study population was in Hardy-Weinberg equilibrium for the A1166C polymorphism at the AT1R locus and for the I/D genotypes of the ACE gene (χ^2 = 0.6 and 0.002, 2 df, $p > 0.4$). Our sample of unrelated Italian individuals did not exhibit Hardy-Weinberg equilibrium for the M235T polymorphism at the AGT locus ($\chi^2 = 6.1$, 2 df, $p < 0.05$). The reason for this absence of equilibrium is unknown. It does not seem to be consequent to technical inaccuracies, but it probably reflects a chance event.

Discussion

Identification of molecular factors that influence the individual susceptibility to essential hypertension is one of the main issues of medical science in the field of cardiovascular diseases. The renin-angiotensin system is an important component of blood pressure regulation, not only for sodium homeostasis but also as a modulator of vascular tone and cardiovascular structure[19]. Genes coding for components of this system may explain the genetic basis of high blood pressure and other cardiovascular disorders. The influence of polymorphic forms at the AGT, AT1R and ACE loci on several clinical features is still disputed[20-22]. Conflicting results may be due to the variable impact that mutations can have on the genetic background of populations. Literature data regarding the distribution of polymorphisms at the renin-angiotensin system genes in different geographical areas have been reviewed and compared, as shown in table II[12,18,20,22-38]. The frequency of the T235 AGT allele is higher in African Americans and Asian people than in Caucasians. The C1166 mutant allele of the AT1R gene shows the lowest frequency among African Americans, Chinese and Japanese. The frequency of the ACE I allele is higher in Chinese and Japanese than in any other population. The allele frequencies at the AGT, AT1R and ACE loci obtained in the present study were similar to those reported for the Italian[28,38] and other Caucasian populations.

Hereditary influences with mutations at several gene loci can be seen in different steps of blood pressure regulation. Permissive mutations for a rise in blood pressure have been identified but their penetrance is influenced by the interaction with environmental factors and the individual genetic background. The environmental impact is thought to be exerted at the level of phenotypic modifications as well as at that of gene expression[39].

The identification of further molecular variants that modulate blood pressure can be expected in the next

Table I. Genotype and allele frequencies.

Angiotensinogen genotypes			Alleles	
MM (no./total)	MT (no./total)	TT (no./total)	M	T
115/300 (38.4%)	157/300 (52.3%)	28/300 (9.3%)	0.64	0.36
Angiotensin II type 1 receptor genotypes			**Alleles**	
AA (no./total)	AC (no./total)	CC (no./total)	A	C
126/300 (42%)	132/300 (44%)	42/300 (14%)	0.64	0.36
Angiotensin-converting enzyme genotypes			**Alleles**	
DD (no./total)	DI (no./total)	II (no./total)	D	I
83/300 (27.7%)	150/300 (50%)	67/300 (22.3%)	0.53	0.47

Table II. Distribution of angiotensinogen (AGT), angiotensin II type 1 receptor (AT1R) and angiotensin-converting enzyme (ACE) gene polymorphisms in populations from different geographic areas.

	AGT genotypes (%)			AGT alleles		References
	MM	MT	TT	M	T	
Northern Europe	36.2	43.5	20.3	0.58	0.42	18, 27, 34
Central Europe	30.4	57.6	12	0.59	0.41	22
Southern Europe	40.2	41.1	18.7	0.61	0.39	29, 38
Middle-East	32.2	41.8	26	0.53	0.47	33
North America	34.7	48.6	16.7	0.59	0.41	23
North America (African Americans)	3.7	26.2	70.1	0.17	0.83	23
China and Japan	5.6	36.1	58.3	0.24	0.76	35, 37
Australia and New Zealand	36.6	49.8	13.6	0.615	0.385	20
	AT1R genotypes (%)			AT1R alleles		
	AA	AC	CC	A	C	
Northern Europe	59.3	34.8	5.9	0.77	0.23	18
Central Europe	48.8	41.6	9.6	0.7	0.3	12, 36
Southern Europe	46.7	42.6	10.7	0.68	0.32	28, 32, 38
Middle-East	57.4	36.6	6	0.76	0.24	33
North America	59	34	7	0.76	0.24	30
North America (African Americans)	91.9	7	1.1	0.95	0.05	30
China and Japan	85.2	14.1	0.5	0.92	0.08	35, 37
Australia and New Zealand	46.4	50	3.6	0.71	0.29	31
	ACE genotypes (%)			ACE alleles		
	DD	DI	II	D	I	
Northern Europe	26.3	51.4	22.3	0.52	0.48	27, 34
Central Europe	24.8	54.4	20.8	0.52	0.48	24
Southern Europe	32.7	44.5	22.8	0.55	0.45	29, 32, 38
Middle-East	39.5	48.8	11.7	0.64	0.36	33
North America	25	58.6	16.4	0.54	0.46	25
North America (African Americans)	37	51	12	0.625	0.375	26
China and Japan	15.5	43.1	41.4	0.37	0.63	35,37
Australia and New Zealand	27.6	49.8	22.6	0.525	0.475	20

When multiple surveys were available, data are shown as mean values.

years. A close interplay between animal modeling and human population studies together with new strategies based on the information provided by the human genome project will be needed. Surveys on genetic factors responsible for blood pressure variability will improve the understanding of the pathophysiology of hypertension and provide tools for an early identification of those individuals at higher risk. Genetic epidemiology methods will allow more specific therapeutic interventions and increasingly effective preventive measures.

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