Table 1

Frequency of SNPs and Standardized Linkage-Disequilibrium Coefficients

SNP	FREQUENCY (n = 192 Chromosomes)	$D' = D/D_{max}$[a]					
		235Thr	11535A	11608T	12058A	12194C	12429T
Met235Thr	.42	...	...	...	...	...	...
C11535A	.31	−.803	...	...	...	...	...
C11608T	.33	−.667	1.000	...	...	...	...
G12058A	.06	.568	−.733	−.750	...	...	...
A12194C	.06	.734	−.754	−.769	.911	...	...
C12429T	.07	.629	−.771	−.786	1.000	1.000	...
T12822C	.39	.816	−.829	−.720	.863	.874	.766

[a] D' is the standardized coefficient of linkage disequilibrium; D is the classical coefficient of linkage disequilibrium; and D_{max} is the maximum D value that is possible given the allele frequencies.

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SNPs at the 3′ End of the Angiotensinogen Gene Define Two Haplotypes Associated with the Common 235Met Variant

To the Editor:

Nakajima et al. (2002) have provided a valuable SNP-based haplotype map of the angiotensinogen gene, *AGT* (MIM 106150), in both Japanese and white American populations. Several linkage and association studies have supported the hypothesis that angiotensinogen plays a role in the pathogenesis of essential hypertension (MIM 145500) and preeclampsia (MIM 189800) (Jeunemaitre et al. 1992; Ward et al. 1993; Hata et al. 1994). An exon 2 SNP that results in a Thr-Met polymorphism at codon 235 has been associated with variation in plasma-angiotensinogen concentrations and with hypertensive disorders. It is not clear whether this is due to either a deleterious effect of a 235Thr-bearing allele or a protective effect of the 235Met allele. In nonpregnant subjects, 235Met is associated with lower concentrations of plasma angiotensinogen, although, in normotensive pregnant subjects, this pattern was reversed in the population that we studied (Jeunemaitre et al. 1992; Morgan et al. 2000). We have genotyped polymorphisms in both the 5′ flanking region (corresponding to G−217A, A−20C, and A−6G) and exon 2 (Thr174Met [C3889T] and Thr235Met [C4072T]) of *AGT,* in 96 healthy white Europeans from Nottingham, United Kingdom, who were recruited sequentially from a blood-donor clinic (Morgan et al. 1996). This investigation demonstrated patterns—both of linkage disequilibrium and of haplotype frequencies—similar to those described for the Utah population that Nakajima et al. (2002) studied. We have confirmed their observation that a single haplotype carrying 235Met (4072T) accounts for more than half of the genes in white Europeans (58% in the Nottingham population). Variants in both the 5′ flanking region and exon 2 were found only on alleles bearing 235Thr.

We have also screened the 3′ end of the gene—including the 3′ UTR of exon 5—and the 1,350 bases of flanking region between exon 5 and the *AGT* dinucleotide repeat polymorphism (Kotelevstev et al. 1991) (EMBL Nucleotide Sequence Database accession number AJ277498). We used SSCP analysis and direct sequencing, to characterize six SNPs (table 1). Three polymorphisms identified in this region have not, to our knowledge, previously been described; the remaining three correspond to SNPs 40 (C11535A), 41 (C11608T), and 42 (G12058A), as observed by Nakajima et al. (2002).

The frequencies of haplotypes that combine these six SNPs and Thr235Met were estimated by the expectation-maximization method, by use of Arlequin software. Strong linkage disequilibrium was observed between all polymorphisms, and five haplotypes accounted for 91% of those observed in this study (table 2). Interestingly, polymorphisms at C11535A and C11608T, which are in complete linkage disequilibrium with each other, defined two common haplotypes bearing 235Met. To our knowledge, these are the only SNPs that have, to date, been described in white Europeans, which split the common

Table 2

Common Haplotypes Defined by the Met235Thr Polymorphism and Six SNPs at the 3′ End of *AGT*

Haplotype	Allele at SNP[a]							Frequency
	Met235Thr	C11535A	C11608A	G12058A	A12194C	C12429T	T12822C	
1	Met	C	C	G	A	C	T	.27
2	Met	A	A	G	A	C	T	.27
3	Thr	C	C	G	A	C	C	.28
4	Thr	C	C	A	C	T	C	.05
5	Thr	C	C	G	A	C	T	.04

[a] Nucleotides are numbered with respect to the transcription start site.

haplotype bearing 235Met; all other variants have been described in association with the 235Thr allele. C11535A lies within the 3′ UTR of exon 5; C11608T lies 30 bases downstream from the 3′ end of exon 5. Whether they have functional effects on angiotensinogen expression requires further investigation, but it is worth noting that in vitro experiments have demonstrated that there is enhancer activity in this region (Nibu et al. 1994*a*, 1994*b*). Given the interest in the Thr235Met polymorphism as a marker for hypertensive disorders, we recommend that genotyping at C11535A or C11608T be included in the haplotyping profile for angiotensinogen in linkage-disequilibrium studies.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Arlequin, http://lgb.unige.ch/arlequin/ (for Arlequin program)
EMBL Nucleotide Sequence Database, http://www.ebi.ac.uk/embl/ (for *AGT* 3′ flanking region [accession number AJ277498])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for EHT [MIM 145500], *AGT* [MIM 106150], and preeclampsia [MIM 189800])

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