A growing chain of evidence linking genetic variation in angiotensinogen with essential hypertension: focus on “A haplotype of human angiotensinogen gene containing −217A increases blood pressure in transgenic mice compared with −217G,” by Jain et al.

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The prevalence of hypertension across the globe continues to rise despite advances in treatment and an ever-growing battery of antihypertensive drugs. Untreated hypertension remains an important risk factor for cardiovascular diseases, including stroke. The renin-angiotensin system (RAS) has long been recognized as a critical mediator of blood pressure regulation, RAS blockers are particularly effective antihypertensive agents. Continued interest in clinically blocking the system is evidenced by the recent FDA approval of the renin inhibitor aliskiren as a first-line antihypertensive (16). Despite the impact of environmental and behavioral factors, it is now widely accepted that there exists a strong genetic component that influences the susceptibility to hypertension.

Angiotensinogen (AGT) is the substrate of angiotensin II (ANG II) and the only known target of the aspartyl protease renin. Numerous studies have offered convincing evidence physiologically linking AGT with hypertension. These include a strong correlation between plasma AGT levels with blood pressure, decreased blood pressure after administration of anti-AGT antibodies, increased blood pressure after injection of AGT, and increased blood pressure in animals with AGT transgenes (10, 20, 23, 27). Perhaps the seminal study mechanistically implicating aberrant AGT gene regulation as a cause for hypertension came from Smithies and colleagues (18, 26) who reported that increasing the number of copies of the mouse AGT gene resulted in increased AGT expression and elevated pressure. Other studies implicate tissue-specific dysregulation of AGT as a potential cause of hypertension (7, 19, 21).

Genetic evidence implicating AGT in essential hypertension in humans was first reported in 1992 by Jeunemaitre et al. (17). AGT was also reported to be associated with preeclampsia (pregnancy-induced hypertension) (28). Like other complex diseases, linkage was confirmed in some populations (3, 4, 28) but was refuted in others (1). At first, the primary variant alleles of AGT investigated were those encoding amino acid changes at positions 174 (T174M, rs4762) and 235 (M235T, rs699) (17, 28). Since then, there have been hundreds of studies by others suggesting that variants at −20 (rs5050) and −217 (rs5049) are potentially the most important physiologically (13, 14, 29). Accordingly, the data accumulated to date support a concept that polymorphisms in the AGT promoter may act cell specifically to differentially regulate AGT transcription in ANG-producing tissues. However, no matter how convincing the in vitro data are, it would take a giant “leap of faith” to conclude, based only on current evidence, that variation at −217 and −20 in the human AGT promoter is physiologically and genetically relevant in humans. Jain et al. (15) present experimental data functionally implicating one of these variants in hypertension.

Jain et al. (15) used a method of gene targeting first described by Bronson et al. (2) and previously employed by us to examine allele-specific AGT expression (5, 6). The experimental strategy entails using gene targeting in mouse embryonic stem cells to insert transgenes differing only at selected polymorphic positions to a defined locus in the genome. The hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus is the one employed because it is 1) permissive for expression of many transgenes, 2) does not effect tissue- and cell-specific expression of the transgene, and 3) importantly, homologous recombination at this locus is easily selected. That independent HPRT-targeted transgenes become inserted at a

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precise location in the genome in a single copy allows researchers to avoid the typical artifacts on expression caused by position effects and copy number differences that plague standard transgenesis. We previously showed that independent lines of mice carrying the human AGT gene are faithfully expressed at the tissue- and cell-specific level, and retain their normal induction by exogenous cues (6). Importantly, the level of protein expression is equivalent across independently generated lines of mice. Therefore, the ability to make a direct comparison among multiple independently inserted transgenes is an important strength of the technique and a strength of their study.

The investigators tested the hypothesis that the G-217A variant in the human AGT promoter increases transcription of the AGT gene leading to increased circulating AGT, increased ANG II, and hypertension (Fig. 1). Supporting this hypothesis was their previous results that G-217A is associated with hypertension in African Americans and is the site for differential binding of glucocorticoid receptor (GR; NR3C1) and transcription factors belonging to the CCAAT/enhancer binding protein (C/EBP) family (13, 14). A recent meta-analysis of 46 published studies supports an possible etiological role for the −217 variant in hypertension (24). They performed gene targeting at the HPRT locus and then generated “humanized” mice by breeding transgenics carrying the HPRT-targeted human AGT (either −217A or −217G) with mice expressing a highly regulated form of the human renin gene (25) to generate a pair of double transgenic strains. The double transgenics are necessary because of the strict species specificity in the enzymatic reaction between AGT and renin (11). They report that mice carrying −217A exhibited increased human AGT mRNA in the liver and kidney and increased circulating human AGT. Both double transgenic strains exhibited increased plasma ANG II when compared with nontransgenic controls, but plasma ANG II was ~25% higher in −217A than −217G mice. Like the increase in plasma ANG II, the levels of endogenous mouse renin mRNA in the kidney of both double transgenic strains was suppressed compared with controls, but the decrease was more robust in −217A-containing mice. Apparently, increased ANG II feedback inhibited expression of the endogenous renin gene. Blood pressure measured by both radiotelemetry and tail cuff was increased in both strains compared with controls, but was elevated to a greater extent in −217A (+15 mmHg) than −217G (+10 mmHg). This augmentation was more evident during the nighttime hours when the mice are most active (+20 mmHg for −217A vs. +10 mmHg for −217G). Consistent with the hypertension being ANG II-dependent, there was an augmented depressor response to losartan in the −217A mice.

The study by Jain et al. (15) provides an elegant proof of principle that variants in the human AGT promoter can functionally modulate the levels of AGT expression and circulating ANG II in vivo, ultimately raising blood pressure. This report provides one more link in a long chain of evidence linking AGT variants with hypertension. Of course, many questions remain. Would the results differ if the mice were fully “humanized”? This could be accomplished by breeding with AGT-deficient knockout mice. This would functionally cripple the endogenous mouse RAS and effectively replace the mouse RAS with the human RAS. Is elevated expression of AGT related to an increase in GR and C/EBP transcription factor binding to −217A vs. −217G in chromatin? Are the increases in AGT expression limited to liver and kidney, or is it evident in other important sites of AGT expression (i.e., adipose tissue and brain)? Is the hypertension primarily due to increased plasma AGT and ANG II, or is the rise in tissue AGT and ANG II functionally important? Does elevated AGT and ANG II translate to increased production of other important ANG peptides such as ANG-(1–7)? Are other polymorphisms in the AGT promoter physiologically relevant? Clearly, further analysis of these and other novel animal models is warranted, which should shed additional light on the mechanisms by which variants in the AGT promoter cause hypertension.

DISCLOSURE
The review of this article submitted by the editor-in-chief of this journal was handled by a consulting editor, Dr. Fred Luft.

REFERENCES


