1.a. Full title: Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and angiotensinogen (AGT) G-6A polymorphism predict stroke case status.

1.b. Abbreviated Title (Length 26): ACE I/D polymorphism, AGT G-6A polymorphism, and stroke

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3. Time Line:  
   Measurement of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and the angiotensinogen (AGT) G-6A polymorphism are complete for the MRI cerebral infarct case-control and incident stroke case-cohort samples. Analyses will be completed subsequently.

4. Rationale:  
   The renin-angiotensin system (RAS) is a principal mediator of blood pressure control, sodium and water homeostasis, and cardiovascular function and structure. The RAS has been extensively studied in the past decades as an important mediator of hypertension and hypertensive end-organ damage. Two components of the RAS that have received considerable attention are the angiotensin-converting enzyme (ACE) and angiotensinogen (AGT).

   Recent studies have concluded that the ACE I/D polymorphism is associated with ischemic stroke in a Japanese hypertensive population (Kario et al., Circulation 93:1630-3, 1996), with thrombic brain infarction in Japanese patients 60 years or younger (Doi et al., Atheroscler 132:145-50, 1997), with parental history of stroke (PHS) in hypertensive

Studies reporting a negative association between the ACE I/D polymorphism and CVD include analyses in a sample obtained from the combination of two case-referent studies and a cross-sectional study from Denmark (Agerholm-Larsen et al., Ann Intern Med 127:346-55, 1997) and analyses in normotensive stroke subgroups (Ueda et al., J Hypertens 13:1597-601, 1995). Ueda et al. reported a weak association between the DD genotype and ischemic stroke in a hypertensive subgroup. Additionally, the ACD I/D polymorphism was not found to influence risk of incident stroke in a large prospective study (Zee et al., Stroke 99:340-3, 1999). Notsu et al. (Stroke 30:1881-6, 1999) did not find any significant association between the ACE I/D polymorphism and silent brain infarction (SBI) or symptomatic subcortical infarction (SSI).

Inoue et al. identified a common variant in the proximal promoter of angiotensinogen (AGT), 6bp upstream from the initiation site of transcription, where an adenine is substituted for a guanine (G-6A) (JCI 99:1786-97, 1997). The authors suggested that the nucleotide substitution affects basal transcription rate of the AGT gene. They tested promoter function in cultured cells and studied AGT oligonucleotide binding with nuclear proteins. Inoue et al. concluded that the interaction of at least one trans-acting nuclear factor is affected by the A-6 variant. Additionally, Inoue et al. reported that the A-6 variant is in tight linkage disequilibrium with the T235 variant, and the A-6 variant demonstrated significant association with essential hypertension.

There are few reports evaluating the contribution of the G-6A polymorphism to risk of cardiovascular disease. Brugada et al. reported that the G-6A polymorphism was not associated with expression of LVH in patients with hypertrophic cardiomyopathy (HCM).

To the best of our knowledge, there are no reports that evaluate a possible association between the G-6A polymorphism and CVD and only a few studies have evaluated the contribution of the ACE I/D polymorphism to risk of CVD, with controversial conclusions. We propose to evaluate the association of the ACE I/D and AGT G-6A polymorphisms with incident clinical and subclinical stroke in the MRI cerebral infarct case-control and incident stroke case-cohort groups of the ARIC study.

5. **Main Objectives:**

a. Ability of the ACE I/D polymorphism to predict MRI cerebral infarct and incident stroke case status, both individually and after considering the predictive ability of traditional risk factors. Subgroup analyses will be carried out separately for clinical and subclinical strokes, and divided by stroke type. Race-specific effects will also be explored.

b. Ability of the AGT G-6A polymorphism to predict MRI cerebral infarct and incident stroke case status, both individually and after considering the predictive ability of traditional risk factors. Subgroup analyses will be carried out separately for clinical and subclinical strokes, and divided by stroke type. Race-specific effects will also be explored.
6. **Data (variables, time window, source, inclusions/exclusions):**

   ARIC’s MRI cerebral infarct case-control and incident stroke case-cohort groups will be used for these analyses. The primary dependent variable is clinical or subclinical stroke case status. Independent variables include, but are not limited to, ACE I/D polymorphism, AGT G-6A polymorphism, race, age, gender, BMI, smoking status, plasma lipid levels, blood pressure, blood pressure treatment and hypertension status.