ARIC Study Manuscript Proposal #717

PC Reviewed: 02/04/00	**Status: Approved**	**Priority: 1**
SC Reviewed: 02/25/00	**Status: Approved**	**Priority: 1**

1.a. Full Title: Adducin (ADD) Gly460Trp polymorphism and G-protein β3 subunit (GNB3) C825T polymorphism predict stroke case status.

1.b. Abbreviated Title (Length 26): ADD Gly460Trp, GNB3 C825T & stroke

2. Writing Group (list individual with lead responsibility first):

Lead: Eric Boerwinkle
Address: Human Genetics Center
University of Texas - Houston Health Science Center
PO Box 20334
Houston, TX 77225
Phone: 713-500-9816 Fax**:** 713-500-0900;
E-mail: eboerwin@gsbs.gs.uth.tmc.edu

First: Alanna Morrison Peter Doris Aaron Folsom Javier Nieto

3. Time Line:

Measurement of the adducin (ADD) Gly460Trp polymorphism and the G-protein β3 subunit (GNB3) C825T polymorphism are complete for the MRI cerebral infarct case-control and incident stroke case-cohort samples. Analyses will be completed subsequently.

4. Rationale:

First purified from human erythrocytes (Gardner et al., J Biol Chem 261:1339-48, 1986), adducin (ADD) is an alpha/beta heterodimeric cytoskeleton protein involved in transmembrane ion transport. The alpha-ADD gene has been localized to 4p16.3 and the beta-ADD gene has been localized to 2p13 (Joshi et al., J Cell Biol 115:665-75, 1991; Lin et al., Genomics 25:93-9, 1995; Gilligan et al., Genomics 43:141-8, 1997). Recent findings support the hypothesis that variation in the ADD protein may affect ion transport through modification of actin cytoskeleton assembly and modulation of sodium pump activity (Manunta et al., J Nephrol 10:172-8, 1997). These studies suggest that alpha-ADD plays a role in both human and rat hypertension.

The Milan hypertensive rat (MHS) develops a genetic form of renal hypertension in which it is possible to reconstruct the sequence of events leading from a single point mutation in the alpha-ADD gene to the complex phenotype of essential hypertension (Cusi et al., Ped Nephrol. 7:865-70, 1993). A polymorphism in the alpha-ADD gene was found

to be responsible for up to 50% of the blood pressure difference between MHS rats and the normotensive control strain (MNS) (Bianchi et al., Clin & Exp Pharm & Physiol 22:S7-9, S399-405 1995). It is hypothesized that modulation of kidney function is a result of variation in the ADD proteins affecting the overall capacity of tubular epithelial cells to transport ions, thus leading to induction of hypertension (Cusi et al., Kidney Intrnatl 49:1754-9, 1996; Manunta et al., J Human Hypertension 10:649-56, 1996). It has also been proposed that upregulation of Na-K pump activity in MHS is linked to alteration in the ADD proteins (Ferrandi et al., Hypertension 28:1018-25, 1996).

In humans, significant linkage was detected in hypertensive sib pairs for three markers located at different distances from the alpha-ADD gene (Cusi et al., Lancet 349:1353-7, 1997). Of the three markers used in the study, the most significant level for linkage was found with the marker closest to the alpha-ADD locus. Another study using four multiallelic markers surrounding the alpha-ADD locus detected significant association between the markers and hypertension, where the independent contribution of marker genotypes to variability in blood pressure decreased exponentially with the increase in distance between the maker and the alpha-ADD locus (Bianchi et al., Clin & Exp Pharm & Physiol 22:S7-9, 1995; Casari et al., Hypertension 25:320-6, 1995). Cusi et al. also reported significant association between the alpha-ADD gene Gly460Trp polymorphism and hypertension, and demonstrated that hypertensive patients with the Trp460 allele have greater sensitivity to changes in sodium balance. Additionally, Castellano et al. reported a positive association between the Trp460 allele and blood pressure when blood pressure was considered as a dichotomous variable, but a only a weak association was found when 24h ambulatory blood pressure values were analyzed. In this population based study, no association was found with either left ventricular mass or carotid wall thickness and the Gly460Trp polymorphism (J Hypertension 15:1707-10, 1997). Finally, the Gly460Trp polymorphism was found to be relatively common in a Japanese population as compared to the previously reported mutation frequencies in Caucasian populations (Sugiyama et al., Hypertension 31:730-3, 1998). The authors did not exclude the involvement of alpha-ADD in the pathogenesis of hypertension, but suggested that the Trp460 polymorphism may have little effect on hypertension in Japanese populations. To the best of our knowledge, no study has evaluated the alpha-ADD Gly460Trp polymorphism with regard to stroke.

Studies on immortalized lymphoblasts from patients with essential hypertension indicate that enhanced sodium-proton exchanger (NHE) activity is genetically fixed and associated with increased cell proliferation (Rosskopf et al.; JCI 92:2553-9, 1993; Cardio Res 29:254-9, 1995). Additional studies suggest that these phenomena are a result of enhanced activation of pertussis toxin (PTX)-sensitive G proteins (Siffert et al.; JCI 96:759-66, 1995; Hypertens 26:649-55, 1995). Siffert et al. (Nat Gen 18:45-8, 1998) reported a novel polymorphism (C825T) in exon 10 of the gene encoding the β3 subunit of heterotrimeric G proteins (GNB3). An association was observed between the T allele and generation of a splice variant, GNB3-s, in which the nucleotides 498-620 of exon 9 are deleted. The product of this in-frame deletion, Gβ3-s, was found to be a functional protein, predominantly expressed in cells from individuals carrying the T allele.

While analyses performed by Siffert et al. suggest a significant association of the T allele with essential hypertension (Nat Gen 18:45-8, 1998), to our knowledge no studies have evaluated an association of the C825T polymorphism with risk of cerebrovascular disease (CVD).

5. Main Objectives:

a. Ability of the ADD Gly460Trp polymorphism to predict MRI cerebral infarct and incident stroke case status, both individually and after considering the predictive ability of traditional risk factors, particularly blood pressure levels and hypertension status. 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 GNB3 C825T polymorphism to predict MRI cerebral infarct and incident stroke case status, both individually and after considering the predictive ability of traditional risk factors, particularly blood pressure levels and hypertension status. 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 this analysis. The primary dependent variable is clinical or subclinical stroke case status. Independent variables include, but are not limited to, the ADD Gly460Trp polymorphism, GNB3 C825T polymorphism, race, age, gender, waist-to-hip ratio, smoking status, fibrinogen and plasma lipid levels, von Willebrand factor, ECG left ventricular hypertrophy, blood pressure, blood pressure treatment and hypertension status.