1.a. Full Title: The association of myostatin genotypes with insulin resistance and hypertension in African Americans from the Atherosclerosis Risk in Communities Study

1.b. Abbreviated title: Myostatin genotype, insulin resistance, and hypertension

2. Writing group:
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3. Timeline:
   Measurement of both the myostatin A55T (AluI) and K153R (BanII) sequence polymorphisms has been completed in the African American portion of the ARIC cohort. A draft manuscript will be distributed for internal circulation by November 2001.

4. Rationale:
   Myostatin (MSTN; growth and differentiation factor 8; GDF8) was first identified by McPherron et al. (1997) as a negative regulator of skeletal muscle growth. The investigators created a myostatin null mouse and demonstrated 3-fold increases in skeletal muscle mass with no increase in fat mass compared to wildtype animals. Higher levels of myostatin have been observed in runt piglets (Ji et al. 1998) and in HIV-infected men with muscle wasting (Gonzalez-Cadavid et al. 1998), consistent with myostatin’s role as a negative regulator of growth. Moreover, several groups have described mutations in the bovine homolog of the myostatin gene resulting in a “double-muscle” phenotype, best exemplified by the Belgian Blue strain (Grobet et al. 1997, 1998; Kambadur et al. 1997; McPherron and Lee 1997). These increases in muscle mass are apparently the result of muscle fiber hypertrophy rather than hyperplasia (Zhu et al., 2000). Both Carlson et al. (1999) and Wehling et al. (2000) have independently reported that both myostatin expression and protein content are greater in fast-twitch compared to slow-twitch skeletal muscle fibers. These observations indicate that myostatin’s growth inhibiting action is likely to be more prominent in fast-twitch muscle fibers, which may be explained in part by posttranslational modifications of the myostatin protein (Wehling et al. 2000).
The proportion of skeletal muscle fiber types has been associated with glucose disposal, obesity and insulin resistance. Fast twitch fibers, especially type IIb fibers have been associated with NIDDM, obesity and insulin resistance in several independent investigations (Eriksson et al. 1994; Gaster et al. 2001; Hickey et al. 1995; Kriketos et al. 1996; Lillioja et al. 1987; Marin et al., 1994; Nyholm et al. 1997). Slow twitch, or type I, fibers are associated with enhanced glucose disposal (Gaster et al. 2001). Thus, an increase in type IIb skeletal muscle fibers either as a result of physical inactivity or prenatal development would by hypothesized to increase risk of insulin resistance and the development of NIDDM and diabetes. Moreover, several investigations have determined that higher proportions of type IIb muscle fibers are associated with hypertension (Endre et al. 1998; Hedman et al. 2000; Houmard et al. 2000; Toft et al. 1999), likely the result of reduced capillarization associated with those fibers compared to types I and IIa (Endre et al. 1998; Houmard et al. 2000).

In 1999, we (Ferrell et al.) examined sequence variation in the human myostatin gene and reported several sequence variations, two of which (A55T and K153R) were present in >10% of African Americans. The rare allele of each of these polymorphisms was present in fewer than 4% of Caucasians, a significant difference compared to African Americans. While we did not observe significant associations with skeletal muscle mass in that report, the A55T and K153R variants are highly conserved among mammals and birds indicating a possible influence on myostatin function. Such an influence could then impact on skeletal muscle fiber type proportions, thereby impacting insulin sensitivity and possibly hypertension.

The aims of the present investigation are to determine the association of the myostatin A55T and K153R allele polymorphisms in relation to insulin resistance, obesity, body composition, and hypertension in African Americans from Jackson, MS and Forsyth County (n = 4,212). Based on these analyses, we will investigate the hypothesis that insulin resistance may be mediated by the association between the A55T and/or K153R polymorphisms and physical activity and obesity status. A secondary hypothesis is that myostatin genotype will be related to blood pressure as a consequence of insulin resistance, after adjusting for age, gender, physical activity and obesity status.

5. Main Issues/Hypotheses to be addressed:
   a. Influence of both the myostatin A55T and K153R polymorphisms on insulin resistance status. Analysis will include age, gender, BMI, smoking, and physical activity as covariates in the analysis. Response variables will include fasted glucose and insulin. Multiple regression analysis will be used to determine the proportion of variance explained by each variable.
   b. Influence of the haplotypes of the A55T and K153R polymorphisms on insulin resistance as outlined in ‘a’ above, by examining 9 possible myostatin haplotypes based on the three possible genotypes for each of the two variants.
   c. Influence of myostatin A55T and K153R polymorphisms (and haplotypes) on body composition and obesity. Analysis will include age, gender, smoking, and physical activity as covariates in the analysis. Response variables will include BMI and waist-to-hip ratio. Multiple regression analysis will be used to determine the proportion of variance explained by each variable.
d. Influence of myostatin A55T and K153R polymorphisms (and haplotypes) on blood pressure. Analysis will include age, gender, obesity, smoking, and physical activity as covariates in the analysis. Response variables will include brachial systolic and diastolic blood pressure (SBP and DBP), as well as presence or absence of hypertension as defined by the current use of hypertension medications and/or a SBP > 160 mmHg or a DBP > 95 mmHg. Multiple regression analysis will be used to determine the proportion of variance explained by each variable.

e. For all analyses, gender-specific effects will be explored.

6. Data (variables, timeline, source, inclusion/exclusion):
Both the myostatin A55T and K153R variants have been genotyped in the African American portion of the ARIC cohort as part of an ancillary study examining gene-environment interactions. Relationships between the myostatin genotypes (and haplotypes) and insulin resistance, body size, and blood pressure measures will be tested using multiple regression and mixed models procedures. A draft manuscript will be distributed for internal circulation by November 2001. All non-African Americans and African Americans from Minnesota and Washington County will be excluded from the study.

7.a. Will the data be used for non-CVD analysis in this manuscript? _X_ Yes ___No

7.b. If Yes, is the author aware that the file ICTER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = ‘CVD Research” would be used? _X_ Yes ___No
(This file ICTDER01 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? _X_ Yes ___No

8.b. If Yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER01 must be used to exclude those with the value RES_DNA = “No use/storage DNA”? _X_ Yes ___No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: [http://bios.unc.edu/units/csc/ARIC/stdy/studymem.html](http://bios.unc.edu/units/csc/ARIC/stdy/studymem.html)

_X_ Yes ______ No