1. **Full Title:** Peroxisome Proliferator-Activated Receptor γ (PPARG) and Neuropeptide Y (NPY) Polymorphism and Retinal Vessel Diameters in African-Americans.

2. **Abbreviated Title (Length 26 characters):** PPARG and Retinal Diameter

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

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4. **Timeline:**

   The intent of this analysis is to investigate the association of the peroxisome proliferator-activated receptor γ (PPARG) and Neuropeptide Y (NPY) gene and retinal vessel diameters in African-Americans in the ARIC study, as part of a proposed series of analyses using Ancillary study 1995.07 genetic data to investigate associations of retinal microvascular disease. Initial analyses and writing will take place between August and Oct 2005, and final writing and manuscript submission between Nov 2005 and Jan 2006.

5. **Rationale:**

   Changes in retinal vessel calibers may reflect systemic microvascular processes. Recent population-based studies show that narrower retinal arteriolar diameters are associated with blood pressure (1), and predict the incidence of type 2 diabetes (2,3) and hypertension (4,5), independent of other risk factors. Wider retinal venular diameters have been further shown to predict gross proteinuria (6) and progression of diabetic retinopathy (7), suggesting that wider venular diameter may also be marker of diabetes severity.

   There is some evidence that genetic factors may influence the calibers of retinal blood vessels (8). In the Beaver Dam Eye Study, the between siblings correlation (95% confidence interval) for arteriolar and venular diameters were 0.23 (0.16, 0.31) and 0.20 (0.12, 0.28), respectively, whereas the spousal correlations were not significantly different from 0. However, the exact genetic determinants of retinal vessel diameters are unknown.

   **Peroxisome proliferator-activated receptor γ (PPARG)**

   One of the most promising genetic risk factor for insulin resistance and type 2 diabetes is a polymorphism in the peroxisome proliferator-activated receptor γ (PPARG) gene (9-11).
PPARG is a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily and is expressed by macrophages, endothelial cells, and vascular smooth muscle cells. It regulates gene expression of key proteins involved in lipid metabolism, vascular inflammation, and proliferation contributing to atherogenesis, and is the target for the thiazolidinedione class of antidiabetic drugs (11). PPARG may play a role in blood pressure (12) and lipid regulation (13), and has been linked with incident myocardial infarction (14), although not all studies have been consistent (15).

The common codon 12 proline to alanine (Pro12Ala) substitution polymorphism produces PPARG protein with lower transcriptional activity (11). A number of studies suggest that carriers of the 12Ala variant allele are at reduced risk of type 2 diabetes (10,16-19), as well as diabetic complications, such as nephropathy (20). In the Nurses Health Study, carriers of the PPARG variant 12Ala allele had a reduced risk of type 2 diabetes compared with noncarriers, with unadjusted and adjusted odds ratios of 0.74 (95% CI 0.55–1.00) and 0.72 (0.52–0.99), respectively (18).

In the ARIC study, PPARG polymorphism at codon 12 was characterized in 1,441 African-American participants (21). Consistent with previous findings in whites, African Americans with the 12Ala allele were less likely to have type 2 diabetes than those without (OR 0.64, 95% CI 0.34–1.20), although this was not statistically significant. Among nonobese individuals, the 12Ala allele was associated with significantly lower insulin (P=0.001), lower HOMA-IR (P= 0.002), higher fasting glucose-to-insulin ratio (P=0.005), and lower diastolic blood pressure (P=0.02). This suggests that among nonobese African Americans, the Pro/Ala genotype may be associated with markers of greater insulin sensitivity.

We have previously demonstrated variation in the relationship of retinal vessel diameter and incident diabetes in the ARIC study (2), being possibly stronger in African-Americans (OR 1.47, 95% CI 1.05-2.02, comparing a standard deviation decrease in retinal AV ratio) than in whites (OR 1.21, 95% CI 1.02-1.43, p-value for interaction 0.15). We have also found that the relationship of retinal arteriolar narrowing and incident diabetes was stronger in individuals with lower BMI of less than 28kg/m² (OR 1.50, 95% CI 1.09-2.07) than those with BMI 28 and higher (OR 1.21, 95% CI 1.02-1.42, p-value for interaction 0.05).

The current proposal will allow us to test the hypothesis that PPARG polymorphism may be related to retinal vessel diameters in African-American participants of the ARIC study.

**Neuropeptide Y (NPY)**

Neuropeptide Y (NPY) is a 36-amino acid polypeptide mainly present as an intact peptide in the central and peripheral nervous system, several peripheral organs, and plasma (22). The gene that codes NPY is located on chromosome 7 p15.1. A functional leucine 7 to proline 7 (Leu7Pro) polymorphism was recently observed. This polymorphism has been associated with variation in blood pressure (23), serum total and LDL cholesterol (24), serum triglycerides (25), and accelerated progression of carotid atherosclerosis in both obese healthy subjects and patients with type 2 diabetes (26).

The Pro7 substitution has also been linked, inconsistently, with diabetic retinopathy (27-29). In one study on 86 subjects (27), the frequency of retinopathy in patients with the Leu7Pro polymorphism was 25% (2 out of 8) compared to those without Leu7Pro polymorphism, 6.4% (5 out of 78) (p=0.126). At 10-year follow-up, the cumulative prevalence of retinopathy was 88% in patients with Leu7Pro polymorphism and 50% in those without (p=0.040).

The current proposal will examine the association of NPY polymorphism and retinal vessel diameters in African-American participants of the ARIC study.
5. Main Hypothesis/Study Questions:

(1) To describe the association of retinal vessel diameter with polymorphism of PPARG gene in African-Americans, to examine if this association is independent of diabetes status, obesity, and blood pressure, and if association is different in subgroups stratified by BMI

- Hypothesis: Polymorphisms of PPARG gene are related to retinal vascular caliber, independent of other factors, and the association may be stronger in individuals with lower BMI. Specifically, the 12Ala variant allele is associated with wider retinal arteriolar diameters and this association is more pronounced in persons with lower BMI.

(2) To describe the association of retinal vessel diameter with polymorphism of NPY gene in African-Americans and to examine if this association is independent of diabetes status, and blood pressure

- Hypothesis: Polymorphisms of NPY gene are related to retinal vascular caliber, independent of other factors. Specifically, the Pro7 variant allele is associated with narrower retinal arteriolar diameters and wider venular diameters.

6. Data (variables, time window, source, inclusions/exclusions):

(1) Retinal variables: retinal arteriolar and venular diameters, AV ratio, retinopathy, focal arteriolar narrowing, artery-venous nicking

(2) PPARG genotype (Pro12Ala) and NPY (Leu7Pro7) genotype

(3) Covariates: age, sex, center, education, socio-economic status, prevalent CHD and MI, diabetes and hypertension status, blood pressure, cigarette smoking, alcohol consumption, body mass index, waist hip ratio, hemostatic function indicators (von Willebrand factor, factor VIII, fibrinogen, WBC), variables from ARIC visit 3, except for von Willebrand factor, factor VIII, and WBC, ARIC visit 1 only

(4) Exclusion criteria: From ARIC visit 3, exclude non-African-Americans, those with no genetic data on PPARG and NYP, and no retinal photographs or ungradable photographs.

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

   ____ Yes  

   _ X_ No

7.b. If Yes, is the author aware that the file ICTDER01 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?  

   ____ Yes  

   _ X_ 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 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/cscc/ARIC/stdy/studymem.html

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

   X Yes   No

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?

   X Yes   No

11.b. If yes, is the proposal

   X A. primarily the result of an ancillary study (list number*) 1995.07
   ___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

References
13. Zietz B, Barth N, Spiegel D, Schmitz G, Scholmerich J, Schaffler A. Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 (PPARgamma2) is associated with higher levels of total


