1.a. Full Title: Associations of hereditary hemochromatosis risk variants in the HFE gene with incident ESRD

b. Abbreviated Title (Length 26 characters): HFE and ESRD

2. Writing Group:
Writing group members: Adrienne Tin, Linda Kao, Joe Coresh, Meredith Atkinson, James Pankow, Eric Boerwinkle, and any other investigators interested in the topic.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _AT_ [please confirm with your initials electronically or in writing]

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3. Timeline:
Starting Analyses: October, 2012
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4. **Rationale:**

Over ten years ago, two missense variants in the hemochromatosis gene (*HFE*), rs1800562 (C282Y) and rs1799945 (H63D), were identified as risk variants for the most common form of hereditary hemochromatosis (HH), also known as Type 1 hemochromatosis (OMIM #235200) (1). Hereditary hemochromatosis is a consequence of iron overload, which can react with organic molecules to form highly reactive oxygen species in the body. The minor allele frequencies (MAF) of these two variants are approximately 5% for C282Y and 14% for H63D in individuals with European ancestry. The association of C282Y with HH behaves in a recessive manner with penetrance estimates varied between 1% to over 10% depending on the sampled population and the phenotype definition (2). Compound heterozygote of C282Y/H63D is associated with HH with much lower penetrance (3). These two *HFE* risk variants are also associated with liver cirrhosis and cardiomyopathy, possibly as a result of organ damage due to iron overload (4). Little is known about the effects of these two *HFE* variants on kidney function or end stage renal disease (ESRD).

Studies on the association of *HFE* in the context of kidney disease have mostly been limited to the association of these *HFE* variants with requirements for iron and erythropoietin dosages in dialysis patients (5-7). Recently genome-wide association studies, using an additive genetic model, identified the associations of these two *HFE* risk variants with increased hematocrit and hemoglobin concentration (8) and the associations of H63D with increased diastolic and systolic blood pressures and risk of hypertension (9). Hence these two *HFE* risk variants may potentially influence kidney function through multiple pathways, from oxidative stress due to iron overload, increased blood viscosity through increased hematocrit, and hypertension, a known risk factor of ESRD (10). Increased blood viscosity has also been associated with higher blood pressure (11). Complex interplays may also exist between these pathways.

In addition, it has been hypothesized that the burden of iron overload might be exacerbated by age due to the effect of senescence on the response to reactive oxygen species (12). As a result, the effect of these two *HFE* variants on kidney function may be more salient and available for detection in the older age groups. Therefore, we propose to test the hypothesis that the C282Y and H63D mutations in *HFE* are associated with increased risk of incident ESRD in the ARIC study, which has incident ESRD outcome up to 2008 when the mean age of the participants were over 70. Moreover, we will test for the association of these two *HFE* risk variants with incident ESRD stratified by diabetes status. It has been shown in African Americans, the genetic risk factors for ESRD differ between individuals with type 2 diabetes and those without (13-15). **Figure 1** shows the possible relations between these *HFE* variants and ESRD.
Figure 1. Possible relations between HFE risk variants and ESRD.

For kidney function measures, the ARIC study has urinary albumin creatinine ratio (UACR) for most participants at visit 4, in addition to other indicators of kidney function, including glomerular filtration rate estimated from creatinine and cystatin C. Therefore, in addition to using visit 1 as baseline, we will perform a sub-analysis using visit 4 as baseline for the association of C282Y and H63D with incident ESRD.

5. Main Hypothesis/Study Questions:

Our hypothesis is:

The minor alleles of rs1800562 (C282Y) and rs1799945 (H63D) will be associated with increased risk of incident ESRD, and this association will be stronger in individuals without diabetes prior to the onset of ESRD. Several genetic models will be tested (see analysis plan).

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

Inclusion:

1. All white ARIC participants with consent for use of DNA
2. The allele frequencies of the HFE variants in African Americans are low. According to Pankow et al. 2008, 8% (=296/3685) of the individuals in the African American cohort have one or more copies of the minor allele of either variants. If at least 100 of these 296 individuals have covariate data, including eGFR and UACR, then we will include the African American cohort in the analysis

Outcome: Incident ESRD hospitalization by 2008. Incident ESRD hospitalization events were ascertained by active surveillance (annual telephone calls, screening all known hospitalizations, as well as local obituaries) and is defined as having ESRD or chronic kidney disease stage 5 hospitalization events (ICD-9 code 585.5-585.6 or ICD-10 code: N18.5-N18.6). Individuals with acute renal failure code (ICD-9 584) in the same event
without prior history of chronic kidney disease were not classified as ESRD cases. Individuals without an event will be censored administratively.

Exposure: rs1800562 (C282Y) and rs1799945 (H63D) genotypes

Covariates: age, sex, center, eGFR, log transformed UACR or albuminuria with sex-specific threshold (≥ 17 mg/g in men and ≥ 25 mg/g in women)

Analysis Plan

1. Summary statistics on the number of incident ESRD cases by diabetes status and genotype. Association between diabetes status and loss to follow-up was found in ARIC (16). Therefore, we will define diabetes status using the last available data before the ESRD event or censoring, including the data obtained from annual follow-ups (AFU).

2. On the association with incident ESRD, we will first conduct proportional hazard regression analyses stratified by diabetes status, then combined individuals with and without diabetes and test for the interaction between diabetes status and genotype. Additional adjustment for baseline kidney function will also be performed. In the combined analysis, diabetes status will be included as a time-varying covariate. This analysis will be first performed using visit 1 as baseline, then using visit 4 as baseline controlling for UACR.

3. Previous studies have shown individuals with 2 copies of C282Y are at much higher risk for HH (recessive manner) compared to those with just one copy; however, individuals who are compound heterozygotes of C282Y/H63D were also at higher risk for HH, albeit with much lower penetrance (3). On the other hand, the associations of these two missense variants with hematocrit, blood pressure, and hypertension were identified using an additive genetic model (8-9). Therefore, in the analyses of the associations of these two variants with incident ESRD, several genetic models will be tested. First, we will test for the association of C282Y and H63D separately using the additive genetic model, then we will combine the two risk variants into one variable to test for the effects of having an increasing number of risk variants and the effects of having at least one copy of the risk variants in either SNPs. We will also test for the effect of C282Y homozygote and C282Y/H63D compound heterozygote vs. the wild type.

4. Multivariate models will also be constructed to examine 1) potential mediation effects and 2) potential heterogeneity of association by menopausal status in women

   a. Based on the currently available data in ARIC, we can test for the possible mediating effect of hypertension or blood pressure. The possible mediating effect of blood viscosity can be tested by using an equation to estimate blood viscosity (17). Some other possible mediating effects
cannot be tested. We cannot test for whether the effects of these HFE risk variants on ESRD may be mediated through iron overload because iron biomarkers, such as transferrin and ferritin is only available in less than 100 individuals (18).

b. Within women, premenopausal women are known to have lower risk of iron overload (19). We will repeat the analysis by menopausal status if over 10% of women differed by menopausal status.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes  _ _ No

b. If Yes, is the author aware that the file ICTDER02 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 ICTDER02 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 ICTDER02 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://www.cscc.unc.edu/ARIC/search.php

__X__ Yes  ____ No

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)?

Lead authors of related proposals are in the writing group.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  _ _ Yes  __ ? _ No Do the genotype data used in Pankow et al. 2008 belong to an ancillary study?

11.b. If yes, is the proposal

___  A. primarily the result of an ancillary study (2004.10) ___

___  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


