

Genome-Wide Scan for Estimated GFR in Multi-Ethnic Diabetic Populations: The Family Investigation of Nephropathy and Diabetes

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Running Title: Diabetic Nephropathy, eGFR and Genome Scan

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ABSTRACT

Objective: Diabetic nephropathy (DN), the most common cause of end stage renal disease, aggregates in families and specific ethnic groups. Deconstructing DN into intermediate, quantitative phenotypes may increase feasibility of detecting susceptibility loci by genetic screens. Glomerular filtration rate (GFR), which characterizes DN, was employed as a quantitative trait in a preliminary whole genome scan.

Research Design and Methods: Estimated GFR (eGFR) was calculated for 882 diabetic sib pairs (mean age 57 years) of African American (25.6% of total), American Indian (8.6%), European American (14.2%), and Mexican American (51.6%) descent enrolled in the initial phase of the Family Investigation of Nephropathy and Diabetes (FIND). A whole genome scan was performed using 404 microsatellite markers (average spacing = 9 cM) and model-free linkage analysis.

Results: For all ethnicities combined, strong evidence for linkage was observed on chromosomes 1q43 ($P = 3.6 \times 10^{-3}$), 7q36.1 ($P = 2.1 \times 10^{-4}$), 8q13.3 ($P = 4.6 \times 10^{-4}$) and 18q23.3 ($P = 2.7 \times 10^{-3}$). Mexican American families, which comprised the major ethnic subpopulation in FIND, contributed to linkage on chromosomes 1q43, 2p13.3, 7q36.1, 8q13.3, and 18q23.3, whereas African American and American Indian families displayed linkage peaks on chromosomes 11p15.1 and 15q22.3, respectively.

Conclusions: We have demonstrated multiple chromosomal regions linked to eGFR in a multiethnic collection of families ascertained by a proband with diabetic nephropathy. Identification of genetic variants within these loci that are responsible for the linkage signals could lead to predictive tests or novel therapies for subsets of patients at risk for DN.

KEY WORDS. eGFR, diabetic nephropathy, renal failure, genetic, ESRD

End stage renal disease (ESRD) is the final outcome for multiple chronic kidney diseases (CKD), including one-third of patients with type 1 and type 2 diabetes. Nearly 45% of incident ESRD cases in the U.S. are due to diabetic nephropathy (DN) (www.usrds.org/2005/pdf/02_incid_prev_05), and less than 5% of ESRD cases are caused by Mendelian inheritance of known susceptibility genes. ESRD is therefore considered a complex disease in the remaining 95% of cases, with hereditary and environmental factors contributing to clinical outcomes. Evidence to support a genetic component to DN is abundant, including concordance of DN between diabetic sibs (1;2), as well as clustering of DN within families and specific ethnic groups (3-5).

Whole genome screens have been conducted to identify DN genes in Pima Indian and African American (AA) populations (6;7), and each study employed composite phenotype definitions for DN. These screens have yielded candidate loci, but no major susceptibility genes to date. A potential disadvantage of this study design is that establishing a categorical phenotype definition can be difficult, and inaccurate phenotype classification can reduce the likelihood of identifying candidate loci. An alternative approach is measurement of a quantitative trait, which represents an intermediate phenotype to predict an aspect of the complex disease. Although intermediate, quantitative traits may also be heterogeneous, some potential advantages to this strategy are that these traits may be more proximal to underlying genes that regulate CKD, controlled by fewer loci, affected by fewer environmental factors, and more likely to reflect disease pathophysiology (8-10). Importantly, analysis using continuous, quantitative traits can increase power to detect genetic effects. Although most often applied to model organisms, this strategy has been successfully implemented to identify complex human disease loci (8;11-14).

Several groups have assessed heritability (h^2) of eGFR as a quantitative trait. Hunt et al reported $h^2 = 0.33$, 0.36 , and 0.53 for three successive creatinine clearance examinations in a healthy Utah pedigree (15). These investigators

subsequently analyzed GFR estimates from a similar (healthy, European American [EA]) population at three time points over a 10 year period, demonstrating h^2 ranging from 0.25 to 0.31 (16). DeWan et al calculated heritability of 0.17 in AA and 0.18 in EA hypertensive cohorts (17). In the Framingham Heart Study, Fox et al determined that eGFR heritability was 0.36 (18). In a study of type 2 diabetic EA subjects, Langefeld et al calculated h^2 of eGFR, by MDRD equation, to be 0.75 after adjusting for age, gender, blood pressure, medications, and hemoglobin A1c (HbA1c) levels (19). Bochud et al estimated heritability in families with hypertension, similarly adjusting for age and gender, and revealing $h^2 = 0.41$ for inulin clearance, 0.52 for creatinine clearance, and 0.82 for creatinine clearance estimated by the Cockcroft-Gault formula (20). More recently, the Joslin group demonstrated significant eGFR heritability ($h^2 = 0.45$) in families enriched for type 2 diabetes (21). Collectively, these data indicate that GFR is heritable, and it is reasonable to screen for genes regulating GFR and/or renal disease progression.

Many of these heritability studies simultaneously demonstrated linkage with quantitative eGFR phenotypes. In the HyperGEN study involving 1,100 hypertensive EA and AA subjects, a locus on 3q27 was identified (17). Follow-up studies by this same group, with added subjects and denser genotyping, revealed linkage to 3p (22) and 7q (23) in the AA cohort. In two studies by Hunt et al, which examined pedigrees from EA families in Utah, loci on chromosome 2q21.3 (16) and 10q23.3 (15) were identified. The Framingham Heart Study identified linkage on chromosomes 3q26.3 with creatinine clearance and on 4q23.3 with eGFR (18). However, these populations were largely devoid of diabetic subjects and mean eGFR of the study populations were in the normal range. The FIND consortium was established to identify DN susceptibility genes in four ethnic groups (Hispanic American [HA], AA, EA and American Indian [AI]) (24). In contrast to other studies, families were ascertained for diabetic ESRD or severe DN and many participants have low eGFR. A whole

genome scan was performed with microsatellite markers. Here, we report the results of a linkage analysis of eGFR.

RESEARCH DESIGN AND METHODS

Study Population. The FIND family study population and study design have been described in detail (24). Briefly, samples were collected from the eight participating investigation centers. The predominant recruitment strategy in the family-based linkage portion of FIND was to enroll probands with DN, as well as diabetic sibs with or without DN. A total of 941 subjects, comprising 882 sib pairs were included in this analysis (Table 1). Approval for recruitment was obtained from Institutional Review Boards at each participating institution. Subjects were recruited and samples collected according to the declaration of Helsinki principles, and a certificate of confidentiality was filed at the National Institutes of Health.

Diabetes definition. Diabetes was self-reported and corroborated in most subjects by prevalent treatment with insulin and/or oral hypoglycemic agents. Diabetes duration was obtained from the medical history, and confirmed by medical record review. In diabetic patients not treated with hypoglycemic agents, diabetes was diagnosed by HbA1c screening and fasting plasma glucose confirmation at study entry. Subjects with HbA1c >7.0 were considered diabetic, whereas those with HbA1c <6.0% were considered non-diabetic. Subjects with HbA1c concentration in the range from >6.0% to <7.0% underwent fasting plasma glucose and/or oral glucose tolerance testing. Assay results for diabetes diagnosis were interpreted according to American Diabetes Association criteria (25). Subjects with either type 1 or type 2 diabetes were included, though nearly all enrollees were believed to have type 2 diabetes (Knowler, WC, Ipp, E, for the FIND Research Group. *J.Am.Soc.Nephrol.* 16:149A, 2005).

Proband phenotype criteria. Detailed criteria have been published previously (24). Briefly, parameters for inclusion in the FIND family study required diagnoses of DN, as defined by (1) kidney biopsy revealing DN plus overt proteinuria (>0.5 g protein/g creatinine), or (2)

ESRD due to DN, as defined by (a) onset of diabetes ≥ 5 years prior to renal replacement therapy plus diabetic retinopathy (documented microaneurysms, proliferative diabetic retinopathy, macular edema or prior retinal laser photocoagulation), or (b) onset of diabetes ≥ 5 years prior to renal replacement therapy with documented proteinuria (>3.0 g protein/g creatinine) or (c) diabetic retinopathy with proteinuria (>3.0 g protein/g creatinine), or (3) CKD due to DN (but not yet ESRD), based upon either (a) diabetic retinopathy with proteinuria (>1.0 g protein/g creatinine) or (b) diabetes duration ≥ 10 years with proteinuria (>3.0 g protein/g creatinine). Demographic and clinical characteristics are shown in Table 2.

Diabetic sib phenotype criteria. Diabetic sibs were considered to be either affected or unaffected by DN. Criteria for sibs affected for DN include: (1) kidney biopsy consistent with DN or (2) microalbuminuria (>30 mg albumin/g creatinine) or (3) serum creatinine concentration ≥ 1.6 mg/dl for men, ≥ 1.4 mg/dl in women. Diabetic sibs were classified as unaffected by DN by virtue of diabetes duration ≥ 10 years, serum creatinine concentration <1.6 mg/dl (for men), <1.4 mg/dl (for women) and urine albumin:creatinine <30 mg/g. Diabetic sibs with normal urine albumin excretion and diabetes duration <10 years were considered indeterminate, but included in the linkage analysis.

Glomerular filtration rate estimation (eGFR). For most subjects, eGFR was calculated by modified MDRD equation (26): $eGFR \text{ (ml/min/1.73m}^2\text{)} = 186 \times (\text{Plasma creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$. eGFR was assumed to be 5.0 ml/min/1.73m² for patients receiving hemodialysis or transplant recipients (n = 333).

Genotyping and analytic methods. Genotyping and linkage analyses were conducted on 882 diabetic sib pairs. DNA was extracted for genotyping from either lymphoblast cell lines or leukocyte buffy coats. Genotyping was performed at the Center for Inherited Disease Research (CIDR), including 404 microsatellite markers on 22 autosomes and 2 sex chromosomes, using the

