Linkage of Serum Creatinine and Glomerular Filtration Rate to Chromosome 2 in Utah Pedigrees


Background: Serum creatinine and creatinine clearance are used as indicators of renal function and may indicate a propensity for development of end-stage renal disease. Identifying genes related to future decreases in renal function could be important in assessing risk and defining abnormal mechanisms amenable to preventive measures. Although creatinine clearance is a better measure of renal function than serum creatinine, proper and complete urine collections in large population studies are sometimes problematic. This can lead to a loss in power to detect linkage. Therefore, in this study we also investigated serum creatinine and estimated glomerular filtration rates (GFR), both of which are more reliably measured.

Methods: Linkage was tested in a genome scan using 49 large Utah pedigrees examined three times over 10 years to detect regions harboring genes related to reduced renal function.

Results: Heritability of serum creatinine ranged from 25% to 31% across three examinations, and heritability of GFR ranged from 37% to 42%. The highest log of the odds (LOD) score for serum creatinine was found on chromosome 2 at 145 cM on the Marshfield map (D2S1334). Consistent nonparametric linkage for serum creatinine was found for all three examinations (LOD = 3.15, 2.75, and 2.00, respectively). Estimates of GFR also showed linkage to this region.

Conclusions: The consistency of linkage to chromosome 2 over longitudinally repeated measurements increases the likelihood that this region harbors a gene influencing phenotypic variation in serum creatinine and GFR. Identification of this gene could help to predict which individuals are most likely to progress to renal disease. Am J Hypertens 2004;17:511–515 © 2004 American Journal of Hypertension, Ltd.

Key Words: Creatinine clearance, glomerular filtration rate, linkage analysis, pedigrees, renal disease.
investigate further whether linkage was more apparent without consideration of urine creatinine. This study reports the genome-wide linkage analyses of serum creatinine and estimated GFR as well as creatinine clearance obtained from three different clinical examinations over 10 years.

Methods
Study Population

The pedigrees included have been previously described. Briefly, 2500 members of 98 Utah pedigrees were examined at the Cardiovascular Genetics Clinic of the University of Utah up to three times from 1980 to 1992. Most pedigrees were ascertained for two or more early deaths from coronary heart disease or two or more stroke-related deaths in the sibship of the founding generation of the pedigree. A few pedigrees were ascertained for the presence of hypertension in a proband. Further details of variables collected may be found in other references. The Mammalian Genotyping Service provided marker genotypes (set 10) on 1855 individuals from the 49 largest pedigrees submitted for genotyping. There were 1516 persons >17 years of age with both marker and serum creatinine data from examination 1, 1193 from examination 2, and 850 from examination 3. Informed consent was obtained from all participants, and the clinical protocols were approved by the University of Utah Institutional Review Board.

Study Variables

Blood was drawn in the morning after an overnight 12-h fast. The clinical laboratory of the University of Utah Hospital measured serum creatinine. At examination 1, timed 12 h, overnight urine samples were collected on three different days. Two samples were collected from weekdays and one sample from the weekend. Urine volume and creatinine were measured from each sample, with the resulting measurements averaged and converted to amount creatinine per 24 h. Creatinine clearance was measured as the ratio of 24-h urine to serum creatinine amounts. Examinations 2 and 3 collected only a single 12-h overnight sample. The GFR was estimated from a formula from the Modification of Diet in Renal Disease study: GFR = 170 × serum creatinine$^{-0.999}$ × age$^{-0.176}$ × serum urea nitrogen$^{-0.17}$ × serum albumin$^{0.318}$ × 0.762$^{sex}$, where sex = 1 if female or 0 if male.

Statistical Analysis

Serum creatinine, creatinine clearance, and GFR values were adjusted for gender, baseline age, age$^2$, age$^3$, and BMI by multiple regression analysis. The unadjusted mean was added to the residuals. All subjects <18 years of age at examination 1 and individuals with diabetes or a previous history of kidney disease at examination 1 were excluded from analysis in this study. Five subjects with serum creatinine levels >2.2 mg/dL and 12 subjects with creatinine clearance >300 mL/min were also excluded, as they appeared to be outliers in the distribution. Persons with elevated serum creatinine levels that were <2.2 mg/dL but who had no prior kidney disease diagnosis were retained in the sample. Skewness for the three serum creatinine measurements was −1.1, −0.5, and 0.3, respectively.

Pedigree relationships were assessed by the ASPEX program (ftp://lahmed.stanford.edu/pub/aspx/index.html) and a modified version of PAP and marker compatibilities were checked by PEDCHECK. Multipoint haplotypes were estimated by the Markov chain Monte Carlo method of Thomas et al to help differentiate identity-by-descent from identity-by-state sharing at each marker. A nonparametric statistic of linkage was calculated at each marker using these haplotypes following the method of Kruglyak et al but was extended for quantitative traits by Camp et al. Details of the 393 markers analyzed and the genetic map can be obtained from the Mammalian Genotyping Service website (http://marshmed.org/genetics).

Results

Table 1 lists clinical characteristics of the subjects. The gender distribution at examination 1 was 51% female and 49% male. All subjects were of white ethnicity. There were three men with a serum creatinine >1.6 mg/dL (one at examination 2 (1.9 mg/dL) and two at examination 3 (1.7 and 2.2 mg/dL)). One woman had a serum creatinine >1.4 mg/dL (1.7 mg/dL at examination 3). The GFR was significantly decreased at examination 3 compared to examination 1 in a paired analysis for both men and women ($P < .0001$). Heritability estimates of serum creatinine were 0.25, 0.31, and 0.27 for the three examinations. Heritabilities of GFR were 0.37, 0.38, and 0.42, respectively.

Figure 1 shows the linkage plots across all chromosomes for serum creatinine for each of the three examinations. The LOD scores were >2.0 for chromosomes 2, 10, 18, and 20 for one or more examinations. Chromosome 20 was not consistently linked for all three examinations, whereas the other chromosomes had at least some evidence of linkage for all three examinations. A genome search using creatinine clearance as the phenotype showed LOD score peaks of 2.0 to 2.6 for chromosomes 2 (145 cM), 10 (101 cM), and 18 (89 cM) near the same locations as for serum creatinine, but only for examination 3 (data not shown).

Figure 2 shows the nonparametric LOD score plot on chromosome 2 for serum creatinine and examination 3 creatinine clearance. Examination 1 shows the best signal for linkage (3.15) at marker D2S1334 located at 145 cM on the Marshfield map. Examination 2 showed a LOD score of 2.75 at the same marker, whereas examination 3 had a LOD score of 2.09 at that marker. Creatinine clearance for examination 3 also showed linkage to chromosome 2 at D2S1334 but examinations 1 and 2 had maximal LOD scores <0.5 (curves not shown).
Figure 3 shows nonparametric LOD scores on chromosome 2 for estimated GFR. The peak LOD score occurred at the same location as for serum creatinine, although the magnitude of the scores at each examination was somewhat reduced (1.37, 1.23, and 1.88, respectively).

**Discussion**

This study analyzed multiple serum creatinine measurements from a collection of healthy pedigree members of white ethnicity. Estimated GFR was also obtained from serum creatinine, serum urea nitrogen, and serum albumin. Consistency of linkage across multiple time points can be taken as an indication that the results are robust to measurement error and longitudinal changes. Because we found that creatinine clearance showed linkage to chromosome 2 only for examination 3, we investigated other measures of renal function either to support or to refute the linkage. Moderate LOD scores were obtained on chromosome 2 from a linkage analysis of both serum creatinine

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<th>Table 1. Subject characteristics</th>
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<td>Glomerular filtration rate (mL/min/1.73 m²)</td>
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Data are given as mean ± SD. Sample sizes for examination 1, 2 and 3 are 1516, 1193, and 850. For examination 1, there were 741 male and 775 female subjects.

BMI = body mass index.
and estimated GFR at each of three examinations over a 10-year period. This suggests that the creatinine clearance linkage peak is more likely to reflect a true genetic contribution. The lack of linkage for creatinine clearance at examinations 1 and 2 likely indicates that: 1) this locus shows an age effect seen only as the subjects get older; 2) problems with urine collection confounded the linkage signal; or 3) the smaller set of pedigree members who had examination 3 showed greater evidence for linkage than the larger set examined at examination 1. If the result was due to an age effect, it is not likely that both serum creatinine and GFR would have shown linkage for examinations 1 and 2. This argument also applies to analyzing a smaller number of individuals for examination 3. It is more likely that urine collection problems resulted in the lack of replication by examinations 1 and 2. These problems evidently did not affect our ability to detect linkage of creatinine clearance for all three examinations to chromosome 10, and the confounding on chromosome 2 may have been due to other factors. However, the heritability estimate for creatinine clearance in white subjects was only 0.18 in this study. The HyperGEN study reported a genome scan for creatinine clearance but did not find a peak on chromosome 2. Their best linkage signals were on chromosomes 1, 3 and 6 in white subjects.

Our heritability estimates for GFR increased across examinations, as did the LOD scores on chromosome 2. Higher heritabilities provide a greater chance for a genetic locus to explain a greater proportion of the trait variation, resulting in a higher LOD score. However, these linkages were all lower than those for serum creatinine, despite the facts that heritabilities for GFR were greater and that the GFR estimate is a more sensitive indicator of renal function. This may indicate that the linkage signals for serum creatinine are inflated. However, both phenotypes give approximately the same region size when using pedigree-specific recombinants in the linked pedigrees, so candidate genes may be selected using either phenotype.

The 1-LOD support interval spans an apparently gene-poor region of about 12 cM or nine megabases. There are only 22 annotated genes listed in this interval, none of which is a compelling candidate. Other genes in this region may yet be discovered, or one of the 22 genes listed may have an unknown function that would relate to renal function. In addition, the IL-1 gene cluster lies approximately 25 to 30 cM from the peak LOD score, which includes at least three IL-1 genes that have been related to inflammatory processes. In particular, a VNTR polymorphism in intron 2 (allele 2) of IL-1RN has been related to diabetic nephropathy, and it is possible that it could explain the linkage found. It is possible that locus heterogeneity across pedigrees may have shifted the localization of the linkage peak away from this gene. Identification of the responsible gene could lead to understanding a mechanism whereby renal function in currently healthy subjects begins to deteriorate. Whether this underlying gene would actually cause future end-stage renal disease is obviously unknown, but the hypothesis could be tested if mutations influencing serum creatinine and GFR were identified.

References