Endothelial Nitric Oxide Synthase Gene Variation Associated With Chronic Kidney Disease After Liver Transplant

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OBJECTIVE: To identify single nucleotide polymorphisms (SNPs) associated with risk of developing chronic kidney disease (CKD), a prevalent comorbidity, after liver transplant (LT).

PATIENTS AND METHODS: This study consists of a cohort of adult (≥ 18 years) primary-LT recipients who had normal renal function before LT and who survived 1 year or more after LT at a high-volume US LT program between January 1, 1990, and December 31, 2000. Patients with adequate renal function (estimated glomerular filtration rate, ≥ 40 mL/min per 1.73 m² during follow-up; $n=308$) and patients with incident CKD (estimated glomerular filtration rate, <40 mL/min per 1.73 m² after LT; $n=92$) were identified. To investigate the association of 6 candidate genes with post-LT CKD, we selected SNPs that have been associated with renal function in the literature. Hazard ratios were estimated using Cox regression, adjusted for potential confounding variables.

RESULTS: The variant allele (298Asp) of the Glu298Asp SNP in the endothelial nitric oxide synthase gene (*NOS3*) was significantly associated with CKD after LT ($P=.05$; adjusted for multiple comparisons). The 5-year incidence of CKD was 70% among patients homozygous for the *NOS3* variant allele (298Asp) compared with 42% among those not homozygous for the *NOS3* variant allele. Specifically, homozygosity for the *NOS3* variant allele conferred a 2.5-fold increased risk of developing CKD after LT ($P=.005$, adjusted for confounding variables).

CONCLUSION: Homozygosity for the variant allele of *NOS3* (298Asp) is associated with CKD after LT and may be useful for identifying recipients at higher risk of post-LT CKD.

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BMI = body mass index; CI = confidence interval; CKD = chronic kidney disease; CNi = calcineurin inhibitor; eGFR = estimated glomerular filtration rate; eNOS = endothelial nitric oxide synthase; GFR = glomerular filtration rate; HR = hazard ratio; LT = liver transplant; NO = nitric oxide; PCR = polymerase chain reaction; SNP = single nucleotide polymorphism

Liver transplant (LT) is a widely accepted therapy for patients with end-stage liver disease and is an established means of restoring health in these patients by extending survival and improving quality of life. However, there remain opportunities to continue to optimize outcomes of LT. Although effective immunosuppression is critical for graft survival after transplant, prolonged exposure of transplant recipients to these immunosuppressive agents can contribute to the development of long-term medical complications.¹⁻³ Chronic kidney disease (CKD) is a notable example and is increasingly recognized in long-term survivors of LT.⁴ Up to 18% of LT recipients develop renal failure within 5 years after LT, and those who develop CKD that requires dialysis support have a very poor survival (27% at 6 years).^{5,6}

Renal failure is a complex disorder with both genetic and environmental components. A unique environmental risk factor for renal failure in LT recipients is prolonged exposure to calcineurin-based immunotherapies (the calcineurin inhibitors [CNi] cyclosporine and tacrolimus). These drugs produce intense vasoconstriction of afferent and efferent glomerular arterioles, reducing renal blood flow and glomerular filtration rate (GFR). The exact mechanism of vasoconstriction is unclear, but there appears to be substantial impairment of endothelial cell function, leading to reduced production of vasodilators (such as nitric oxide [NO]) and enhanced release of vasoconstrictors (endothelin and thromboxane).^{7,8} Additionally, transforming growth factor beta-1, endothelin-1, and reactive oxygen and nitrogen species may contribute.⁹⁻¹¹

Although some reduction in renal function is common among LT recipients, some maintain intact renal function many years after LT. Thus, there is variability in the degree of individual susceptibility to CKD after LT that may not be fully explained by environmental or treatment-related factors. We hypothesize that genetic predisposition plays a role in an individual's susceptibility to CNi-induced CKD. Previously published reports in nontransplant populations have suggested a relationship between vasomodulatory factors, CKD, and genetic variation occurring in the form of single nucleotide polymorphisms (SNPs) in the following vasomotor pathways: NO (gene symbol, *NOS3*), endothelin (gene symbol, *EDN1*), kinin (gene symbol, *BDKRB1*), angiotensin (gene symbol, *AGTR1*), adrenergic system (gene symbol, *ADRB2*), and transforming growth factor, beta-1 (gene symbol, *TGFB1*). Thus, we evaluated for associations between 7 common SNPs occurring in the 6 aforementioned genes and the risk of CKD after LT.

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PATIENTS AND METHODS

Research resources at Mayo Clinic in Rochester, MN, including an extensive prospective database and biorepository of all LT recipients initiated in 1985, were used for this study. The Mayo Clinic Institutional Review Board approved this study, and only patients with research authorization in the form of written informed consent were included. Study patients were drawn from adult primary-LT recipients at Mayo Clinic using the following eligibility criteria¹: age, 18 years or older²; primary LT between January 1, 1990, and December 31, 2000³; survival, 1 year or more after LT (with the goal of including patients with longer-term survival after LT); and adequate renal function before LT (estimated GFR [eGFR], ≥ 40 mL/min per 1.73 m², as estimated by the Modification of Diet in Renal Disease equation, at initial evaluation for LT). Patients with acute liver failure and hepatic malignancies were excluded. Patients undergoing transplants before 1990 were not included because of the paucity of biospecimens available. All patients meeting study eligibility criteria (n=400) were genotyped for the 7 candidate SNPs.

To ensure adequate ascertainment of the development of CKD after LT, we required that eligible patients have at least 2 laboratory assessments of serum creatinine at least 4 months after LT (to avoid iatrogenic-induced creatinine elevations that commonly occur proximate to the transplant surgery) and not within 6 months before death or second LT (to avoid creatinine elevations secondary to a terminal event). Chronic kidney disease was defined as an eGFR less than 40 mL/min per 1.73 m² on 2 or more separate occasions 6 or more months apart. Glomerular filtration rate lower than 40 mL/min per 1.73 m² was used as the threshold for CKD because it is low enough to prompt considerations of alterations in clinical management. Renal biopsy was not routinely performed for evaluation of CKD after LT because of its invasiveness; thus renal histology was not available. The incidence of CKD after LT was compared across genotypes of the 7 polymorphisms considered.^{12,13}

MEASUREMENTS

Predictor Variables and Covariates. Demographic and pre-LT variables were recorded, including age, sex, height, weight, race/ethnicity, liver disease etiology (categorized as alcohol, hepatitis C, hepatitis B, cholestatic, or other), diabetes, blood pressure measurements, antihypertensive medications, and serum creatinine levels. Patients who had a mean arterial pressure of 107 mm Hg or higher or who were receiving antihypertensive medications (except β -blockers and diuretics for management of portal hypertensive complications) before LT were identified as having hypertension at baseline. Body mass index (BMI)

was calculated by dividing an individual's body weight (in kilograms) by the square of his or her height (in meters). Individuals taking antidiabetic medications (oral hypoglycemic agents or insulin) or having persistently elevated fasting blood glucose (≥ 126 mg/dL; to convert to mmol/L, multiply by 0.0555.) before LT were identified as having diabetes at baseline.

After LT, all available serum creatinine levels and CNI-based immunosuppressive drug (cyclosporine and tacrolimus) levels were extracted. After LT, the timing and frequency of serum creatinine and immunosuppressive drug level measurements were dictated by clinical necessity. To assess CNI exposure after LT, we extracted all cyclosporine and tacrolimus serum levels over the duration of clinical follow-up after LT for each patient and used these data to generate a summary CNI variable for each patient that represented the cumulative cyclosporine and tacrolimus exposure at each time point.

Outcomes Variable. We evaluated eGFR as a categorical outcome, defined as less than 60 mL/min per 1.73 m², 31 to 60 mL/min per 1.73 m², and 30 or less mL/min per 1.73 m². The spread of the eGFR results in our patient population did not allow us to analyze the traditional 5 stages of CKD (stage 1, GFR >90 mL/min per 1.73 m²; stage 2, 60-89 mL/min per 1.73 m²; stage 3, 30-59 mL/min per 1.73 m²; stage 4, 15-29 mL/min per 1.73 m²; and stage 5, <15 mL/min per 1.73 m²).

Data during the first 4 months after LT were ignored to avoid inclusion of iatrogenically induced fluctuations in renal function commonly seen in the immediate post-LT period. Similarly, data within 6 months of a patient's death or second LT were excluded to avoid inclusion of renal insufficiency induced by impending death or allograft failure.

Selection of Candidate Genes and SNPs. Hypothesizing that SNPs in genes in vasomodulatory pathways are associated with the development of CKD after LT, we reviewed the literature to identify relevant candidate genes and SNPs. Published medical literature relating to vasomodulation, vasoconstriction, vascular disease, renal insufficiency, gene mutations, and SNPs was reviewed in English using MEDLINE and PubMed. Additional searches were performed through the National Center for Biotechnology Information Web site (<http://www.ncbi.nlm.nih.gov/Entrez/index.html>) and the SNP Consortium Web site (<http://snp.cshl.org/>). We identified 7 SNPs of interest occurring in the following 6 genes implicated in vasoregulation: angiotensin II receptor, type 1 (*AGTR1*); endothelin 1 (*EDN1*); nitric oxide synthase (endothelial cell) (*NOS3*); adrenergic, beta-2-, receptor, surface (*ADRB2*); bradykinin receptor B1 (*BDKRB1*); and transforming growth factor, beta-1 (*TGFB1*).¹⁶⁻³¹ Priority was given to SNPs demonstrated to be common, functional, and/or repeatedly associated with renal function in the literature in different populations sampled.

DNA EXTRACTION AND GENOTYPING

Genomic DNA was isolated from peripheral blood leukocytes using the Gentra Puregene Blood Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Detection of SNPs was performed using Applied Biosystems (Carlsbad, CA) genotyping products. Allele-specific DNA probes for the Lys198Asn polymorphism (rs5370) in *EDN1* were available through Applied Biosystems Assays-on-Demand. Allele-specific probes for the following were obtained using Applied Biosystems Assays-by-Design: Arg16Gly (*ADRB2* gene, rs1042713), Gly27Glu (*ADRB2* gene, rs1042714), A1166C (*AGTR1* gene, rs5186), G-699C (*BDKRB1* gene, rs4905475), Glu298Asp (*NOS3* gene, rs1799983), and Leu10Pro (*TGFB1* gene, rs1800740). Allelic discrimination was performed using the Applied Biosystems 5' nuclease assay, combining polymerase chain reaction (PCR) amplification and detection using fluorogenic probes. Assays were performed in duplicate using TaqMan Universal PCR Master Mix in 5- μ L total volumes and run in 384-well microtiter plates in the Applied Biosystems 7900HT Sequence Detection System.

STATISTICAL ANALYSES

Continuous variables are summarized as mean and standard deviation. Discrete variables are presented as frequencies and group percentages. Genotype and allele frequencies were determined using the gene-counting method. Deviations from Hardy-Weinberg expectations were examined by comparing the observed to expected allele frequencies in patients who did not develop CKD.

Associations between the SNP genotypes and the development of CKD after LT were determined using the Fisher exact test for the dominant and recessive models and using the Cochran-Armitage test for trend for the additive model. Using Cox proportional hazards regression analysis, hazard ratios (HRs) for CKD after LT and their 95% confidence intervals (CIs) were calculated. Models were also adjusted for potential confounding variables, including age, diabetes, hypertension, race/ethnicity, liver disease etiology, BMI, pre-LT renal function, and cumulative CNI exposure. We also used the bootstrap method to reduce the potential for spurious findings due to multiple testing and to validate the results in our sample. The bootstrap approach selects random samples of size N with replacement from the original data. In our study, within each bootstrap replicate (1000 replications), we chose a random sample of study patients, which allowed for any one individual to be chosen once, more than once, or not at all for each replicate. We then calculated the odds ratio for each replicate and constructed an empirical distribution for the odds ratio. We calculated the mean odds ratio across all replicates and constructed a

95% CI for the odds ratio. $P \leq .05$ was considered statistically significant (global significance test). With respect to analyzing eGFR as a categorical outcome, ordinal logistic regression analyses were conducted to test for an association between SNP genotype and renal function after LT. All analyses were conducted using a SAS statistical software package (Cary, NC).

RESULTS

During the study period, 763 adults underwent primary LT at Mayo Clinic. Of these, 400 met the study inclusion criteria and had available DNA samples. The mean $\pm$ SD age of the LT population was 51 ± 10 years; 54% were men. Pre-LT patient characteristics are given in Table 1. Mean $\pm$ SD serum creatinine and eGFR before LT were 0.9 ± 0.2 mg/dL (to convert to μ mol/L, multiply by 88.4) and 86.0 ± 29.8 mL/min per 1.73 m^2 , respectively. Fifty-five patients (14%) had diabetes, and 23 patients (6%) were diagnosed as having hypertension before LT. The most common liver disease etiologies in our patients were cholestatic liver disease (42%), hepatitis C (17%), and alcoholic liver disease (9%). Most (91%) of our LT recipients were white. During follow-up, 92 patients (23%) had developed CKD. The probability of developing CKD at 1, 3, and 5 years after LT was 2.6%, 16.4%, and 44.4%, respectively. The univariate Cox regression analyses testing for associations between dif-

TABLE 1. Baseline Characteristics of Liver Transplant Recipients

Patient variable	All patients (N=400) ^a
Age (y), mean $\pm$ SD	51 $\pm$ 10
Male	215 (54)
Diabetes	55 (14)
Body mass index, mean $\pm$ SD ^b	27.2 $\pm$ 9.9
Pre-liver transplant serum	
creatinine (mg/dL), ^c mean $\pm$ SD	0.9 $\pm$ 0.2
Estimated GFR (mL/min/1.73 m ²), mean $\pm$ SD	86.0 $\pm$ 29.8
Hypertension	23 (6)
Liver disease etiology ^d	
Alcohol	37 (9)
Cholestatic (PBC, PSC)	167 (42)
Hepatitis B virus	11 (3)
Hepatitis C virus	67 (17)
Other	103 (26)
Missing data	15 (4)
Racial/ethnic group	
White	364 (91)
African American	5 (1)
Latino	8 (2)
Other	23 (6)

^a Values presented as number (percentage) of patients, unless indicated otherwise. GFR = glomerular filtration rate; PBC = primary biliary cirrhosis; PSC = primary sclerosing cholangitis.

^b Data available in 375 patients.

^c To convert serum creatinine values to μ mol/L, multiply by 88.4.

^d Total of liver disease etiology percentages exceed 100% due to rounding.

ferent pre-LT clinical characteristics and the development of CKD demonstrated that increasing age and decreasing pre-LT renal function were significantly associated with increased risk of CKD ($P=.01$ and $P=.009$, respectively). There was no significant association between CKD and race/ethnicity, liver disease etiology, BMI, or preexisting hypertension or diabetes.

CANDIDATE GENE ASSOCIATION STUDY OF CKD AFTER LT

Genotyping was successful in all 400 patients (100%) for 5 of the 7 SNPs (Table 2). Two exceptions (due to assay failures) were *TGFB1*, for which genotyping data were available for 392 patients (98%), and *EDN1*, for which genotyping data were available for 399 patients (99%). Distribution of genotypes for the SNPs among patients in whom renal function was maintained after LT did not differ significantly from that expected under the Hardy-Weinberg equilibrium (data not shown).

Of the 7 SNPs tested for association with CKD, only 1, the *NOS3* SNP (Glu298Asp, ref SNP ID: rs1799983), was significantly associated with post-LT CKD ($P=.05$, adjusted for multiple comparisons using the global significance test; Table 2). The 5-year incidence of CKD was 70% among individuals homozygous for the *NOS3* variant allele (298Asp) compared with 42% among individuals not homozygous for the *NOS3* variant allele. This association between the *NOS3* variant allele and CKD was formally examined with Cox models, and under the additive genetic model, homozygosity for the *NOS3* variant allele remained statistically significantly associated with risk of CKD after LT (HR, 2.5; 95% CI, 1.3-4.7; $P=.005$) after adjusting for other confounding variables, including pre-LT serum creatinine value, age, diabetes mellitus, hypertension, and heterozygosity for the *NOS3* variant allele. This finding remained significant when nonwhites ($n=36$) were removed from the analyses. We further found no difference between men and women with respect to the *NOS3* variant allele and the development of CKD. In sensitivity analyses, we included post-LT immunosuppression (CNI) exposure in the model and, as expected, immunosuppression exposure was significantly associated with CKD after LT. More importantly, even with inclusion of post-LT CNI exposure in the model, homozygosity for the *NOS3* variant allele remained strongly significant, conferring a HR of 2.3 (95% CI, 1.2-4.3; $P=.01$) for CKD. In addition to the Cox regression analyses, the development of CKD during 5 years after LT was assessed using Kaplan-Meier analyses, which demonstrated a statistically significant increase ($P=.02$) in the development of CKD among individuals homozygous for the *NOS3* variant allele compared with individuals not homozygous for the *NOS3* variant allele (Figure 1).

TABLE 2. Genotype Frequencies for Each SNP in Patients With and Without CKD After Liver Transplant^a

SNP (gene)	Patient group	Homozygous wild-type (%)	Heterozygous (%)	Homozygous variant allele (%)	<i>P</i> value ^b
Glu298Asp (<i>NOS3</i>)	No CKD CKD	50 41	44 42	6 16	.05
Arg16Gly (<i>ADRB2</i>)	No CKD CKD	41 37	47 49	12 14	.8
Gly27Glu (<i>ADRB2</i>)	No CKD CKD	29 30	54 52	17 17	.9
Lys198Asn (<i>EDN1</i>)	No CKD CKD	61 57	33 33	6 10	.3
Leu10Pro (<i>TGFB1</i>)	No CKD CKD	37 40	44 46	19 14	.6
A1166C (<i>AGTR1</i>)	No CKD CKD	52 54	40 40	9 5	.6
G-699C (<i>BDKRB1</i>)	No CKD CKD	82 85	16 14	2 1	.8

^a CKD = chronic kidney disease; SNP = single nucleotide polymorphism.

^b *P* values calculated on the basis of the χ^2 test of the 2x3 table and adjusted for multiple comparisons.

An additional analysis using ordinal logistic regression was undertaken to further investigate the association between the *NOS3* variant allele and eGFR, in which eGFR was categorized as less than 30 mL/min per 1.73 m², 31 to 60 mL/min per 1.73 m², and more than 60 mL/min/1.73 m², rather than using a cutoff of 40 mL/min per 1.73 m². The results of this analysis were consistent with the aforementioned findings in that the *NOS3* variant allele was significantly and positively associated with a decline in eGFR ($P=.04$).

EFFECT OF THE *NOS3* GLU298ASP (rs1799983) SNP ON PRE-LT RENAL FUNCTION

We conducted a post hoc analysis evaluating the effect of the *NOS3* variant allele on change in renal function while patients were on the LT waiting list. Estimated GFR at initial LT evaluation was significantly lower in individuals homozygous for the variant allele (mean $\pm$ SD, 77 $\pm$ 21 mL/min per 1.73 m²) compared with those with other genotypes (87 $\pm$ 30 mL/min per 1.73 m²; $P=.03$). This difference widened at the time of LT surgery, with an even lower eGFR among individuals homozygous for the variant allele (66 $\pm$ 30 mL/min per 1.73 m²) compared with those with other genotypes (78 $\pm$ 29 mL/min per 1.73 m²; $P=.02$). There was no significant difference in time elapsed from initial LT evaluation to LT surgery between individuals homozygous for the variant allele and those with other geno-

FIGURE 1. Kaplan-Meier curves comparing the development of chronic kidney disease after liver transplant by *NOS3* genotype. There is a statistically significant difference in the cumulative incidence of chronic kidney disease between individuals who are (solid line) and are not (broken lines) homozygous for the *NOS3* variant allele ($P=.02$; log rank test).

types (192 vs 194 days, respectively; $P=.58$), indicating that the lower eGFR was not attributable to a longer time on the LT waiting list with deteriorating renal function.

DISCUSSION

One of the most serious complications in LT recipients is CKD. The most common cause of CKD after LT is the nephrotoxicity of CNIs, which produce intense vasoconstriction of glomerular arterioles, leading to reductions in renal blood flow and GFR. The vasoconstriction is thought to result from impaired endothelial cell function, leading to reduced production of vasodilators.^{7,8} The results of sustained renal vasoconstriction include vascular angiopathy, altered mesangial cell contraction, tubular atrophy, and eventually interstitial fibrosis, leading to permanent reduction in renal function.^{6,32}

The principal finding of the current study was an association between the Glu298Asp SNP of the *NOS3* gene and an increased risk of CKD after LT. This increased risk was highest in patients homozygous for the variant allele (298Asp), which conferred a 2.5-fold increased risk of CKD compared with LT recipients not homozygous for the *NOS3* variant allele (after adjusting for age, diabetes, hypertension, and immunosuppression levels). Although the homozygous state for this variant was less frequent (8.5% of the cohort), those who were affected had a 70%

cumulative incidence of de novo CKD during the first 5 years after LT.

The *NOS3* gene is located on chromosome 7, spans 4.4 kilobases of DNA, and comprises 26 exons that encode a 135-kDa protein, endothelial nitric oxide synthase (eNOS), which contains 1203 amino acids. The major function of eNOS is the synthesis of NO from L-arginine and molecular oxygen. NO mediates a host of biological actions in a wide array of cell types, including relaxant effects on vascular smooth muscle tone.³³ In addition, in the kidney, NO has been shown to relax mesangial cells and to affect renal sodium handling and the release of renin.

NO is a highly diffusible molecule that is released immediately on its formation. Because NO itself is not stored, regulation of this molecule is thought to occur through alterations in *NOS3* gene expression or *NOS3* activity or associated cofactors of the molecule. The *NOS3* SNP of interest in this investigation (rs1799983, Glu298Asp) is a G-to-T substitution at position 894 in exon 7 of the *NOS3* gene, which results in a glutamate-to-aspartate substitution at codon position 298 of the eNOS protein. Given the location of this SNP in a coding region of the gene and the resultant alteration in the protein amino acid sequence, this SNP has potential to influence enzyme activity or stability. However, empirical data about the functional importance of this particular SNP have been conflicting. Previous studies have failed to demonstrate significant differences in the eNOS enzyme activity in proteins that have Glu or Asp at location 298.^{34,35} Recently, however, Tesauero et al³⁵ demonstrated that the eNOS enzyme resulting from homozygous Asp298 is more vulnerable to enzymatic cleavage in vitro than the enzyme encoded by homozygous Glu298. Thus, although the Glu298Asp SNP does not affect enzymatic activities directly, it may increase susceptibility of the Asp298 protein to proteolytic cleavage and thus contribute to lower NO generation.³⁶ Additionally, a previously published report demonstrated that the Glu298Asp mutation in *NOS3* appears to alter signaling mechanisms in response to shear stress in the eNOS pathway, which may affect endothelial function.³⁷ Such genetically encoded alterations in the NO pathway could confer added susceptibility to environmental stressors, such as vasoconstricting medications, including the immunosuppressive CNIs.

Numerous studies have shown that NO plays an important role in CNI nephrotoxicity.^{8,38-49} In animals, increased NO production by agents such as L-arginine or molsidomine has been shown to ameliorate the CNI nephrotoxicity, whereas depletion of NO by L-NAME, a competitive inhibitor of eNOS, augments the nephrotoxicity.⁴⁸ These data indicating the importance of NO in the pathogenesis of CNI nephrotoxicity suggest that the *NOS3* SNP found in this study may have a mechanistic role.

Portal hypertension in the setting of liver cirrhosis in general is known to be a vasodilated state, whereas renal vasoconstriction is the key event in the pathogenesis of hepatorenal syndrome. Our data demonstrating an association between the *NOS3* SNP and pre-LT renal dysfunction may be extrapolated to the patient with end-stage liver disease inasmuch as the resultant decrease in NO in the renal vascular beds may make them more susceptible to renal insults that occur commonly in this setting (ie, prerenal azotemia, hepatorenal syndrome, drug-induced nephrotoxicity from nonsteroidal anti-inflammatory drugs or diuretics). Of note, because patients with moderate to severe pre-LT renal insufficiency were excluded by design from the current study, we had limited ability to examine the effect of polymorphisms on the degree of renal dysfunction. Despite this, we found significant differences in renal function at the time of initial LT evaluation and in deterioration in renal function among patients awaiting LT, which lends further support to the potential pathogenetic role of the *NOS3* SNP and also suggests that the magnitude of this association may be underestimated in the current study.

Candidate gene studies have some inherent limitations that need to be acknowledged. Validity of candidate gene analyses depends on background knowledge of the gene polymorphism and the characteristics that make it a "good candidate" for the disease in question. Chronic kidney disease is a complex disease process that is likely determined by a number of genetic and environmental factors. Therefore, with respect to our findings of an association between the *NOS3* SNP and CKD after LT, one inference alternative to the presumed causal relationship is that the *NOS3* SNP is in linkage disequilibrium with an unmeasured causal variant, which is contributing to CKD. The fact that the physiologic effect of the *NOS3* Glu298Asp SNP has been questioned may increase the importance of this issue. Our study findings provide the necessary evidence to pursue this possibility in independent study populations.

Other limitations of the current study include the lack of renal histologic data, which were unavailable because renal biopsy is not routinely performed in patients with end-stage liver disease awaiting LT because of the high-risk nature of the procedure in patients with severe coagulopathy. Additionally, we used calculated estimates of GFR derived from the commonly used and readily accessible Modification of Diet in Renal Disease equation, rather than using a more direct measurement of GFR, such as iothalamate clearance, because iothalamate clearance is an involved and expensive measurement that is not widely available. Finally, our patient population may not be entirely representative of the national US experience in that most of our patients were white, hepatitis C was present in 17%, and cholestatic liver disease was the most common indication for LT.

CONCLUSION

Homozygosity for the variant allele of the Glu298Asp polymorphism of the *NOS3* gene was significantly associated with the development of CKD after LT in a predominantly white population of patients with end-stage liver disease undergoing LT. Long-term CNI exposure is necessary after LT to prevent rejection and maintain viability of the hepatic allograft, but CNIs are also well known to cause nephrotoxicity due in part to intense renal vasoconstriction. Because no effective treatment currently exists for renal injury induced by long-term CNI exposure, developing a means for identifying patients who may be at risk of this serious complication is an important part of clinical management to optimize outcomes among long-term survivors of LT. These findings may also have broader implications, given that CKD is not unique to LT recipients but is also common among other solid organ (heart, lung, intestine) transplant recipients. The discovery of risk factors for the development of CKD in the transplant setting should ultimately allow for a priori identification and risk stratification of patients at increased risk.

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