Genetic analysis of fluvastatin response and dyslipidemia in renal transplant recipients[S]

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Abstract The Assessment of Lescol in Renal Transplantation clinical trial demonstrated the efficacy of fluvastatin in reducing cardiovascular (CV) disease in renal transplant recipients. The study included a voluntary pharmacogenetic component, enrolling 1,404 patients, which allowed association testing of baseline measures and longitudinal analysis of the 707 fluvastatin-treated and 697 placebo-treated individuals. A candidate gene approach, examining 42 polymorphisms in 18 genes, was used to test for association between selected polymorphisms and major adverse cardiac events, graft failure, change in LDL and HDL cholesterol, and baseline LDL and HDL cholesterol. Reported associations between cholesteryl ester transfer protein (CETP) and baseline HDL cholesterol were replicated, with four previously implicated single nucleotide polymorphisms significantly associated in males and one in females; tests of reported associations between CETP and CV disease yielded varying results. We found no evidence for genetic factors affecting fluvastatin response. Polymorphisms in 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) previously reported to affect the efficacy of pravastatin did not show a similar effect on the reduction of LDL cholesterol by fluvastatin.—Singer, J. B., H. Holdaas, A. G. Jardine, B. Fellstrøm, I. Os, G. Bermann, and J. M. Meyer on behalf of the Assessment of Lescol in Renal Transplantation (ALERT) Study Investigators. **Genetic analysis of fluvastatin response and dyslipidemia in renal transplant recipients. *J. Lipid Res.* 2007.** 48: **2072–2078.**

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Premature cardiovascular (CV) disease is the single most important contributor to the reduced life expectancy of renal transplant recipients (RTRs) (1, 2). The increased risk in RTR is dependent on age, sex, preexisting CV disease, and lipid subfractions (3). Recently, the Assessment of Lescol in Renal Transplantation (ALERT) Study (4, 5) has shown that statin therapy reduces the risk of CV events in RTRs (6). This study enrolled 2,102 male and female RTRs, aged 23–74 years, from northern Europe and Canada, all with stable graft function and all receiving cyclosporine, none already receiving statins. Patients were randomized to receive 40–80 mg of fluvastatin per day, or placebo, and followed for 5–6 years. In this study, fluvastatin treatment was associated with a significant reduction in myocardial infarction (MI) and cardiac death but not in the primary composite end point of major adverse cardiac events (MACE; defined as cardiac death, nonfatal MI, or coronary revascularization). A 10.7% rate of MACE was seen in fluvastatin-treated patients, versus 12.7% in the placebo arm. However, in an open-label 2 year extension to the main study (5), MACE was significantly reduced by allocation to statin therapy, with a 13.0% rate in the fluvastatin arm versus 16.5% in the placebo arm.

Development of CV disease, regulation of CV risk factors (such as hyperlipidemia), and response to intervention (such as statins) have all been linked to genetic polymorphisms. Specifically, variants in genes encoding cholesteryl ester transfer protein (CETP) (7–10), LPL (11, 12), matrix metalloproteinase 3 (13), and angiotensin-converting enzyme (14–16) have been associated with cholesterol levels and overall CV risk. Two single nucleotide polymorphisms (SNPs) in the statin target, 3-hydroxy-3-methylglutaryl-

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Abbreviations: ALERT, Assessment of Lescol in Renal Transplantation; CETP, cholesteryl ester transfer protein; CV, cardiovascular; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MACE, major adverse cardiac events; MI, myocardial infarction; PRINCE, Pravastatin Inflammation/CRP Evaluation; RTR, renal transplant recipient; SNP, single nucleotide polymorphism.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one table.

coenzyme A reductase (HMGCR), have been associated with differential efficacy of statin treatment (17). Genetic polymorphism has also been investigated in connection with allograft nephropathy and with apoptotic cell death (18, 19).

The associations of genetic polymorphisms with CV disease and mortality and posttransplantation mortality have been studied extensively, but separately. The ALERT trial included an optional collection of DNA for retrospective genetic analyses, providing the opportunity to look at the two phenotypes in the context of a single study. To discover possible effects of genetic variation on the efficacy of fluvastatin as a posttransplantation treatment, we undertook an analysis of 42 polymorphisms in 18 candidate genes previously reported to affect fluvastatin metabolism, cholesterol regulation, CV disease, allograft nephropathy, and cell death (**Table 1**). We examined these loci for association with MACE and graft failure, baseline LDL and HDL cholesterol levels, and change in LDL and HDL in response to treatment during the study.

MATERIALS AND METHODS

Sample collection

Details of the ALERT trial and the eALERT extension, their inclusion/exclusion criteria, design, and conduct have been published (4, 5). Laboratory measurements of fasting lipids were performed by Medinet (Breda, The Netherlands). Participants in the genetic analysis provided an additional written informed consent. The trial adhered to the International Conference on Harmonization guidelines for good clinical practice and was conducted in accordance with the Declaration of Helsinki. The ethics committee at each participating center approved both the trial and the optional genetic analysis.

Blood samples from each consenting patient were collected at the individual trial sites, and genomic DNA was extracted by

TABLE 1. Tested polymorphisms

Gene	Rationale	Reference	Description
ABCB1	Chronic allograft nephropathy	rs1045642	Synonymous (I1145I)
ABCB1	Chronic allograft nephropathy	rs2032582	Missense (T893P)
ACE	Cardiovascular events	X62855.1 (1,451)	Intron 16 insertion/deletion
ADRB2	Cardiovascular events	rs1042713	Missense (R16G)
AGER	Chronic allograft nephropathy	rs2070600	Missense (S82G)
AGT	Cardiovascular events	rs4762	Missense (T207M)
AGTR1	Cardiovascular events	rs5183	Synonymous (P354P)
AGTR1	Cardiovascular events	rs5186	3′ UTR
BCL2	Apoptosis	rs2051423	Intron
BCL2	Apoptosis	rs1381548	Intron
BCL2	Apoptosis	rs1481031	Intron
BCL2	Apoptosis	rs1545812	Intron
BCL2	Apoptosis	rs1531697	Intron
BCL2	Apoptosis	rs1564483	3′ UTR
BCL2L1	Apoptosis	rs1484994	Intron
BCL2L1	Apoptosis	rs1994251	Intron
BCL2L1	Apoptosis	rs6060621	Intron
CCL2	Chronic allograft nephropathy	rs1024611	Promoter
CETP	Cholesterol-related	rs17231506	SNP 1 (21)
CETP	Cholesterol-related	rs1800776[a]	Promoter
CETP	Cholesterol-related	rs1800775[a]	Promoter (−629C>A)
CETP	Cholesterol-related	rs708272	Intron (TaqI)
CETP	Cholesterol-related	rs289714	SNP 6 (21)
CYP2C9	Primary metabolizer of fluvastatin	rs1799853	Missense (C144R)
CYP2C9	Primary metabolizer of fluvastatin	rs1057910	Missense (L359I)
HMGCR	Target of fluvastatin	rs17244841[a]	SNP 12 (17)
HMGCR	Target of fluvastatin	rs5908	Missense (V638I)
HMGCR	Target of fluvastatin	rs17238540	SNP 29 (17)
HMGCR	Target of fluvastatin	rs12916[a]	3′ UTR
HMGCR	Target of fluvastatin	Deletion[a]	3′ UTR
HMOX1	Apoptosis	rs1078979	Promoter
HMOX1	Apoptosis	rs2285112	Intron
HMOX1	Apoptosis	rs2071748	Intron
IL6	Chronic allograft nephropathy	rs1800795	Promoter
IL6R	Chronic allograft nephropathy	rs8192284	Missense (A358D)
LPL	Cholesterol-related	rs328	Missense ([a] 474S)
MMP3	Cholesterol-related	rs3025058	Promoter
SLCO1B1	Linked to statin transport	rs2306283	Missense (D130N)
SLCO1B1	Linked to statin transport	rs4149056	Missense (A174V)
SLCO1B1	Linked to statin transport	rs2291075	Synonymous (F199F)
SLCO1B1	Linked to statin transport	rs4149069	Intron
SLCO1B1	Linked to statin transport	rs4149087	3′ UTR

ACE, angiotensin-converting enzyme; CETP, cholesteryl ester transfer protein; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MMP3, matrix metalloproteinase 3; SNP, single nucleotide polymorphism; UTR, untranslated region.

[a] Indicates a polymorphism assayed by sequencing.

Covance (Princeton, NJ) using the Puregene D-50K DNA Isolation Kit (Gentra, Minneapolis, MN). Ultimately, 1,404 ALERT samples were genotyped: 707 from the fluvastatin group (of 1,050 patients) and 697 from the placebo group (of 1,052 patients). The distribution of demographics, treatment group, and baseline cholesterol in the genotyped sample (**Table 2**) closely resembles that in the overall ALERT population, and randomization provided good matching between the fluvastatin and placebo arms (4).

Genotyping

Initial genotyping was performed using TaqMan Assays-by-Design and Assays-on-Demand (Applied Biosystems, Foster City, CA) using 10 ng of genomic DNA, according to the manufacturer's instructions. Certain loci failed TaqMan genotyping because of neighboring polymorphisms or intractable flanking sequences and were instead assayed by direct sequencing of genomic DNA (Table 1). No polymorphisms deviated significantly ($P < 0.05$ in a Chi-square test) from Hardy-Weinberg equilibrium. Limited quantities of some DNA samples restricted the total number of genotype calls for certain assays.

Statistical analysis

Association tests were performed using SAS (SAS Institute, Cary, NC). Genotype was the independent variable, no assumption was made about dominance, and the various clinical end points in the trial were dependent variables. Continuous dependent variables were assessed by analysis of covariance; logistic regression was used for categorical-dependent variables; treatment group, sex, treatment center, body mass index, smoking status, and apolipoprotein E genotype were used as covariates. The presence of a pharmacogenetic effect (defined here as a treatment-specific effect of genotype on outcome) was tested by adding a term for genotype-treatment group interaction to the appropriate model to examine the difference between associations in the fluvastatin and placebo arms. Additional statistical models, including proportional hazards tests and models that allowed for genetic dominance, were used to replicate findings reported by others.

To address the effect of multiple tests on statistical significance, permutation analysis was performed: the pairing of clinical and genotype data was randomized 5,000 times, and the lowest P value of the 252 P values (6 end points $\times$ 42 polymorphisms) for each randomization was recorded. The 250th lowest value of the 5,000 low values was deemed the threshold for study-wide significance. Power calculations were performed by randomly generating genotypes and outcomes for a given relative risk value and performing Monte Carlo analysis to determine the probability of reaching study-wide significance.

Analysis of allele frequencies and conformance to Hardy-Weinberg equilibrium was performed in Excel (Microsoft, Redmond, WA), and linkage disequilibrium and haplotype analyses were performed with Haploview (20) using default parameters for linkage disequilibrium and data quality.

TABLE 2. Demographic comparison of genotyped and overall study populations

Variable	Genotyped	Overall
Total	1,404	2,102
Age (years)	50.0 ± 0.29	49.7 ± 0.24
Sex		
Male	66%	66%
Female	34%	34%
Race		
Caucasian	97.60%	97.00%
Asian	2.30%	2.30%
Black	0.10%	0.40%
Other	0.10%	0.30%
Treatment		
Placebo	50%	50%
Fluvastatin	50%	50%
Smokers		
Current	18%	19%
Former	36%	35%
Baseline LDL (mmol/l)	4.16 ± 0.03	4.14 ± 0.02
Baseline HDL (mmol/l)	1.35 ± 0.01	1.34 ± 0.01
Baseline body mass index (kg/m^2)	25.8 ± 0.12	25.8 ± 0.10

RESULTS

We examined the relationships between the tested polymorphisms and CV disease, renal, baseline, and pharmacogenetic outcomes. Primary results are provided in **Table 3**, and breakdowns by subgroup and results of tests of pharmacogenetic effect are shown in supplementary Table I. The following findings were of particular interest.

CETP and cholesterol

After correction for multiple testing, associations between one polymorphism in CETP and baseline HDL cholesterol exceeded the threshold ($P < 0.00015$) for study-wide statistical significance (**Table 4**), with another falling slightly short of significance. Both of these SNPs are located in the upstream regulatory region of the gene and were associated previously with HDL levels: SNP 1 (21) and −629A (8). Two of the other three SNPs examined in CETP [TaqI, SNP 4 (21), and SNP 6 (21)] were also associated with baseline HDL with nominal P values $\ll 0.05$. A test of pharmacogenetic effect did not provide any results meeting study-wide thresholds for significance. The results for association with baseline HDL were primarily driven by the male patients. Whereas a test of association of SNP 1 and baseline HDL results in a P value of 2.04×10^{-4} in males and 5.26×10^{-3} in females, none of the other SNPs in CETP gave a nominally significant P value in females. For example, a test of association of −629A and baseline HDL results in a P value of 3.37×10^{-5} in males and 0.48 in females (see supplementary Table I).

Haplotypes of CETP have been reported to be associated with magnitude of change in HDL after treatment with a variety of statins in a study in which individual SNPs had no significant association (21). The genotypes at SNP 1, SNP 4, and SNP 6 in this work provided an approximation of that analysis, which did not improve over single-point analysis in the association with baseline HDL or with treatment-specific effects on change in HDL. Similar tests were performed with the only observed haplotype block to meet the criteria described previously (22), a three-point haplotype between rs1800776, −629A, and SNP 4, again with no improvement over single-point results. Finally, log-transformation of the cholesterol data also did not significantly change the results (data not shown).

TABLE 3. Association testing results

Gene	Reference	Variant	Obtained *P* Value					
			MACE	Graft Failure	Change in LDL	Baseline LDL	Change in HDL	Baseline HDL
ABCB1	rs1045642	I1145I	0.45	0.41	0.12	0.24	0.67	0.46
ABCB1	rs2032582	T893P	0.31	0.35	0.28	0.33	1	0.16
ACE	X62855.1 (1,451)	Insertion/deletion	0.37	0.05	0.81	0.43	0.43	0.7
ADRB2	rs1042713	R16G	0.66	0.35	0.98	0.99	0.16	0.53
AGER	rs2070600	S82G	0.49	0.24	0.37	0.57	0.67	0.88
AGT	rs4762	T207M	0.39	0.92	0.91	0.87	0.59	0.67
AGTR1	rs5183	P354P	0.27	0.78	0.04	0.5	0.32	0.93
AGTR1	rs5186	3′ UTR	0.36	0.77	0.29	0.93	0.86	0.31
BCL2	rs2051423	Intron	0.85	0.86	0.74	0.12	0.72	0.87
BCL2	rs1381548	Intron	0.69	0.06	0.21	0.38	0.78	0.89
BCL2	rs1481031	Intron	0.27	0.39	0.47	0.1	0.66	0.3
BCL2	rs1545812	Intron	0.92	0.5	0.84	0.45	0.36	0.93
BCL2	rs1531697	Intron	0.71	0.05	0.69	0.53	0.84	0.74
BCL2	rs1564483	3′ UTR	0.83	0.62	0.53	0.45	0.67	0.51
BCL2L1	rs1484994	Intron	0.9	0.41	0.08	0.74	0.89	0.83
BCL2L1	rs1994251	Intron	0.11	0.56	0.07	0.88	0.68	0.58
BCL2L1	rs6060621	Intron	0.93	0.83	0.21	0.86	0.82	0.54
CCL2	rs1024611	Promoter	0.93	0.77	0.14	0.42	0.16	0.48
CETP	rs17231506	SNP 1	0.18	0.12	0.61	0.65	0.68	**3.49×10^{-06}**
CETP	rs1800776	Promoter	0.55	0.99	0.56	0.21	0.08	0.87
CETP	rs1800775	−629C>A	0.02	0.13	0.28	0.6	0.62	1.82×10^{-04}
CETP	rs708272	TaqI	0.1	0.24	0.23	0.99	0.14	3.56×10^{-03}
CETP	rs289714	SNP 6	0.1	0.15	0.38	0.95	0.46	5.49×10^{-03}
CYP2C9	rs1799853	C144R	0.11	0.63	0.32	0.57	8.80×10^{-03}	0.39
CYP2C9	rs1057910	L359I	0.97	0.14	0.7	0.25	0.25	0.33
HMGCR	rs17244841	SNP 12	0.61	0.22	0.15	0.29	0.64	0.83
HMGCR	rs5908	V638I	0.16	1	0.98	0.87	0.26	0.88
HMGCR	rs17238540	SNP 29	0.81	0.17	0.04	0.13	0.88	0.92
HMGCR	rs12916	3′ UTR	0.39	0.07	0.48	0.5	0.44	0.51
HMGCR	Deletion	3′ UTR	0.25	0.14	0.42	0.49	0.72	0.72
HMOX1	rs1078979	Promoter	0.16	0.58	0.76	0.56	0.44	0.26
HMOX1	rs2285112	Intron	0.25	0.35	0.75	0.68	0.67	0.32
HMOX1	rs2071748	Intron	0.79	0.19	0.71	0.76	0.59	0.1
IL6	rs1800795	Promoter	0.67	0.37	0.98	0.29	0.03	5.46×10^{-03}
IL6R	rs8192284	A358D	0.6	0.44	0.44	0.46	0.59	0.87
LPL	rs328	*474S	0.36	0.49	0.34	0.6	0.44	6.97×10^{-03}
MMP3	rs3025058	Promoter	0.58	0.78	0.85	0.29	0.45	0.33
SLCO1B1	rs2306283	D130N	0.23	0.74	0.54	0.76	0.36	0.54
SLCO1B1	rs4149056	A174V	0.08	0.81	0.18	0.02	0.83	0.5
SLCO1B1	rs2291075	F199F	0.07	0.71	0.48	0.94	0.86	0.89
SLCO1B1	rs4149069	Intron	0.06	0.42	0.68	0.47	0.31	0.81
SLCO1B1	rs4149087	3′ UTR	0.09	0.44	0.79	0.85	0.38	0.69

MACE, major adverse cardiac events. Results in boldface are significant study-wide.

An association between the SNP 4/TaqI polymorphism in CETP and CV disease (MI, angina, or other fatal or nonfatal CV event) in a survival analysis of statin-treated patients with familial hypercholesterolemia has been reported (23). A similar analysis of time to MACE in the fluvastatin-treated patients of ALERT found a consistent but nonsignificant ($P = 0.31$) effect, with a hazard ratio of 1.38 (95% confidence interval, 0.74–2.55) for noncarriers of the C (B1) allele relative to carriers. [The statistical method and model of dominance used for this analysis were selected to most closely match those described previously (23) and were not tested in all combinations of polymorphism and phenotype.]

Increased coronary risk has been associated with the homozygous or heterozygous A allele at −629A (24) in a survival analysis. A comparable analysis of time to MACE in ALERT found a nonsignificant ($P = 0.06$) hazard ratio of 1.51 (95% confidence interval, 0.99–2.31) for carriers of the allele. [Again, the statistical method and model of dominance were selected to most closely match those used previously (24) and were not tested in all combinations of polymorphism and phenotype.]

HMGCR and response to fluvastatin

HMGCR, the rate-limiting enzyme of cholesterol synthesis, is the target of statins, including fluvastatin (25, 26). The minor alleles at two SNPs in HMGCR, termed SNP 12 and SNP 29 (17), have been associated with smaller reductions in LDL cholesterol after pravastatin therapy (i.e., decreased pravastatin efficacy) during the Pravastatin Inflammation/CRP Evaluation (PRINCE) clinical trial (27), differences that were not seen in patients taking a placebo.

We examined five SNPs in the HMGCR gene, including SNP 12 and SNP 29. An attempt to develop a TaqMan assay for rs12916 led us to sequence that region of the 3′ untranslated region, discovering a previously undescribed 17 bp deletion nearby at position chromosome 5, 74,692,265–74,692,283 [*Homo sapiens* May 2004 assembly (28)]. None of the tested loci yielded a nominally significant ($P < 0.05$)

TABLE 4. Genotyped polymorphisms in CETP and association with baseline HDL cholesterol

Polymorphism	n	Baseline HDL	*P* Value (Study-wide)
		mmol/l	
SNP 1/rs17231506			
CC	630	1.29 ± 0.02	**3.49×10^{-6} (0.003)**
CT	567	1.38 ± 0.02	
TT	173	1.45 ± 0.03	
rs1800776			
CC	1,107	1.34 ± 0.01	0.87 (–)
AC	126	1.33 ± 0.04	
AA	4	1.48 ± 0.13	
−629A/rs1800775			
CC	327	1.28 ± 0.03	1.82×10^{-4} (0.06)
AC	588	1.33 ± 0.02	
AA	321	1.43 ± 0.02	
SNP 4/TaqI/rs708272			
CC	381	1.30 ± 0.02	0.004 (0.59)
CT	543	1.35 ± 0.02	
TT	253	1.41 ± 0.03	
SNP 6/rs289714			
AA	776	1.38 ± 0.02	0.005 (0.73)
AG	327	1.29 ± 0.02	
GG	37	1.30 ± 0.08	

Results in boldface are significant study-wide.

association with change in LDL, whether tested in the fluvastatin-treated arm only (**Table 5**), in the full patient set with treatment group as a covariate, or in the full patient set with a genotype-treatment interaction as the independent variable (Table 3; see supplementary Table I). In fact, the results for SNP 12 and SNP 29 trend in the opposite direction from that reported (Table 5). The results after 6 months, the time period examined in PRINCE (17), trended in the same direction as reported in that study, but again, none of the tested loci yielded a nominally significant ($P < 0.05$) association with change in LDL, whether tested in the fluvastatin-treated arm only (results for SNP 12 and SNP 29 in Table 5), in the full patient set with treatment group as a covariate, or in the full patient set with a genotype-treatment interaction as the independent variable (see supplementary Table I).

MACE and graft failure

No genetic associations with the primary outcome measures of MACE and graft failure were seen at levels that reached study-wide significance. (The lowest nominal *P* value was 0.016 for an association between −629A and MACE.) To place these negative findings in context, power estimations were made for a hypothetical SNP with a 15% minor allele frequency, genotype distribution in accord with Hardy-Weinberg equilibrium, a 97% genotyping call rate, and an additive effect on a binary outcome measure with the prevalence of MACE in genotyped patients (10.5%). The calculated power to reach study-wide significance is 53% at a relative risk of 2.5, 87% at a relative risk of 3.0, and >99% at a relative risk of 3.6. Analysis of graft failure, with a 15.2% rate in genotyped patients, would have provided additional power.

eALERT results

Data from the open-label extension phase of ALERT were subjected to similar evaluation for MACE, graft failure, and change in LDL and HDL. Results were similar to those from the core study, with no significant evidence of any association between the tested SNPs and outcome.

DISCUSSION

A variety of existing hypotheses were available to guide our analysis. Polymorphisms in angiotensin-converting enzyme and angiotensin II receptor, type 1 have been associated with chronic allograft dysfunction and chronic inflammatory state in renal transplant patients (29, 30). Genetic associations with dyslipidemia and CV health are numerous, and a growing literature (31, 32) addresses the impact of genetics on the action of statins.

This analysis strongly supports the association of CETP polymorphism with baseline HDL level in male patients. Four polymorphisms with previously reported links to HDL were tested in this study: SNP 1, −629A, SNP 4/TaqI,

TABLE 5. Effect of HMGCR genotype on lowering of LDL cholesterol by treatment with fluvastatin (ALERT) and pravastatin (PRINCE) (17)

Study	SNP 12 Genotype	Mean Change in LDL (6 Months)	Mean Change in LDL (Full Study Period)
ALERT	AA	−1.06 mmol/l (n = 558)	−1.44 mmol/l (n = 498)
	AT	−0.97 mmol/l (n = 28)	−1.67 mmol/l (n = 24)
		$P = 0.35$	$P = 0.34$
PRINCE	AA	−0.88 mmol/l	–
	AT	−0.71 mmol/l	–
		$P = 0.005$	
	SNP 29 genotype	Mean Change in LDL (6 Months)	Mean Change in LDL (Full Study Period)
ALERT	TT	−1.04 mmol/l (n = 630)	−1.43 mmol/l (n = 564)
	TG	−0.93 mmol/l (n = 30)	−1.70 mmol/l (n = 27)
		$P = 0.46$	$P = 0.21$
PRINCE	TT	−0.88 mmol/l	–
	TG	−0.71 mmol/l	–
		$P = 0.003$	

ALERT, Assessment of Lescol in Renal Transplantation; PRINCE, Pravastatin Inflammation/CRP Evaluation. PRINCE results were originally presented in units of mg/dl and have been converted to mmol/l for comparison with ALERT values. ALERT association *P* values were calculated as described in Materials and Methods; PRINCE values are presented as reported.

and SNP 6. One showed significant association with baseline HDL even after extensive correction for multiple testing; all four had nominal *P* values $\ll 0.01$. None showed a study-wide significant association with MACE, and analysis of MACE by CETP genotype (**Table 6**) indicated a biologically implausible overdominance for almost all combinations of end point and SNP. [The replication of the findings from earlier studies (23, 24) obscured that overdominance by combining heterozygotes with one of the homozygous classes.]

The relationship between CETP polymorphism and CV risk is complex, with apparently opposite results reported (23, 33) for the effect of SNP 4/TaqI, for example. The ALERT population has some additional features that may affect comparisons with results from other studies. As has been noted (24), the "paradoxical" effects of HDL polymorphism may be very different in a high-risk population than in the general population. ALERT also had a relatively high rate of some of the minor CETP alleles, with, for example, a minor allele frequency of 0.46 for SNP 4/TaqI compared with the 0.42–0.43 seen in more heterogeneous populations (33). Given the predominance of northern Europeans in the ALERT sample (33% Swedish, 19% Norwegian, 13% Finnish, and 9% Danish), those high minor allele frequencies are consistent with those reported previously (34) and may yield results different from those in cases in which the risk alleles are rarer.

What is certainly clear from these various studies is the absence of the straightforward increase in CV disease that might be expected from the robust effects of CETP variation on HDL level. This might be attributable to changes in the nature of the HDL particles that weaken the benefits of increase or to some other mechanism that acts in parallel with and opposite to the HDL increase.

Despite a previous report (17) finding a significant association between two SNPs in HMGCR and efficacy of pravastatin in decreasing LDL cholesterol, we found no evidence for it in the polymorphisms we tested, including the two in that report. This may simply reflect a difference in the action of fluvastatin compared with pravastatin. Statins, although structurally related, are also characterized by notable differences. Fluvastatin is metabolized primarily by CYP2C9, for example, whereas pravastatin is not notably metabolized by the P450 system. This and other differences may account for the failure to see an effect of HMGCR polymorphism on fluvastatin efficacy. Alternatively, the much longer time between baseline and final measurement in ALERT [5–6 years, compared with 24 weeks in PRINCE (4, 17)] may be a factor if the response difference associated with HMGCR polymorphism declines after chronic statin therapy. However, an analysis with comparable time points also found no nominally significant associations.

No significant associations were seen between any of the tested SNPs and the primary clinical end points of MACE and graft failure. Simulation suggests that a moderately rare SNP (5% minor allele frequency) of large effect size would yield a study-wide significant association in the large majority of tests, indicating that large effects on MACE and graft failure of the tested SNPs in this RTR population can be excluded. This does not, of course, eliminate the possibility of such effects in other, untested genes or in the tested genes at loci with weak or no linkage disequilibrium with the genotyped polymorphisms. The results in Table 3 and supplementary Table I will allow all of the obtained association results, in the entire genotyped population and in subgroups, to be used for replication and hypothesis generation by other researchers.

The conclusion drawn by the investigators in the eALERT trial (5) was that statin therapy should be considered standard for all RTRs. This pharmacogenetic analysis found no evidence that variation at any of the tested candidate loci should affect that recommendation, at least with regard to fluvastatin.

TABLE 6. MACE prevalence by CETP genotype

Polymorphism	Overall	Fluvastatin	Placebo	Male	Female
SNP 1/rs17231506					
CC (n = 641)	9.8%	9.0%	10.7%	12.7%	4.2%
CT (n = 581)	12.1%	9.7%	14.4%	13.2%	10.0%
TT (n = 174)	8.6%	13.2%	3.6%	10.3%	5.2%
rs1800776					
CC (n = 1,126)	10.8%	10.2%	11.3%	12.7%	7.2%
AC (n = 130)	13.1%	8.8%	17.7%	15.6%	7.5%
AA (n = 4)	0.0%	0.0%	0.0%	0.0%	0.0%
−629A/rs1800775					
CC (n = 334)	8.1%	8.7%	7.4%	10.3%	2.9%
AC (n = 600)	13.7%	9.6%	17.5%	16.7%	8.6%
AA (n = 334)	9.0%	12.1%	5.3%	9.2%	8.4%
SNP 4/TaqI/rs708272					
CC (n = 388)	9.3%	8.6%	10.0%	11.6%	5.1%
CT (n = 556)	12.6%	9.4%	15.8%	15.0%	8.6%
TT (n = 257)	9.0%	12.5%	5.5%	9.4%	7.9%
SNP 6/rs289714					
AA (n = 793)	9.3%	10.2%	8.4%	11.1%	6.0%
AG (n = 333)	13.2%	9.4%	16.8%	16.1%	7.8%
GG (n = 37)	13.5%	6.7%	18.2%	21.1%	5.6%

Fluvastatin-placebo and male-female ratios are all approximately proportional to their ratio in the overall patient set.

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