Supporting Information

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SI Materials and Methods

Study Subjects. HIV-infected volunteers followed at Wilford Hall Medical Center (WHMC) initially as part of the Military HIV Program Natural History Project and more recently as part of the Tri-Service AIDS Clinical Consortium (TACC) HIV Natural History Study participated in this investigation. WHMC is the referral hospital for all HIV-infected U.S. Air Force (USAF) personnel. We determined *APOE* $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ alleles in 1,267 HIV-seropositive (HIV+) adults and 1,132 ethnically comparable HIV-seronegative (HIV-) controls that were also from WHMC. The voluntary, fully informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169–9, and extensive details about this cohort have been described previously (1–4). The protocol and consent for this study were approved by the Institutional Review Boards at the WHMC and UTHSCSA.

Genotyping Assays. Two SNPs, T2060C (rs429358) and C2198T (rs7412), were genotyped using TaqMan-based allelic discrimination assays. For genotyping T2060C, the sense and antisense primers were 5′-GGGCACGGCTGTCCAA-3′ and 5′-CACCTCGCCGCGGTACT-3′, respectively, and fluorogenic probes with BHQ modification were 5′FAM-CGGCCGCgCACGTCCT-3′ and 5′-TET-CGGCCGCaCACGTCC-3′. For genotyping C2198T, the sense and antisense primers were 5′-CTCCGCGATGCCGATGA-3′ and 5′-CTCGCGGATGGCGCTGA-3′, respectively, and the fluorogenic probes with MGB modification were 5′-FAM-AGAAGcGCCTGGCA-3′ and 5′-VIC-CAGAAGtGCCTGGCA-3′. Genotyping of polymorphisms in *CCR5* and copy number of the gene encoding *CCL3L1*, its major agonist (and potent HIV-suppressive chemokine) as well as generation of the low, moderate and high *CCL3L1-CCR5* genetic risk groups (GRG) were as described previously (1–3).

Cell Lines and Tissue Culture. 293T cells were used to produce HIV reporter virions expressing luciferase or containing BlaM-Vpr. MAGI-R5 is a HeLa-derived cell line engineered to constitutively express human CD4 and CCR5, as well as the beta-galactosidase gene under control of the HIV long terminal repeat (LTR) promoter (5). 293T and MAGI-R5 cells were maintained in DMEM (Gibco BRL) containing 10% FBS and 1% penicillin-streptomycin (Gibco BRL) at 37°C in 5% CO_2. SupT1-CCR5 cells were maintained in RPMI (Gibco BRL) supplemented with 10% FBS and 1% penicillin-streptomycin. For luciferase experiments, MAGI-R5 cells were cultured in DMEM supplemented with 10% mixed AB-human serum (DMEM-ABHS) or in DMEM with 10% human lipoprotein depleted serum (DMEM-LPDS; LPDS was kindly provided by the Gladstone Institute of Cardiovascular Disease).

Virus Production. HIV reporter virions expressing luciferase were produced as described previously (6) by transfecting 293T cells (10×10^6) using calcium phosphate and 20 μg of pEnv-NL4–3 and 60 μg pNL-luc-E-R-proviral DNA (obtained from the National Institutes of Health AIDS Research and Reference Reagent Program). Cells were incubated for 12–16 h at 37°C in DMEM-ABHS, after which the medium was replaced with DMEM-LPDS media to minimize exogenous lipoprotein incorporation into virion. After 24 h of culture at 37°C, virus-containing supernatants were collected, centrifuged at low speed to remove cellular debris, aliquoted, and stored at −80°C. The infectivity of the viral preparation was assessed by luciferase quantification as described below.

HIV virions containing BlaM-Vpr were generated as described (7). Briefly, 293T cells (20×10^6) were transfected with either pNL4–3 or 81A proviral DNA, pCMV-BlaM–Vpr, and pAdVAntage vectors (Promega). After 48 h of culture at 37°C, virus-containing supernatants were centrifuged at low speed to remove cellular debris and then ultracentrifuged at $72{,}000 \times g$ for 90 min at 4°C to sediment viral particles. Viral stocks were normalized by $p24^{Gag}$ content, measured by ELISA (NEN Life Science Products).

Infectivity Assays in MAGI-R5 Cells. MAGI-R5 cells were plated in 96-well flat bottom tissue or 24-well culture plates at a density of 2.5×10^4 or 1×10^5 cells per well, respectively in DMEM-LPDS and incubated for 16–24 h at 37°C. The medium was replaced by DMEM with or without increasing concentrations of purified recombinant human apoE3 or apoE4 protein (provided by Dr. Karl H. Weisgraber of the Gladstone Institute of Neurological Disease). After 2 h of incubation at 37°C, 0.2 ml of HIV luciferase reporter virions pseudotyped with NL4–3 envelope (825 ng p24/ml) was added (without changing the pretreatment media) for 16–24 h. The media was replaced again with fresh DMEM-FBS. After 3 days, cells were harvested and luciferase activity was measured using the Promega Luciferase Assay System, according to the instructions provided by the manufacturer.

HIV Virion-Based Fusion Assay. The measurement of HIV fusion to SupT1-CCR5 cells was performed with the flourescence resonance energy transfer (FRET) based fusion assay, as described (7). In this assay, virus is prepared that incorporates a fusion protein of HIV vpr with an active beta-lactamase (BlaM) domain (BlaM-vpr). Upon fusion of the viral envelope with the target cell membrane, the BlaM is transferred to the target cells and is able to cleave the substrate and fluorescent dye (CCF2-AM). When target cells are loaded with CCF2-AM, infection with virus carrying BlaM-vpr results in substrate cleavage, causing a shift in the emission spectrum of the dye from green to blue. This shift can be detected by flow cytometry and is a sensitive marker for cells that have undergone fusion. Briefly, SupT1-CCR5 cells (5×10^5) were incubated with apoE3 or apoE4, for 1 h at 37°C. NL4–3 or 81A virions containing the BlaM-Vpr (125–250 ng $p24^{Gag}$) were added for 1 h at 37°C. The cells were washed in CO_2-independent medium (Gibco BRL) and loaded with CCF2-AM dye (0.5 mM; Invitrogen) for 1 h at room temperature. After two washes with DMEM, BlaM cleavage of CCF2 was allowed to proceed for 16 h at room temperature in 200 μl of DMEM supplemented with 10% FBS and 2.5 mM probenecid, an inhibitor of anion transport. The cells were next washed once in PBS and fixed in a 1.2% solution of paraformaldehyde. The change in emission fluorescence of CCF2 after cleavage by the BlaM-Vpr chimera was measured by flow cytometry using a BD LSRII (Becton Dickinson). Data were collected using FACSDiva software (Becton Dickinson) and analyzed with FlowJo software (Treestar).

Statistical Analysis. We first assessed whether the *APOE* alleles were in Hardy-Weinberg equilibrium using the exact test for multiallelic loci. The association between *APOE* alleles or genotypes and the risk of acquiring HIV or specific AIDS-defining illnesses was determined using multinomial logistic regression

analysis. The association between *APOE* alleles or genotypes and the rate of disease progression was assessed using Cox proportional hazard regression models. Disease endpoints were time to AIDS (1987 CDC criteria), time to death, and time to developing specific AIDS-defining illnesses. Logistic regression analyses were also used as described below in the **Supplementary Note**. We determined the association between *APOE* genotypes and the initial viral load by analysis of variance (ANOVA). All statistical analyses were conducted using Stata 7.0 (Stata Corp.).

Supplementary Note. To confirm the lack of association between the $\varepsilon4/\varepsilon4$ genotype and HAD, we conducted a stepwise logistic regression model in which possession of the $\varepsilon4/\varepsilon4$ genotype was used as the dependent variable and AIDS-defining illnesses were used as the independent variables, all placed in a single model. In the final stepwise regression model an association between risk of developing HAD and possession of the $\varepsilon4/\varepsilon4$ genotype was not observed (odds ratio = 0.90, 95% C.I. = 0.12 – 6.84, P = 0.923) whereas an association for risk of developing *Mycobacterium avium* complex (odds ratio = 5.91; 95% C.I. = 2.34 – 14.93; $P < 0.0001$, Fig. S2) and *Coccidiodes immitis* (odds ratio = 32.07; 95% C.I. = 2.76 – 373.10; P = 0.006; Kaplan-Meier plots not shown) was detected.

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Fig. S1. *APOE* alleles and genotypes influence rate of disease progression in HIV-infected individuals from the WHMC cohort. Kaplan-Meier plots depict the association between the indicated *APOE* alleles/genotypes and rate of progression to AIDS (1987 CDC criteria). Data are for the entire WHMC cohort. RH, relative hazards; CI, confidence interval. *P* values in *C* are by log-rank test, whereas they are by Cox proportional hazard regression models in *A*, *B*, and *D*. Color-coded numbers adjacent to each plot are the corresponding number of subjects in each study group.

Fig. S2. *APOE* genotypes influence rate of disease progression to *Mycobacterium avium* complex. RH, relative hazards; CI, confidence interval. *P* values are by Cox proportional hazard regression models. Color-coded numbers adjacent to each plot are the corresponding number of subjects in each genotypic group.