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Differential course of HIV-1 infection and apolipoprotein E polymorphism

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Abstract: We studied the course of infection with human immunodeficiency virus type 1 (HIV-1) in relation to apolipoprotein E (*APOE*) polymorphism found for 209 Italians treated at Infectious Disease Clinics in Rome and Modena. Clinically, patients were classified into four groups according to the yearly rate of decline in CD4+ cell count (LTNP: long-term non-progression; SLOW, 'NORMAL' or RAPID). Patients at both extremes of the clinical spectrum, i.e. those who rapidly progressed to AIDS and those with stable high CD4 cell counts, had few *APOE* $\varepsilon 4$ and $\varepsilon 2$ alleles ($P = 0.04$). Detailed clinical information was then used to construct four model-based clinical profiles using grade-of-membership analysis (GoM), predictive of *APOE* genotypic frequencies: 1. The clinical profile associated with good long-term prognosis lacked $\varepsilon 2$ (P=0.01); 2. Disease progression to AIDS was associated with $\varepsilon 4$ and $\varepsilon 2$, most evident for zidovudine-lamivudine regimens without a protease inhibitor ($P = 0.03$); and, 3. AIDS patients had low $\varepsilon 4$ and $\varepsilon 2$ frequencies, consistent with a high mortality rate among $\varepsilon 4+$ and $\varepsilon 2+$ AIDS patients. These findings suggest allele-specific immunomodulatory effects involving inherited *APOE* isoform important enough to alter the clinical course of HIV infection and, possibly, drug efficacy. They imply a connection between lipid metabolism and immunity potentially relevant to common disorders.
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Keywords: HIV-1 infection, APOE, genetic epidemiology, grade-of-membership analysis, immunomodulation

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1 Introduction

Apolipoprotein E (apoE) is a 34 kDa glycosylated protein originally described in the context of cholesterol transport [1]. There is a growing body of experimental evidence implicating apoE in the interplay between lipid metabolism and immune response. *In vitro*, apoE suppresses lymphocyte proliferation, generation of cytolytic T-cells, and stimulation of cultured neutrophils [2–5]. ApoE-deficient (APOE $^{-/-}$) mice have impaired immunity after bacterial challenge [6, 7], significantly higher levels of antigen-specific IgM after immunization with tetanus toxoid, and impaired delayed type hypersensitivity responses [8]. Moreover, double knockout apolipoprotein (APOE $^{-/-}$) and low-density lipoprotein receptor (LDLR $^{-/-}$) mice were hypercholesterolemic and had slower clearance of hepatotropic lymphocytic choriomeningitis virus compared to single knockout LDLR$^{-/-}$ mice [9].

There are three common human isoforms of apoE, designated E2, E3, and E4 differing by single amino acid interchanges at residues 112 and 158: E3 ($Cys_{112}Arg_{158}$); E4 ($Arg_{112}Arg_{158}$); E2 ($Cys_{112}Cys_{158}$). The encoding APOE alleles are designated $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. Epidemiologic studies have firmly established the 4 allele as a risk factor for vascular [10–12] and also neurologic disorders including Alzheimer's disease (AD) [13–15] and a poor prognosis in multiple sclerosis (MS) [16–18]. Both AD and MS are chronic progressive disorders likely involving focal inflammatory processes: reactive microglia, complement fixation and expression of inflammatory cytokines [19–21]. ApoE is the primary apolipoprotein produced in the CNS, and is up-regulated after injury. The potential importance of isoform-specific interplay between lipid metabolism and immunity/inflammation in CNS disorders is further indicated by Itzhaki *et al.* [22] who found herpes simplex virus type 1 in brain regions affected by AD for patients who carried $\varepsilon 4$, i.e. a gene-viral interaction, and by Sorbi *et al.* [23] who found that $\varepsilon 4+$ accident victims were less likely to wake from post-traumatic coma.

Following the clinical course of human immunodeficiency virus type-1 (HIV-1) infection for subjects having differing combinations of inherited APOE alleles, i.e. genotypes, represents another opportunity to demonstrate isoform-specific interplay between lipid metabolism and immunity. To date, one small prospective study ($n = 24$) found more frequent mild dementia and peripheral neuropathy early in the course of infection for subjects who carried $\varepsilon 4$ [24], and CD4 count tended to decline more rapidly for $\varepsilon 4+$ subjects. Interestingly, the HIV-1 Tat protein competes with APOE, and amyloid precursor protein (derived peptides are the major constituent of senile brain plaques found in AD), to gain access to neurons via LDLR [25]. In the periphery, AIDS patients, particularly those carrying the $\varepsilon 2/3$ genotype, show tripled plasma triglyceride and doubled apoE protein levels compared to uninfected control subjects [23]. Increased sialylation of apoE was correlated with the increase in apoE levels.

In this study we investigated 209 Italians infected with HIV-1. They were divided into four clinical groups according to the yearly rate of decline in CD4+ cell count: LTNP, i.e. long term non-progressor having stable high counts, as well as SLOW, 'NORMAL' and

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RAPID decline. The clinical groups are contrasted in terms of APOE genotypic frequencies using standard data analytic approaches, e.g. chi-square tests. Next, detailed clinical information on the subjects was used to construct model-based clinical profiles, including treatment information, to more specifically relate disease state to APOE polymorphism. This was accomplished via grade-of-membership analysis or GoM.

GoM identifies model-based extreme pure type groups representing important features of rich datasets. Specifically, the extreme pure type groups are each represented by frequencies for the variables which can be interpreted in light of existing knowledge of the topic. They can be considered as stereotypes which many, few, or, even, none, of the subjects exactly match in every detail. Individuals have membership scores in the extreme pure type groups which range from zero, i.e. not at all like the group, to one, i.e. an exact match. Thus individuals can resemble aspects of several groups. In this instance, four GoM groups represent the detailed data related to rate of decline in CD4+ cell count and treatment. Note that APOE genotype was included in the model as a sort of by-stander - where the clinical groups identified without APOE information were, nonetheless, found to differ in terms of APOE genotypic frequencies.

2 Statistical methods and Experimental Procedures

2.1 Study subjects

209 HIV-positive patients treated at Infectious Disease Clinics in Rome and Modena were investigated. APOE genotype [34] information was available for each subject. Age, CD4+ levels, and treatment information pertained to the date of ascertainment. Informed consent was given by all participants to this study.

2.2 Statistical methods

Chi-square tests were used to compare genotypic frequencies among subject groups. Grade-of-membership analysis was used identify clinical profiles and their corresponding APOE frequency distributions. Chi-square tests were used to compare genotypic frequencies according to likeness to the profiles.

2.3 Grade-of-membership analysis

GoM can be described after first identifying four indices. One is the number of subjects I $(i = 1, 2, ..., I)$. Here $I = 209$ subjects were identified. The second index is the number of variables J $(j = 1, 2, ..., J)$. There are $J = 14$ variables each representing one of the clinical variables displayed in Table **??** (see Results). Our third index is Lj: the set of response levels for the J^{th} variable. The Lj are shown in the second column of Table 1.

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Table 1 Results of various techniques used for the detection of Parvovirus B19 induced fetal hydrops and distribution of sections evaluated in the study.

Variable	Categories	Sample	I 'LTNP'	II 'Slow'	III 'Normal'	IV 'Rapid'
Group	LTNP	21.1	100	0	0	0
	Slow progression	12.4	0	42	0	0
	Normal progression	46.4	0	58	100	0
	Rapid progression/AIDS	20.1	0	0	0	100
CD4+ cell count/mm3	1000+	3.0	11	0	0	0
	750-999	9.6	34	0	0	0
	500-749	23.1	33	28	60	0
	250-499	31.2	23	72	40	12
	100-249	25.1	0	0	0	67
	28-99	8.0	0	0	0	21
	30-39	17.1	72	0	0	0
	20-29	26.8	0	64	73	0
	10-19	34.2	0	36	27	58
	4-9	15.2	0	0	0	42
Reverse	Stavudine/D4T	49.5	0	100	0	100
Transcriptase	Didanosine/ddI	26.0	0	100	0	100
Inhibitors	Lamivudine/3TC	51.0	0	0	100	0
	Zidovudine/AZT	31.2	0	0	100	0
	Zalcitabine/ddC	3.7	0	0	16	0
	Nevirapine/NEV	2.6	0	0	11	0
Protease	Ritonavir/RTV	25.5	0	0	0	77
Inhibitors	Indinavir/INV	9.9	0	0	0	34
	Nelfinavir/NFV	6.8	0	25	0	0
	Saquinavir/SQV	3.7	0	0	0	16
Route of	IV drug use	57.4	56	64	53	61
Transmission	Heterosexual sex	22.6	16	30	47	0
	Homosexual sex	11.8	21	0	0	25
	Bisexual sex	7.2	7	0	7	14
Age (years) for		M F	M F	M F	M F	M F
Males and Females	17-24	16 9	0 8	0 25	66 7	0 0
	25-29	16 10	9 5	32 27	0 10	23 0
	30-34	14 8	14 11	17 0	0 17	24 3
	35-65	21 8	26 28	0 0	0 0	49 0
Profile size		209	49.70	51.15	50.56	57.58

This leads to the definition of the basic GoM model where the probability that the i^{th} subject has the Lj^{th} level of the J^{th} variable is defined by a binary variable (i.e., $y_{ijl} = 0, 1$). The model with these definitions is,

$$Pr\ ob(y_{ijl} = 1.0) = \sum_k g_{ik}\lambda_{kjl}$$

where the g_{ik} are convexly constrained scores (i.e., $0.0 < g_{ik} < 1.0; \sum_k g_{ik} = 1.0$) for subjects and the λ_{kjl} are probabilities that, for the K^{th} latent group, the Lj^{th} level is found for the J^{th} variable. The procedure thus uses this expression to identify K profiles representing the pattern of $J \times Lj$ responses found for I subjects. GoM models were constructed using GoM software developed at the Center for Demographic Studies, Duke University [35].

The parameters g_{ik} and λ_{kjl} are estimated simultaneously using the likelihood function (in its most basic form) [36].

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$$L = \prod_i \prod_j \prod_l \left(\sum_k g_{ik} \cdot \lambda_{kjl} \right)^{y_{ijl}}$$

In the likelihood y_{ijl} is 1.0 if the Lj^{th} level is present and 0.0 if it is not present. GoM models specifying from $K = 3$ to 5 groups, i.e. clinical profiles, were constructed. The significance of adding the $K+1$ profile was tested as an independent increment in the fit of the model adjusting for the larger number of degrees of freedom in the larger model. Akaike Information Criterion [37] was calculated as

$$A\ I\ C = -2l\hat{\theta} + 2P$$

where l is the likelihood value and P is the number of estimated parameters. However, for parameters on the boundary, i.e. value= 0, only one is penalized. The rationale for subtracting only one for parameters on the boundary is that the distribution for those parameters is $\frac{1}{2}\chi^2$(central). The lowest value of the AIC designates the best model, i.e. the model with the best fit and least bias.

GoM models specifying either 3, 4, or 5 clinical profiles, i.e. $K = 3$ to 5, had $AIC = -357.4, -420.2$, and -330.7, respectively. The 4-group model is reported.

Information on APOE genotype was not used to construct the groups used to clinically characterize the subjects. One option in the likelihood is to separate calculations for "internal" (here, clinical) and "external" (here, APOE genotype) variables. For internal variables M.L.E. of g_{ik} and λ_{kjl} are generated and the information in internal variables used to define the K groups. For external variables the likelihood is evaluated (and M.L.E. of λ_{kjl}; generated) but the information is not used to redefine the K groups, i.e., the likelihood calculations for likelihood equations involving the g_{ik} are disabled for external variables so that the g_{ik}, and the definition of the K groups, is not changed.

3 Results

3.1 The clinical groups and their APOE frequencies

The subjects were divided into four groups based on the rate of decline in $CD4^+$ cell count: 1) LTNP: Long-term-non-progressors were infected for 10 or more years and have a $CD4^+$ count over 500 cells/μL, stable in recent years, no symptoms and never took any antiretroviral therapy ($n = 44$). 2) SLOW: Patients with slow progression, i.e. a loss of < 50 $CD4^+$ T cells/μL/per year ($n = 26$). 3) 'NORMAL': Those infected for many years showing a "normal" yearly loss of $80-100$ $CD4^+$ cells during the last five years ($n = 97$). 4) RAPID: Patients loosing > 150 $CD4^+$ cells/μL/year, and developing AIDS or having less than 200 $CD4^+$ T cells/ L within 5 years of infection ($n = 42$). Importantly, the subjects were classified before the start of HAART (highly active antiretroviral therapy). Thus the groups reflect the natural time courses of HIV infection and host-virus interactions.

APOE genotypic frequencies for the NORMAL group (Fig. 1) were indistinguishable from those found for Italian adults ($n = 179$) [27]: 13.4% for $\varepsilon 2$/- (i.e. $\varepsilon 2/3$ or $\varepsilon 2/2$)

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was found versus 12.8% expected, 67.0% vs 69.8% for ε3/3, and 19.6% vs 17.3% for ε-/4 (ε3/4, ε2/4, or ε4/4). Curiously, ε4+ and ε2+ genotypes were a third less frequent for both ends of the progression spectrum (chi-square testing: $P = 0.04$). Stable and slowly progressing patients had relatively few ε4 and ε2 alleles. Subjects who had rapidly progressed to AIDS and in the clinic series, i.e. still alive, also had relatively few ε4 and ε2 alleles.

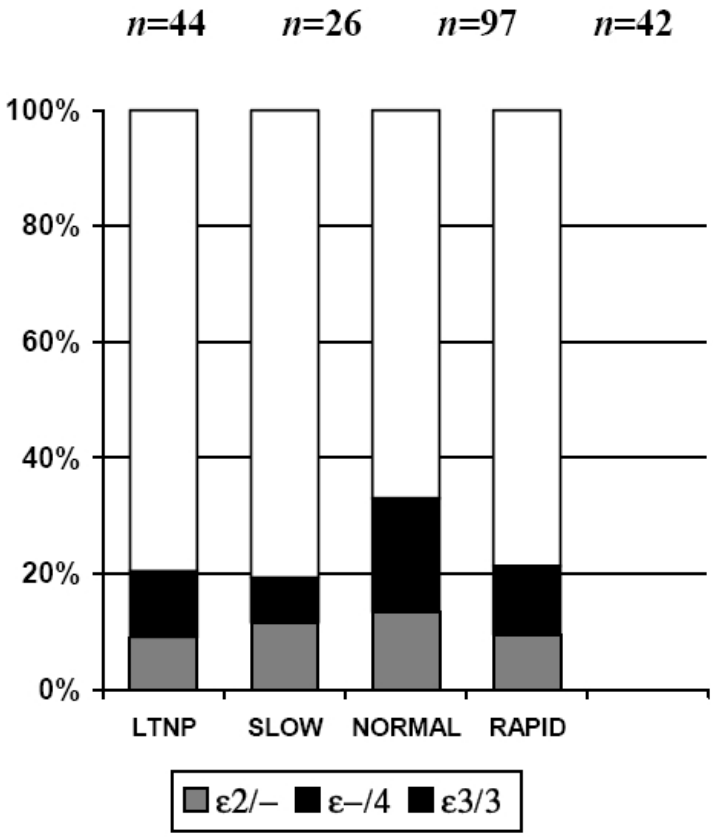

Fig. 1 APOE genotypic frequencies for Italian HIV-1+ patients according to rate of CD4+ cell loss.

One interpretation of this finding is that both ε2 and ε4 1) speed disease progression to AIDS, i.e. those who had a high stable or slowly declining CD4+ cell count were relatively unlikely to be ε2+ or ε4+, and 2) increase the risk of opportunistic infection and death for immune compromised patients, i.e. ε2+ or ε4+ AIDS patients frequently died before ascertainment as part of the study.

This interpretation is consistent with the adequate CD4 cell count (mean 598) (Fig. 2a) and percentage (34%) (Fig. 2b) found for the LTNP group compared to the NORMAL group: Few ε2+ and ε4+ subjects remained stable over many years. At the other end of the spectrum, the RAPID group of survivors to the point of ascertainment had inadequate CD4 levels (mean cell count 197; 13.7%) and frequently ε2- and ε4-.

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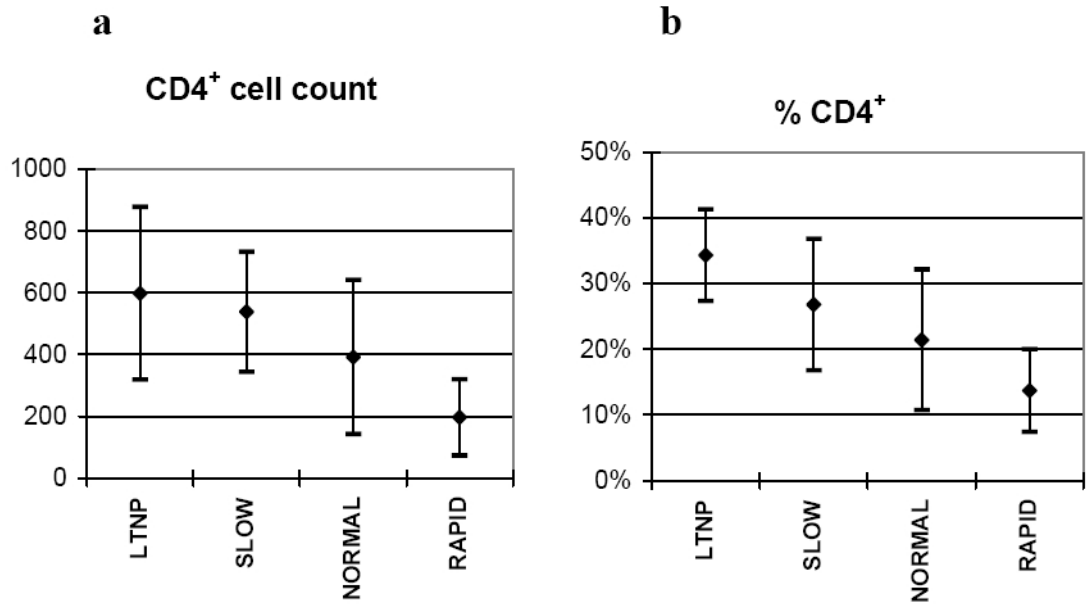

Fig. 2 Mean CD4+ cell count/mm3 and percentage for the HIV+ patients.

3.2 Model-based clinical profiles

Detailed clinical profiles were then identified using GoM (Table 1). The predictive information consisted of subject group, age, sex, CD4+ cell count and percentage, route of transmission, and antiretroviral treatment. The four profiles are defined by the frequencies of responses for the variables, akin to the sample percentages, distinct for each profile.

The profiles were labeled I to IV based on subject group composition, and corresponded closely to the subject groups. That is, a subject exactly like Profile I would be in the LTNP group. Profile II was consistent with either SLOW or NORMAL groups. A subject exactly like Profile III or IV would be in the NORMAL or RAPID group, respectively. CD4+ cell count and percentage were highest for Profile I (in fact, higher than for the LTNP group as a whole!), lowest for Profile IV, and intermediate (and comparable) for Profiles II and III. Males predominated in Profiles III (transmission via heterosexual sex or IV drug use) and IV (transmission via homosexual/bisexual sex or IV drug use).

The profiles differed in terms of antiretroviral therapy: Profile I did not receive treatment. Profiles II and III received dual nucleoside analog therapy with stavudine/didanosine or lamivudine/zidovudine, respectively. Profile IV added protease inhibitors (stavudine/didanosine plus ritonavir/other).

3.3 Resemblance of individuals to the profiles

As individuals, the study subjects who exactly resembled a single profile contributed one to the size of the relevant profile (Table 1, bottom) and zero to the other profiles.

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Otherwise, the subject contributed a total of one to the sizes of the relevant profiles depending on the extent of resemblance. There were 30 LTNP subjects who exactly resembled Profile I 'LTNP'. This was 60% of the profile size of 49.7. Exclusive membership was lesser for the other profiles: 12 of 51.2 for Profile II, 4 of 50.6 for Profile III, and 15 of 57.6 for Profile IV 'Rapid'. The remaining subjects partly resembled and contributed size to two or more profiles.

3.4 APOE frequencies for the model-based profiles

The profiles based on CD4 level and rate of decline, not APOE information, were more distinctive in terms of genotypic distributions (Figure 3) than the raw data (Figure 1). Profile I 'LTNP' completely lacked $\varepsilon 2/3$. That is, subjects exactly like Profile I did not carry $\varepsilon 2/3$. Comparing the 31 subjects with $> 80\%$ membership in Profile 1, the 132 with less than 20% membership, and the 46 having intermediate membership, the lack of 2/3 was statistically significant (chi-sq= 11.76, 2 d.f., $P = 0.003$). Specifically, subjects with $> 80\%$ membership had no chance of carrying $\varepsilon 2/3$. This finding was robust in that models with 3 and 5 profiles also identified a 'LTNP' profile lacking $\varepsilon 2/3$. Moreover, subjects with $> 80\%$ membership in Profile I were statistically less likely to carry $\varepsilon 2/3$ than the control group of Italians ($P = 0.03$). Note that the 2% of the profile size contributed by subjects carrying 2/4 is combined with the 14% carrying $\varepsilon 3/4$ (Figure 3).

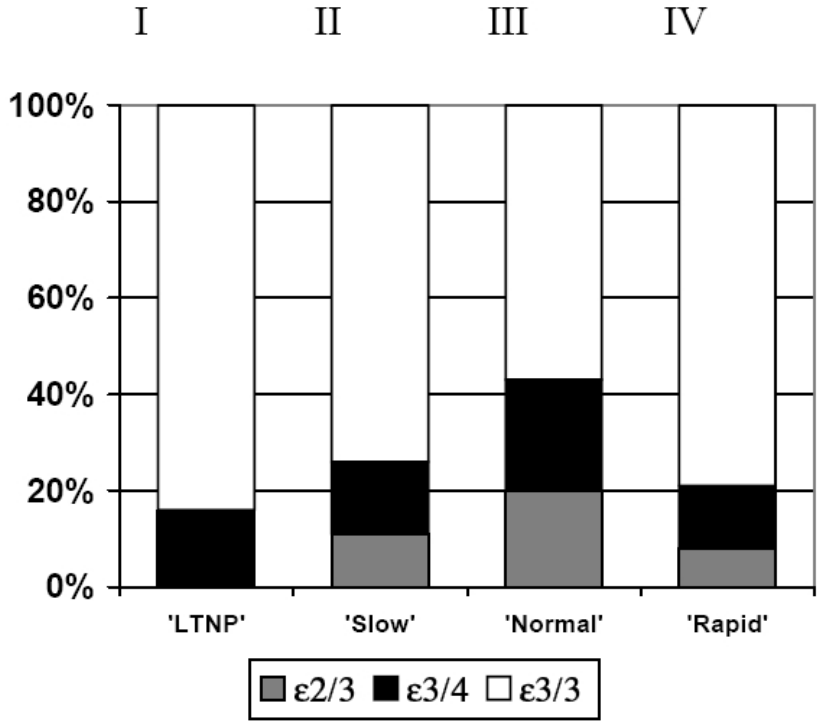

Fig. 3 APOE genotypic frequencies for clinical Profiles I to IV.

Exclusive members of the 'Slowly' progressing Profile II, had few $\varepsilon 2$ and $\varepsilon 4$ alleles. One of 16 (11%) subjects who had exclusive, or, nearly exclusive, i.e. $> 80\%$, membership

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in Profile II carried either $\varepsilon 2$ or $\varepsilon 4$ compared to 54 of 179 (31%) control subjects, providing statistically significant evidence for lower than expected $\varepsilon 2$ and $\varepsilon 4$ frequencies for Profile II ($P = 0.04$).

Profile III 'Normal' had elevated $\varepsilon 2$ and $\varepsilon 4$ frequencies compared to those expected for Italians. The degree of resemblance of subjects to Profile III was correlated with the frequency of $\varepsilon 2+$ and $\varepsilon 4+$ genotypes: 21% for subjects having low $< 20\%$ membership in Profile III, 29% for subjects having intermediate, i.e. 21% to 79%, membership, and 47% for subjects having high $> 80\%$ membership (chi-sq= 4.55, 1 d.f., $P = 0.03$). This finding was robust as the model specifying 5 profiles also identified a 'Normal' profile having elevated $\varepsilon 2$ and $\varepsilon 4$ frequency: 45%, higher than the reported 4-profile model. Finally, Profile IV 'Rapid' at high risk of opportunistic infection had low $\varepsilon 2$ and $\varepsilon 4$ frequency compared to the control group, and half that of Profile III, but the differences did not reach statistical significance.

4 Discussion

We investigated 209 Italians infected with HIV-1 identified in a clinic series and found that those having a typical yearly rate of decline in CD4+ T cell count of 80 to 100 had APOE genotypic frequencies indistinguishable from the Italian population. However, subjects at both extremes of the clinical spectrum – having stably high levels or rapid progression to AIDS – were less likely to carry either the $\varepsilon 2$ or $\varepsilon 4$ APOE alleles ($P = 0.04$).

To better define the process, subjects were represented by four model-based clinical profiles. Good long-term prognosis (no antiretroviral treatment) (Profile I) was inconsistent with $\varepsilon 2$ ($P = 0.01$). Given the adequate CD4+ levels for the profile, the lack of $\varepsilon 2$ reflects progression of the disease to AIDS for $\varepsilon 2^+$ subjects, rather than selectively high mortality. Slow disease progression and initial treatment with stavudine/didanosine, (Profile II) was associated with $\varepsilon 4$ and $\varepsilon 2$ frequencies marginally lower than for the Italian population, also reflecting progression for $\varepsilon 4+$ and $\varepsilon 2+$ subjects. Profile III, which had more rapidly reached marginal CD4 levels comparable to Profile II, was rich in $\varepsilon 4$ and $\varepsilon 2$. Again, this is consistent with both alleles speeding disease progression. Unlike Profile II, Profile III had been treated with zidovudine/lamivudine (no protease inhibitor), i.e. possible gene-drug interaction.

The low $\varepsilon 4$ and $\varepsilon 2$ frequencies for Profile IV 'Rapid' diagnosed with AIDS, half that for Profile III having marginal CD4 levels, are consistent with selectively high mortality for $\varepsilon 4+$ and $\varepsilon 2+$ subjects. The frequent late diagnosis for Italian males [28] and the predominance of males in Profile IV having poor prognosis argues in favor of early diagnosis and aggressive treatment.

The study findings are entirely consistent with previous in vitro and mouse models demonstrating that apoE has immunomodulatory effects [2–9] and extend the results to humans demonstrating isoform-specific effects strong enough to alter the immune course of HIV-1 infection, from early to late stages - and specific enough to suggest differential response to therapy. The depletion of $\varepsilon 2$ and $\varepsilon 4$, rather than $\varepsilon 4$ alone, tends to implicate

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dyslipidemia in determining the immunologic course of HIV-1 infection, given the previous report of ε2/3-related elevation in plasma triglyceride and apoE protein levels [23]. The study offers no explanation for the previous study by Dunlop *et al.* [29] who did not find an association between apoE isoform and mortality, or dementia, in AIDS patients. One speculation is that recent effective therapies have in fact disclosed the genetic disparities by extending survival. The plausibility of the study is supported by the finding that a single amino acid interchange for the major histocompatibility complex class I type HLA-B*35 allele effects the rate of progression to AIDS [30].

The study is limited in that it is cross-sectional: Prospective investigations are needed to confirm the findings, preferably in contexts that consider viral load set point (determined by acute initial infection), the neurologic course of HIV-1 infection and including measures of HLA-B*35, serum apoE level, lipids, and cytokines. A finding of high serum apoE and altered lipid/cytokine levels for ε2+ and/or ε4+ subjects would further implicate lipid metabolism in immune function and begin to explain the demonstrated isoform-specific effects found in this study. It would also motivate management of lipodystrophy associated with, among other things, protease inhibitors [31, 32]. Once confirmed, APOE-related processes could be targeted in HIV-1 treatment potentially mitigating the observed genetic disparity as for simvastatin treatment abolishing 4-related excess mortality following myocardial infarction [33].

In summary, the course of HIV-1 infection, and, perhaps, the efficacy of specific treatments, are determined in part by apoE isoform. These results raise the possibility that immune function in other conditions is influenced by lipid metabolism.

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