The Importance of Assessing Effect Modification  
When Asserting Racial Differences in Associations  
between Human Leukocyte Antigen Class II Alleles  
and Hepatitis C Virus Outcomes

To the Editor—In a recent article, Thio et al. [1] sought to determine whether DQB1*0301 and other HLA class II alleles were associated with hepatitis C virus (HCV) clearance and persistence in 3 multiethnic cohorts. The results of their analyses confirmed the previously documented association of DQB1*0301 and other HLA class II alleles with viral clearance [2]. Furthermore, Thio et al. hypothesized that the inconsistencies in the association between HLA class II alleles and HCV clearance found in earlier studies could be due to ethnic differences [1]. They reported that the association of DQB1*0301 and other HLA class II alleles with HCV clearance or persistence differed by ethnic identity. Finally, they emphasized the importance of studying genetic associations in an ethnically diverse cohort. These authors' hypotheses, conclusions, and recommendations imply that race modifies the association between HLA class II alleles and HCV clearance or persistence, yet they did not present any formal assessment of effect modification.

Formal assessment of effect modification is crucial if the differences between stratum-specific estimates are a primary focus of the study. Because of the race-matched case-control design of this study [1], effect modification by race can be assessed only on the multiplicative scale (the heterogeneity of the odds ratios [ORs]) [3]. When strong evidence of heterogeneity is identified, the estimates of the effect measures—ORs in this case—should be presented after stratification by the modifying factor, that is, race [4].

Thio et al. [1] highlighted differences by race but did not show compelling evidence of heterogeneity. In fact, the evidence for racial heterogeneity provided by the ORs in the results of this study is not strong. For example, using Cochran’s Q test of homogeneity [5] to compare the association between DQB1*0301 and HCV clearance in blacks (OR, 0.65; 95% confidence interval [CI], 0.42–1.00) with that in whites (OR, 0.85; 95% CI, 0.54–1.33), we find no evidence of significant heterogeneity of the ORs (Cochran’s Q, 0.70; df, 1; P = .40). Furthermore, the overlap of the CI functions illustrated in figure 1 suggests that the claimed ethnic difference is not clearly apparent.

Moreover, if the assessment of race as a potentially important effect modifier of the association between DQB1*0301 and HCV clearance was an anticipated study question, avoidance of matching on race would be preferable [6]. Matching on such a factor precludes further assessment of effect modification on the additive scale in relative terms [3, 6]. Assessment on the additive scale provides better evidence of the extent and direction in which the effect modifier alters the main effect [3].

In addition, we want to emphasize that race is a construct that reflects social stratification of groups according to phenotypic and cultural characteristics [7]. The presumption that observed differences between socially defined groups could reveal genetic/racial traits has been widely criticized [8, 9]. For example, heterogeneity of genetic characteristics within socially defined racial groups is far greater than that between groups [10]. Therefore, to attribute observed outcome differences to biological aspects of race warrants at least some minimal evidence of heterogeneity.

We contend that the evidence presented by Thio et al. [1] is not nearly strong enough to justify asserting and publicizing [11] that the association of DQB1*0301 and other HLA class II alleles with HCV clearance differs by ethnicity.

Olga L. Sarmiento,1 Chandra L. Ford,2 Elizabeth C. Newbern,1 William C. Miller,1,3 Charles Poole,1 and Jay S. Kaufman1
Departments of 1Epidemiology and 2Health Behavior and Health Education, School of Public Health, and 3Department of Medicine, School of Medicine, University of North Carolina at Chapel Hill

Figure 1. Confidence interval functions for the association between DQB1*0301 and hepatitis C virus (HCV) clearance in blacks (dotted line) and in whites (continuous line). Each curve illustrates possible confidence limits for the log odds ratio. The point of the peak of the curve corresponds to the estimated log odds ratio. Vertical line shows the null value for the log odds ratio (i.e., the value corresponding to the null hypothesis of no association).

References
AAs are well documented [9]. Pooling ethnic groups for associ-
ations could affect the type of associations identified in the 2
groups. For example, one ethnic group may possess a particular
allele that is associated with an infectious disease outcome,
whereas that allele may be rare or missing altogether in another
group. For example, one ethnic group may possess a particular
allele that is associated with an infectious disease outcome,
whereas that allele may be rare or missing altogether in another
group. For example, one ethnic group may possess a particular
allele that is associated with an infectious disease outcome,
whereas that allele may be rare or missing altogether in another
group. For example, one ethnic group may possess a particular
allele that is associated with an infectious disease outcome,
whereas that allele may be rare or missing altogether in another
group. For example, one ethnic group may possess a particular
allele that is associated with an infectious disease outcome,
Association between Efavirenz and Selected Psychiatric and Neurological Conditions

To the Editor—We read with interest the article by Veldkamp et al. [1] concerning the pharmacokinetics of efavirenz and nevirapine when used in combination for the treatment of human immunodeficiency virus (HIV)–infected persons. The results of their study demonstrated that exposure to efavirenz is significantly decreased when used in combination with nevirapine. The study proved the combination to be safe, and the 14 patients reported few adverse events (AEs). None of the patients reported AEs when efavirenz was used alone during the first 2 weeks of the study, and only 2 patients experienced nausea or peripheral neuropathy after nevirapine was added to the regimen.

We found it interesting that few AEs were reported by Veldkamp et al. [1]. Here, we describe the AEs reported by HIV-infected patients who were prescribed efavirenz. Several phase 3 studies have reported that efavirenz is associated with a variety of psychiatric and neurological conditions, such as anxiety, depression, and confusion [2, 3]. We conducted a retrospective study at the Medical Center of Louisiana HIV Outpatient Clinic (New Orleans), to compare the frequencies of selected psychiatric and neurological conditions that possibly are associated with efavirenz.

Using the Centers for Disease Control and Prevention Adult Spectrum of Disease (ASD) database, HIV-infected patients in Louisiana who were prescribed efavirenz for the first time during the 6 months preceding June 2001 were identified. Demographic information from the ASD database was used to describe the population. The frequencies of the following conditions (clinically diagnosed during the study period but not diagnosed 6 months before) were ascertained: anxiety, depression, psychosis, suicidal ideation, noninjection drug use, injection drug use, alcohol abuse, and any opportunistic condition. Frequencies of the selected variables were compared between patients who were prescribed and those who were not prescribed efavirenz, by χ² analysis. Logistic regression models were used to assess the association between the time when efavirenz was first prescribed and the occurrence of the selected variables, while adjusting for CD4 cell count.

In total, 1897 patients were included, 133 of whom had been prescribed efavirenz for the first time. Most patients were male (67.2%), African American (64.4%), and ≥ 35 years old (64.5%). Mean age was 38.6 years (SD, 9.62 years). The χ² analysis showed that patients who were prescribed efavirenz for the first time were significantly more likely to have a CD4 cell count < 200 cells/dL and an opportunistic condition than were those who were not prescribed efavirenz. HIV dementia and a clinical diagnosis of depression also were significantly associated with prescribed efavirenz. After adjustment for CD4 cell count, the multivariate analysis showed that depression and HIV dementia were significantly associated with prescribed efavirenz. The odds ratio (OR) for depression was 1.74 (95% confidence interval [CI], 1.02–2.99), and the OR for HIV dementia was 4.00 (95% CI, 1.16–13.81).

There were several limitations to our study. We were only able to determine whether the patients had been prescribed efavirenz. Adherence to the prescribed drug was unknown. Most important, the putative relationship between efavirenz and the selected variables in this study requires rigorous prospective studies. The pharmacokinetics of efavirenz in combination with other antiretrovirals and the possibility of AEs should continue to be investigated.

Kathleen J. Welch and Anne Morse,
for the Adult Spectrum of Disease Project in New Orleans
Louisiana Office of Public Health, Centers for Disease Control and Prevention Adult Spectrum of Disease Study, New Orleans

References

Children with Cystic Fibrosis Produce an Immune Response against Exoenzyme S, a Type III Cytotoxin of Pseudomonas aeruginosa

To the Editor—Cystic fibrosis (CF) is a common lethal autosomal genetic disorder among white persons, affecting ~1 in 3500 live births. The lungs of patients with CF frequently are colonized by >1 bacterial pathogen, typically Staphylococcus aureus and Pseudomonas aeruginosa. Chronic colonization of the lungs of patients with CF with mucoid strains of P. aeruginosa is associated with progressive lung destruction and increased mortality [1]. P. aeruginosa produces both secreted and cell-surface virulence determinants. Secreted virulence factors can provide nutrients for growth, enhance invasive potential, or directly damage host tissue. Type III cytotoxins are delivered directly by the bacterium into the eukaryotic cell cytoplasm. In both acute lung infection and in cultured cells, type III cytotoxins act at the site of infection to subvert the local immune response [2]. P. aeruginosa produces several type III secreted cytotoxins, including exoenzyme S (ExoS). ExoS is a bifunctional cytotoxin that inhibits 2 independent eukaryotic signal-transduction pathways [3]. Type III cytotoxins enter the cell through a transport channel formed by 2 bacterial proteins, PopB and PopD, in a process involving another type III protein, PcrV. Immunization with PcrV is an effective intervention in acute pneumonia caused by P. aeruginosa [4]. The presence of an antibody response has been used to implicate the expression of several virulence factors of P. aeruginosa in children with CF, including exotoxin A, lipopolysaccharide, and outer-surface proteins [5, 6]. In a recent study in the Journal, Burns et al. [7] reported a longitudinal assessment of P. aeruginosa in young children with CF, which concluded that most patients had evidence of infection by age 3 years. These results suggest that P. aeruginosa infection occurs early in CF but may be intermittent or undetectable by culture [7] and that combined serologic testing and culturing can effectively detect infection. Here, we present evidence that some children with CF produce antibodies to ExoS and other type III antigens, which suggests that they are infected with acutely cytotoxic strains of P. aeruginosa.

Infants with mutations in the CF transmembrane regulator (CFTR) were identified by genotyping. Serum samples and cough cultures were collected from these infants during routine evaluations, as defined elsewhere [8]. Serum antibodies against the cytotoxin ExoS and components of the type III system, PopB and PcrV, were detected by Western blot, using purified recombinant PcrV and a P. aeruginosa cell extract, which contained ExoS and PopB. In a random screening of serum samples (diluted 1:1000) obtained from 12–24-month-old infants with CF, 7 of 13 serum samples were observed to contain antibodies against ExoS and PopB, and 5 of these 7 positive serum samples...
had a weak response to PcrV. A longitudinal study of 3 infants with positive serum samples was performed, and findings were compared with the growth of *P. aeruginosa* from oropharyngeal cultures obtained during vigorous coughing (figure 1). In serum samples from 1 infant, increasing titers of antibodies against ExoS and PopB were found before a positive oropharyngeal culture for *P. aeruginosa* was obtained, whereas, in serum samples from the other 2 infants, the appearance of antibody against ExoS and PopB coincided with positive oropharyngeal cultures. In each longitudinal study, the titer of antibodies against PcrV was lower than the titer to ExoS or PopB.

There were no differences with respect to gender, CFTR genotype profile, or gastrointestinal disease status among the 7 children whose serum samples tested positive and the 6 children whose serum samples tested negative for type III reactive antibodies between ages 12 and 24 months. Of the 6 children whose serum samples tested positive for type III reactive antibodies, only 1 had a transiently positive *P. aeruginosa* culture result between ages 12 and 24 months, whereas all 7 children whose serum samples tested positive for type III reactive antibodies had early positive *P. aeruginosa* culture results (*P = .005*, Fisher’s exact test).

Type III cytotoxins have been implicated in *P. aeruginosa* disease associated with epithelial cell and lung injury [9] and with expression of ExoS, which is shown to accelerate injury in cultured epithelial cells. This pilot study presents the first determination that some children with CF are infected by *P. aeruginosa* that express the type III cytotoxin ExoS. These results imply that there is a role for type III cytotoxins during initial infection and may present a new diagnostic strategy that uses the immune response to ExoS and new vaccine strategies against the type III antigen PcrV to delay or prevent infection by *P. aeruginosa* in the lungs of children with CF. PcrV is thought to be a protective antigen [4], and the weak response of this antigen in children with CF, relative to other type III antigens, may be the basis for chronic infection by *P. aeruginosa*.

**Bruce Banwart,**1 Mark L. Slaingard,2 Philip M. Farrell,3 Michael J. Rock,1 Peter L. Havens,1 Joel Moss,4 Mary E. Ehrmantraut,1 Dara W. Frank,2 and Joseph T. Barbieri1

1Department of Pediatrics, Children’s Hospital of Wisconsin, and 2Department of Microbiology and Molecular Genetics, Medical College of Wisconsin, Milwaukee, and 3University of Wisconsin Medical School, Madison; 4Pulmonary–Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

**References**


**Infection with Unusual Types of Cryptosporidium Is Not Restricted to Immunocompromised Patients**

**To the Editor**—In their study of *Cryptosporidium* infection in children in Lima, Peru, Xiao et al. [1] report that 5 types of *Cryptosporidium* were identified in stool specimens and that none of the children screened were found to have antibodies against human immunodeficiency virus (HIV) type 1. Recent advances in the molecular identification of *Cryptosporidium* species have permitted not only the differentiation of species otherwise indistinguishable by microscopy from *C. parvum* but also differentiation of subtypes within the genus. It has been reported that species and subtypes other than *C. parvum* infect HIV-immunocompromised patients [2, 3], but there is increasing evidence that these infections are not restricted to HIV-immunocompromised hosts.

From January 2000 through July 2001, we collected, confirmed, and genotyped 3100 clinical isolates of *Cryptosporidium* identified in patients by microscopy at local laboratories throughout England and Wales, using polymerase chain reaction (PCR) to amplify sequences of the 70-kDa heat shock protein (HSP70) [4] and PCR/restriction fragment–length polymorphism analysis to amplify and digest sequences of the *Cryptosporidium* oocyst wall protein (COWP) [5] and small-subunit rRNA [6] genes. A minimum data set was collected for each isolate,
including clinical information about the patient from whom the isolate was obtained. Although the majority of isolates were *C. parvum* genotype 1 or genotype 2, we identified and confirmed by sequence analysis 21 *C. meleagridis* isolates from 19 patients and 2 isolates from 2 other patients that do not match currently recognized species or genotypes. None of these 23 patients were reported to have HIV or to be immunocompromised, although *Campylobacter* species were identified in the same stool specimens that yielded 3 of the *C. meleagridis* isolates. However, *Campylobacter* is the gastrointestinal pathogen most commonly identified in the United Kingdom, and coinfection with *Cryptosporidium* species has been reported in outbreak and sporadic cases, which may reflect common elements in the epidemiology and transmission of these 2 organisms [7, 8]. Isolation of *C. meleagridis*, *C. felis*, and the *Cryptosporidium* species called the “dog type” from immunocompetent individuals has recently been reported [9, 10]. Work is also in progress to identify and characterize the 2 unusual isolates identified in our laboratory, and the epidemiology of these unusual infections is currently being explored.

We support the claim of Xiao et al. [1] that infection in humans with unusual species and subtypes of *Cryptosporidium* is not restricted to immunocompromised patients. Of 29 isolates from 19 immunodeficient patients (5 patients with retroviral infections, 5 with congenital T cell deficiencies, 4 with carcinoma, 2 who had received bone marrow transplantations, and 2 with undefined immunodeficiencies) that were tested in our laboratory, 19 isolates were demonstrated to be *C. parvum* (9 isolates of genotype 1 and 10 of genotype 2), and 8 isolates reacted to amplification with HSP70 *Cryptosporidium* genus–targeted primers but not with COWP primers. It is possible that these 8 isolates are unusual species of *Cryptosporidium*. Thus far, only 2 isolates that were confirmed to be *Cryptosporidium* by immunofluorescence antibody test (TCS Water Sciences) reacted with neither HSP70 nor COWP gene primers. Our data from patients with and without immunocompromise demonstrate that infections with unusual types of *Cryptosporidium* occur in immunocompetent as well as in immunocompromised patients.

Unusual cryptosporidial infections are not restricted to immunocompromised hosts, and further investigation of the pathogenicity and epidemiology of these infections is necessary to establish their public health significance and to identify risk factors for exposure and measures for prevention.

Reprints or correspondence: Dr. Rachel M. Chalmers, Public Health Laboratory Service Cryptosporidium Reference Unit, Swansea Public Health Laboratory, Singleton Hospital, Sgeti Lane, Swansea SA2 8QA, United Kingdom.

The Journal of Infectious Diseases 2002;185:270–1 © 2002 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2002/18502-0022$02.00

**Rachel M. Chalmers, Kristin Elwin, Anne L. Thomas, and David H. M. Joynson**

*Public Health Laboratory Service Cryptosporidium Reference Unit, Swansea Public Health Laboratory, Singleton Hospital, Sgeti Lane, Swansea, United Kingdom*

**References**


