Genetic Determinants of Idiopathic Noncirrhotic Portal Hypertension in HIV-Infected Patients

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Background. Noncirrhotic portal hypertension (NCPH) is a rare but potentially life-threatening complication in patients with human immunodeficiency virus (HIV). Cases of NCPH have been reported in HIV-negative individuals as result of treatment with thiopurines for leukemia or inflammatory bowel disease. Exposure to didanosine, which is also a purine analogue, predisposes to NCPH in the HIV setting. However, it is unclear why NCPH only develops in a small subset of didanosine-treated patients.

Methods. A multicenter, case-control study was conducted to investigate the role of pharmacogenomics in NCPH in HIV patients with prior didanosine exposure. Three controls were chosen for each case, adjusted for sex, age, CD4 counts, plasma HIV-RNA, and site. Tagging 36 single-nucleotide polymorphisms (SNPs) at enzymes involved in the purine metabolism (inosine triphosphatase, 5′-nucleotidase cytosolic-II, purine nucleoside phosphorylase and xanthine oxidase) was performed using SNPlex microarray technology.

Results. Eighty individuals were examined; 22 with NCPH and 58 matched controls. Two SNPs at the 5′-nucleotidase gene were associated with NCPH: rs11191561 (48% CG/GG vs 17% CC; \(P = .003\)) and rs11598702 (40% CC/CT vs 9% TT; \(P = .003\)). SNPs at another 2 loci at the xanthine oxidase gene were also associated with NCPH: rs1429376 (71% AA vs 23% CC/AC; \(P = .015\)) and rs1594160 (71% AA vs 23% CC/AC; \(P = .015\)). There was a cumulative risk of NCPH for these 4 SNPs: 7%, 26%, 42%, 50%, and 100%, respectively, for 0, 1, 2, 3, or all SNPs (\(P = .001\)).

Conclusions. SNPs at the 5′-nucleotidase and xanthine oxidase genes influence the risk of NCPH in HIV patients treated with didanosine.

Keywords. noncirrhotic portal hypertension; HIV; didanosine; pharmacogenomics; gene polymorphisms.

Liver disease associated with antiretroviral therapy has been widely reported, especially for drugs extensively metabolized in the liver, such as some nonnucleoside analogues or protease inhibitors [1, 2]. Nucleoside analogues have been classically linked to drug-induced liver toxicity throughout mitochondrial toxicity, potentially producing liver steatosis and/or lactic acidosis. More recently, cases of idiopathic noncirrhotic portal hypertension (NCPH) have been reported in HIV-infected individuals [3–13]. This is a challenging entity defined by the presence of portal hypertension in the absence of severe hepatic impairment. Even though the overall prevalence of this condition is low in HIV-treated patients (0.5%), the risk of potentially life-threatening complications (ie, gastrointestinal bleeding) emphasizes the importance of early diagnosis. Other signs of portal hypertension such as ascites or encephalopathy are less common in this condition, in which the hepatic function is relatively well preserved.
The mechanism underlying the development of NCPH in HIV remains largely unknown; however, prior exposure to didanosine has been recognized as a predisposing factor in the majority of series [3, 7, 12, 13]. Similarly, cases of NCPH have been reported in HIV-negative individuals as result of exposure to thiopurines, such as azathioprine, mercaptopurine, or thioguanide, used as treatment for leukemias or inflammatory bowel disease [14–16]. Liver biopsy in HIV patients with NCPH typically shows absence of cirrhosis with obliteration of distal portal veins in a large proportion of portal tracts [17], with or without features of nodular regenerative hyperplasia [4]. Whereas the liver parenchyma is minimally affected, signs of severe portal hypertension are clinically manifest, with splenomegaly, esophageal varices, and, occasionally, portal thrombosis [3–10].

We hypothesize that some metabolites emerging during didanosine metabolism might cause damage in the endothelium of portal vessels. In this way, allelic differences at genes coding for enzymes involved in didanosine metabolism, either for activation or degradation, could affect the concentrations of potentially harmful metabolites. Herein, we examined the influence of single-nucleotide polymorphisms (SNPs) at genes involved in the purine metabolic pathway on the development of NCPH in HIV-infected patients with prior exposure to didanosine.

**PATIENTS AND METHODS**

This was a prospective, multicenter, international, case-control study that examined SNPs potentially associated with the development of NCPH in HIV-infected individuals. The study population was identified at several HIV clinics that belong to the European AIDS Trials Network (NEAT), an initiative funded by the European Commission.

Study entry criteria required positivity for HIV infection, prior exposure to didanosine, no cirrhosis, and lack of any identifiable cause of hepatic disease, including hepatitis B or C, alcohol abuse, use of hepatotoxic medications, autoimmunity, and hereditary disorders (eg, hemochromatosis, Wilson disease, coagulopathies). Patients were split into case and control groups, with a 1:3 assignment. Cases were subjects who had developed NCPH, whereas controls were individuals similarly exposed to didanosine without developing NCPH. The 2 groups were matched by length of didanosine exposure (<40, 41–90, or >91 months), age, sex, CD4 count (<200, 201–500, or >501 cells/μL), and plasma HIV-RNA (detectable vs undetectable; lower limit of detection of 50 copies/mL). The study was funded by NEAT and the protocol was approved by the ethics committee of each participating center.

Genomic DNA was isolated from peripheral blood mononuclear cells using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. DNA specimens were then stored for each case and controls, and shipped to Madrid, Spain, where all samples were kept frozen until the time of the study. Genetic examinations were chosen for 4 enzymes involved in the metabolism of physiologic purines. Figure 1 schematically represents the step they are responsible for. A total of 36 SNPs were tested as follows: 4 at the inosine triphosphatase (ITPA), 8 at the 5′-nucleotidase, 2 at the purine nucleoside phosphorylase, and 22 at the xanthine oxidase or xanthine dehydrogenase. All these positions were selected using the resources provided by the HapMap project (www.hapmap.org).

Further genetic testing with characterization of allelic variants was made using SNPlex microarrays technology. Allelic discrimination was performed using an Applied Biosystems 3730xl DNA Analyzer and GeneMapper3.7 software. Pedcheck [18] was used to detect Mendelian inconsistency.

**Statistical Analyses**

The main characteristics of the study population and distribution of allelic variants is reported as absolute values and percentages. Comparisons between SNPs were tested using the $\chi^2$ test. All results are expressed as absolute numbers and percentages. Significant differences were only considered for $P$ values <.05. Tests for multiple comparisons were applied following principles reported elsewhere for genomic studies [19]. All statistical analyses were performed using SPSS version 15.0 (SPSS Inc, North Chicago, Illinois).

**RESULTS**

A total of 88 individuals were initially included in the study, 22 with NCPH and 66 matched controls. Eight controls were retrospectively excluded because they did not match well (n = 5) and/or did not meet entry criteria (n = 3). To limit bias, the presence of at least 2 matched controls for each case of NCPH was then verified. From the final 80 individuals examined, 67% were male, with a median age 47 years (interquartile range [IQR], 44–53); >90% were of white race, and median didanosine exposure was 66 months (IQR, 48–86). No significant differences were found when comparing NCPH and controls apart from the CD4 count, which was a direct consequence of portal hypertension and hypersplenism in the former (Table 1). The intake of antiretroviral agents other than didanosine was similar in cases and controls. Patients did not take chronically any other relevant medication that could have boosted the effect of didanosine or acts as its own.

A total of 36 tagging SNPs were analyzed (Supplementary Table 1). The prevalence of SNPs did not differ significantly at any position when comparing our controls and European HapMap populations. In contrast, 4 SNPs were significantly overrepresented in NCPH cases compared to controls. Two
SNPs located at the 5′-nucleotidase gene were associated with NCPH: 48% of patients with rs11191561CG/GG vs 17% with CC (P = .003) and 40% of patients with rs11598702CC/CT vs 9% with TT (P = .003). Another 2 alleles located at the xanthine oxidase gene were also associated with NCPH: 71% of patients with rs1429376AA vs 23% with CC/AC (P = .015) and again 71% of patients with rs1594160AA compared to 23% with CC/AC (P = .015). Figure 2 summarizes these findings.

The frequency of the minority allelic variants in whites at the 4 SNPs found to influence the risk of NCPH was as follows: rs11191561 = 0.128, rs11598702 = 0.388, rs1429376 = 0.252, and rs1594160 = 0.279.

Interestingly, as shown in Figure 3, there was a cumulative risk of NCPH in the study population considering the presence of the 4 SNPs: 7%, 26%, 42%, 50%, and 100%, respectively, for none, 1, 2, 3, or all SNPs (P = .001). As expected, the isolated and cumulative prevalence of the 4 SNPs associated with NCPH was generally greater in cases compared to controls (Supplementary Table 2).

**DISCUSSION**

The advent of a wide number of potent antiretroviral agents has dramatically improved the prognosis of the HIV population. Individuals with HIV infection now live longer, as they rarely develop opportunistic infections as long as they attend regular medical care and receive proper antiretroviral therapy. However, complications of comorbidities (ie, cardiovascular disease) are still a significant concern.

Table 1. Main Demographics and Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 58)</th>
<th>Controls (n = 22)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>49 ± 8</td>
<td>51 ± 9</td>
<td>.16</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>49 (85)</td>
<td>17 (78)</td>
<td>.33</td>
</tr>
<tr>
<td>Mean duration of HIV infection, y</td>
<td>17 ± 4</td>
<td>18 ± 4</td>
<td>.17</td>
</tr>
<tr>
<td>Mean CD4 count, cells/µL</td>
<td>313 ± 175</td>
<td>645 ± 287</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean CD4 percentage</td>
<td>31 ± 8</td>
<td>27 ± 8</td>
<td>.04</td>
</tr>
<tr>
<td>No. of individuals with plasma HIV RNA &lt;50 copies/mL, %</td>
<td>58 (100)</td>
<td>21 (96)</td>
<td>.53</td>
</tr>
<tr>
<td>Mean duration of ddi exposure, mo</td>
<td>63 ± 27</td>
<td>69 ± 25</td>
<td>.38</td>
</tr>
</tbody>
</table>

Statistically significant data are presented in italics.

Abbreviations: ddi, didanosine; HIV, human immunodeficiency virus.
events) and long-term toxicities of medications are increasingly being reported in this population. Hepatic complications are among the most common and generally attributed to the concomitant presence of chronic viral hepatitis B or C, alcohol abuse, fatty inflammatory hepatic infiltration, or drug-related hepatotoxicity [20].

During the last couple of years, reports from several HIV clinics in Western countries have alerted about a form of severe portal hypertension that develops in patients without any evidence of serious hepatic parenchymal damage [21]. These HIV-infected patients with NCPH may suddenly experience massive gastrointestinal bleeding episodes, as result of esophageal variceal bleeding, with or without portal thrombosis. Common causes of liver damage are typically ruled out, whereas liver biopsies show a distinctive lesion characterized by massive absence of portal veins along with focal fibrous obliteration of small portal veins [17]. Prior exposure to didanosine has uniformly been recognized as a predisposing factor for developing this condition [3, 7, 12, 13].

Although NCPH in the HIV setting may result from etiologies similar to those in the general population, its increased frequency supports that unique factors occur more frequently in the HIV population. Cases of NCPH have been reported in HIV-negative individuals as a result of exposure to chemotherapeutic agents, mainly purine analogues (eg, azathioprine, 6-mercaptopurine, or 6-thioguanine) [14–16, 22, 23]. On the other hand, didanosine, which is also a purine analogue, has been shown to be associated with increased cardiovascular risk in HIV-infected patients [24]. This antiretroviral drug may cause systemic endothelial damage throughout still unclear mechanisms, although it may involve an enhancement of proinflammatory mediators that might create a prothrombotic state and precipitate cardiovascular events [25, 26]. Even though the majority of reports have linked didanosine exposure to the development of NCPH, the prevalence of disease is very low in comparison with the large number of HIV patients.
exposed to the drug, indirectly supporting that a second hit or predisposing etiology is required.

Our rigorous assessment of a genetic predisposition to the risk of developing NCPH among HIV-infected persons is a novel contribution to the field. Patients carrying thiopurine methyltransferase deficiency are prone to experience a higher incidence of azathioprine or 6-mercaptopurine myelosuppression, and specific polymorphisms have been found to predict this complication [27]. In the same way, the ITPA deficiency, which recently has been postulated to provide protection against ribavirin-induced hemolytic anemia [28], may also lead to an increased risk of purine analogue toxicity. Testing specific ITPA polymorphisms may allow prediction of these complications. As shown in Figure 1, genetic polymorphisms affecting the expression and/or function of other critical enzymes in the purine metabolic pathway (ie, 5'-nucleotidase, purine nucleoside phosphorylase, or xanthine oxidase) might also contribute to enhance the toxicity of didanosine or its metabolites over the portal vessels once the oral drug is absorbed in the gut and flows into the portal circulation.

It must be noted that the characteristic obstructive portal venopathy identified in HIV-infected patients with NCPH might also appear superimposed to liver damage resulting from other conditions. In that situation, its recognition might be rather difficult [29]. Given that alcohol abuse, fatty liver disease, and especially chronic hepatitis C affect a substantial proportion of the HIV population, NCPH should be suspected in patients in whom no or only mild liver parenchymal damage is present. In this regard, transient elastometry may be particularly helpful to exclude advanced liver fibrosis.

The relevance of our findings must be interpreted cautiously. First, didanosine is largely been abandoned and is no longer recommended as first-line antiretroviral agent for other reasons, mainly increased risk of mitochondrial toxicity. Therefore, in contrast with the abacavir hypersensitivity reaction, which is largely predicted by a single genetic marker (HLA-B*5701), the clinical implication of our results is limited by the progressive removal of didanosine from the HIV armamentarium. Thus, baseline genetic testing of xanthine oxidase and 5'-nucleotidase SNPs will not move forward before didanosine is prescribed. A second note of caution derives from the inherent difficulties of linking genetic traits with disease [30], and therefore our findings must be validated in a different set of patients, including non-Europeans.

In summary, this is among the first studies reporting genetic determinants of idiopathic NCPH in HIV-infected individuals. We found 4 SNPs at the 5'-nucleotidase and xanthine oxidase genes that significantly influence the risk of NCPH in HIV-infected patients with prior exposure to didanosine. Hypothetically, endothelial damage at portal vessels caused by increased levels of harmful purine metabolites of didanosine within the portal circulation in subjects with susceptible allelic variants might contribute to this complication. We cannot exclude that other SNPs may also determine the risk of NCPH in the HIV population, and genome-wide association studies testing larger populations might help to answer this question.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References