Human leukocyte antigen (HLA) and pharmacogenetics: screening for HLA-B*57:01 among human immunodeficiency virus–positive patients from southern Alberta

Noureddine Berka,*, John M. Gill, Abdelhamid Liacini, Tyler O’Brien, Faisal M. Khan

Abstract

The field of pharmacogenetics is witnessing a growing interest in the role of the human leukocyte antigen (HLA) in manifestation of adverse drug reactions (ADR). Here we report a retrospective analysis of the association of HLA-B*5701 with abacavir hypersensitivity syndrome (AHS) in a large Canadian cohort of 489 human immunodeficiency virus–1-positive patients exposed to abacavir. A total of 3.7% of abacavir-exposed patients had developed AHS. Using polymerase chain reaction sequence-specific primer–based genotyping, the HLA-B*5701 allele was observed in 20 patients (4.1%). Of the 20 HLA-B*5701+ abacavir-treated patients, 18 (90%) had developed AHS. Carriage of the HLA-B*5701 allele indicated a strong association with abacavir hypersensitivity (p < 0.0001; odds ratio = 6.534; 95% confidence interval = 321–149,735). HLA-B*5701 genotyping demonstrated high sensitivity, specificity, and positive and negative predictive values. The data derived from the study highlight the importance of engaging histocompatibility and immunogenetics laboratories in taking a lead in mapping other less characterized HLA and immunogenetic markers associated with ADRs.

Keywords: HLA Pharmacogenetics B*57:01 HIV Abacavir

1. Introduction

The terms pharmacogenetics and pharmacogenomics are often used interchangeably, although pharmacogenetics refers to the science of defining how heritable genetic variations lead to differing responses to drugs. On the contrary, pharmacogenomics refers to the science regarding how the meticulous identification of human genome and related proteins’ inter- and intra-individual variation in their expression and function over time can be used in designing new drugs as well as predicting a personalized treatment for individual patients. In the present study, we have used the term pharmacogenetics because we report the screening of a genetic variation that can minimize adverse drug reactions (ADRs) and promote benefit over harm. Pharmacologically, ADRs are of 2 types, A and B, with the latter being relatively more severe [1]. Type A ADRs are predictable based on pharmacologic action of the drug; however, the predictability of type B ADR is not dependent upon pharmacologic action of the drug or its dose, but rather is driven by host genetics. Type B ADRs appear not to be concentration dependent [2,3] and strong evidences suggest a role of immune mechanisms [4].

Given the immune pathogenesis of these ADRs, the human leukocyte antigen (HLA) genes seem to be prime candidates for their heritability. A major and recent advance has been the revelation of associations between HLA alleles and hypersensitivity against various drugs. Most prominent among them are the association of HLA-B*57:01 with abacavir hypersensitivity against abacavir [15–16]. Direct and indirect HLA-involved mechanisms are suggested to be behind this strong association. Recently, we and others have demonstrated that in the case of abacavir hypersensitivity, drug presentation is major histocompatibility complex class I (more specifically, HLA-B*57:01) restricted, and there is a clonal expansion of CD8 cytotoxic T cells that induces a multifunctional response [15,16]. These studies clearly suggest the direct functional involvement of HLA molecules in the pathogenesis of the ADR.

For many years, histocompatibility and immunogenetics laboratories (HILs) have successfully undertaken testing for HLA-associated diseases. As such, they are well placed to prospectively screen for HLA alleles/haplotypes associated with ADRs. Recent surveys questioning the capacity of HILs to meet the demands of a clinical
pharmacogenetics and the utility of pharmacogenetic testing of HLA-B*57:01 in this Caucasian-dominated multiethnic cohort of HIV-1 patients.

2. Subjects and methods

2.1. Subjects

A total of 489 HIV-1-positive patients were recruited from a multiethnic cohort of over 1,000 patients who had been managed by the Southern Alberta HIV Care Program in Calgary since 1988. All patients had received abacavir at least once before the introduction of HLA testing. The incidence of abacavir hypersensitivity (ABH) was defined with restrictive diagnostic criteria to ensure the inclusion of true hypersensitive cases in the analysis. Clinical diagnostic criteria included the occurrence of classic symptoms (fever, rash, gastrointestinal symptoms, malaise, fatigue, aching, and respiratory symptoms) within 6 weeks of exposure. In cases where confounding reasons other than the clinical diagnosis of ABH were present, epicutaneous patch testing was performed to confirm the diagnosis. Five milliliters of peripheral blood was collected in EDTA vacutainers for retrospective analysis of the HLA-B*57:01 molecular genotyping. The recruited HIV-1 patients had a median age of 45 years and were predominately male and Caucasian (66%). Other ethnic groups represented in this cohort include blacks, aboriginals, Indo-Asians, Hispanics, Metis, and others (Table 1). A written informed consent was obtained from all subjects. The Ethics Committee of the University of Calgary approved the study. The technologists performing the molecular genotyping of HLA-B*57:01 were blind to the clinical end point (incidence of AHS).

2.2. HLA-B*57:01 genotyping

Genomic DNA was extracted from 200 μL buffy coat using a Qiagen QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). HLA-B*57:01 genotyping was performed using sequence-specific primer (SSP)–based amplification [18].

2.3. Sequence-based typing (SBT) of the HLA-B locus

The genotyping of the HLA-B locus by SBT was performed to confirm SSP typing results in 5 patients, including (i) 2 randomly selected patients (1 HLA-B*57:01 and 1 HLA-B*57:01 patient); (ii) 2 HLA-B*57:01 but AHS patients, and (iii) 1 HLA-B*57:01 but suspected AHS patient. SBT was performed using Atria Genetics HLA SBT kits (Atria Genetics, San Francisco, CA). The procedure involves amplification of the HLA-B gene and subsequent sequencing using dideoxynucleotide chain terminators labeled with different fluorescent dyes for exons 2, 3, and 4 of the HLA-B gene. The fluorochrome-labeled DNA fragments were ethanol purified, heat denatured, and electrophoresed by capillary electrophoresis on an ABI 3130 Genetic Analyzer. The sequence analysis was performed using CONEXO™ 3.5+ software (Conexio Genomics, Applecross, Western Australia, Australia).

2.4. Statistical analysis

Statistical analyses were performed using SPSS 15.01 software (SPSS, Inc., Chicago, IL). Fisher’s exact test was performed to investigate the association of HLA-B*57:01 carriage with AHS. Odds ratios (OR) at 95% confidence intervals (CI) were determined to describe the strength of the association using Haldane’s modification, which adds 0.5 to accommodate possible zero counts. P values < 0.05 were considered significant. The sensitivity, specificity, and positive and negative predictive values of HLA-B*57:01 testing were determined using GraphPad (InStat Software, Inc., La Jolla, CA) to assess the predictive diagnostic potential of the test.

3. Results

3.1. Prevalence of AHS

In the cohort of 489 abacavir-exposed HIV-1-positive patients, 18 patients (3.7%) developed AHS. Among these AHS patients there were 15 Caucasians, 2 Indo-Asians, and 1 aboriginal. One patient was excluded from the analysis because of the lack of epicutaneous patch testing to confirm AHS and because symptoms of ABH were overlapping with those of various other medical conditions.

3.2. Prevalence of HLA-B*57:01 and its association with AHS

In the total cohort of 489 patients, the HLA-B*57:01 allele was observed in 20 patients (4.1%), predominantly including Caucasians (n = 16, 3.3%), followed by Indo-Asians (n = 2, 0.4%) and aboriginals (n = 1, 0.2%). Of the 20 HLA-B*57:01 abacavir-treated patients, 18 (90%) developed hypersensitivity. The results of SBT confirmed the results of SSP-based typing for all 5 patients tested with SBT. Carriage of the HLA-B*57:01 allele indicated a strong association with hypersensitivity against abacavir (p < 0.0001; OR = 6.934; 95% CI = 321–149,735; Table 2).

We further tested the sensitivity, specificity, and negative and positive predictive values of HLA-B*57:01 testing. As a predictive diagnostic test for AHS, the HLA-B*57:01 screening yielded high values for sensitivity (100%), specificity (99.6%), positive predictive value (90%), and negative predictive value (100%).

4. Discussion

Abacavir is a US Food and Drug Administration–approved and universally used nucleoside analogue reverse transcriptase inhibitor drug for the treatment of HIV-1 infection. The drug has high efficacy; however, the potentially life-threatening hypersensitivity reactions that affect 2 to 8% of abacavir-treated patients are a major limitation. The association of AHS with the carriage of HLA-B*57:01 has been demonstrated in various reports and we and others have
Table 2

Association of HLA-B*5701 with abacavir hypersensitivity (ABH)

<table>
<thead>
<tr>
<th>HLA-B*5701 status</th>
<th>ABH status (%)</th>
<th>p value[^c]</th>
<th>OR (95% CI limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*5701[1], n = 20</td>
<td>ABH[1], n = 18 (90)</td>
<td>&lt;0.0001</td>
<td>6.934 (321–149,035)</td>
</tr>
<tr>
<td>HLA-B*5701[1], n = 468[^d]</td>
<td>ABH[1], n = 0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parameters for pharmacogenetic utility of HLA-B*5701 testing for the prediction of abacavir hypersensitivity

<table>
<thead>
<tr>
<th>Sensitivity of the test</th>
<th>100%</th>
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<tbody>
<tr>
<td>Specificity of the test</td>
<td>99.6%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>90%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100%</td>
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</tbody>
</table>

OR: odds ratio; 95% CI, 95% confidence interval.

[^a]: A total of 489 HIV-1[^e] patients were treated with abacavir.
[^c]: Calculated by Fisher’s exact test.
[^d]: One patient was excluded from the analysis because of the lack of epicutaneous patch testing to confirm abacavir hypersensitivity syndrome and because symptoms of ABH were overlapping with those of other medical conditions.

reported a putative functional basis of this association. The strong potential of HLA-B*57:01 testing in predicting AHS has also been reported to have the limitations of a false-positive diagnosis of AHS and identification of HLA-B*57:01[^g] abacavir-tolerant (AHS[^h]) patients[^i]. In the present study, we confirmed this association in a Caucasian-dominated multiethnic cohort of 489 patients from southern Alberta, Canada. Our results confirm the utility of HLA-B*57:01 testing as a pharmacogenetics test for HIV-1[^j] patients with strong positive and negative predictive values and using molecular SSP-based testing. Unlike flow-based assays, molecular-based assays have proven to be more sensitive and specific. The ideal screening assays should avoid both false-negative and false-positive results, be cost-effective and accessible, and have an acceptable turnaround time.

Consistent with earlier studies[^k, l, m], we also observed 2 abacavir-tolerant (AHS[^c]) HLA-B*57:01[^l] patients in our study. The HLA typing results were confirmed by sequencing. It has been previously suggested that AHS may require a combination of factors to complete a hypersensitivity reaction[^i]. In that regard, an earlier study indicated that an HLA haplotype rather than an allele is associated with AHS[^n]. However, this result is highly unlikely because all subsequent association reports[^i] and functional studies from our laboratory and other research groups indicated that systemic reactions to abacavir are cytotoxic CD8 T cell mediated and are uniquely restricted to antigen recognition by HLA-B*5701[^o, p]. Chessman et al., however, have demonstrated that recognition of abacavir requires other factors such as transporter associated with antigen presentation and tapasin[^q]. It is possible that diminished function of any of these other factors may abrogate AHS in HLA-B*57:01[^l] individuals.

We also observed 1 HLA-B*57:01[^l] patient with a possible false-positive diagnosis of AHS. The patient, who had symptoms associated with AHS, was removed from the analysis after reassessment because of a lack of results of epicutaneous patch testing and because the symptoms overlapped with those of other medical conditions. Interestingly, when we sequenced this patient for the HLA-B locus, we determined that the patient carries HLA-B^58:01, a naturally occurring variant of HLA-B*57:01. Both HLA-B*57:01 and B*58:01 are part of the HLA-B17 allotypic family that differs only at 4 amino acids and has significant overlap in their peptide repertoire[^r]. Many virus-specific T cells have exhibited cross-reactivity with both of these major histocompatibility complexes[^s]. However, Chessman et al. have demonstrated that abacavir-specific CD8 T cells raised in vitro from an HLA-B^57:01[^t] donor, when restimulated with human lymphoblastoid cell lines expressing HLA-B^58:01 in the presence and absence of abacavir, did not exhibit an abacavir-specific response from CD8 T cells[^u].

The overall estimate of European prevalence of HLA-B*57:01 after weightings were applied was 4.98%, with individual country-specific estimates ranging from 1.53% (Finland) to 7.75% (Switzerland). The prevalence of HLA-B*5701 was observed to be highest in Caucasians (6.49%) and lowest in the black population (0.39%)[^v]. The prevalence of HLA-B*57:01 in our study is comparable with these results. We previously participated in an international multicenter validation study that served as an external quality assessment of HLA-B*57:01 testing[^w]. We strongly believe that there is a need for more such international collaborations to study HLA-associated pharmacogenetics and establish common pathways that can help in understanding the etiology of ADRs. When carrying out these kinds of pharmacogenetic studies, methodological and statistical considerations are important to assure the validity of outcomes and to successfully convert the knowledge into clinical practice[^x]. North American HILs are well placed to conduct genetic testing for HLA-associated pharmacogenetics because most are performing HLA genotyping at the variable level of resolution (high and low). More importantly, HILs have adopted stringent quality assurance practices that are guided by best practices from the American Society of Histocompatibility and Immunogenetics and the College of American Pathologists. It would also be helpful to initiate studies through the International Histocompatibility Workshop to allow a wider geographic and ethnically diverse sampling for the assessment of the role of HLA in pharmacogenetics.

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References