Polymorphism of Transporter Associated with Antigen Presentation 1 as a Potential Determinant for Severity of Disease in Recurrent Respiratory Papillomatosis Caused by Human Papillomavirus Types 6 and 11

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Recurrence respiratory papillomatosis (RRP) is a rare disease caused by human papillomaviruses (HPVs). It is characterized by multiple recurrences of benign neoplasms and has a variable clinical course, ranging from infrequent recurrence to acute airway obstruction. One way in which HPV subverts the immune system in RRP is by interfering with TAP1 (transporter associated with antigen presentation 1). We examined whether a known TAP1 polymorphism in the ATPase domain altered the severity of disease in patients with RRP. The presence of this polymorphism was significantly correlated with severity of disease (P = .015). Because of the proximity of the TAP1 gene to human leukocyte antigen (HLA) class II genes on chromosome 6, we postulated that a linkage disequilibrium may exist. Of the patients with polymorphic TAP1, 36% were positive for HLA-DRB1*0102 (P = .021; P = .147 with Bonferroni's correction). However, this association appeared to mitigate the severity of disease (P = .04). Therefore, severity of disease in a patient with RRP might be determined by sequencing TAP1, in conjunction with HLA class II genes.

Recurrent respiratory papillomatosis (RRP) is a rare disease caused by human papillomaviruses (HPVs) types 6 and 11 [1, 2]. There are approximately equal numbers of patients with juvenile-onset and adult-onset RRP [3]. Although RRP is rare, with a prevalence of 1.69–2.59 cases/100,000 persons for juvenile-onset disease [4], it is associated with a high degree of morbidity and significant mortality. RRP is characterized by frequent recurrences of benign neoplasms of the airway, sometimes interspersed with unexplained periods of relative quiescence. Standard treatment is surgical removal, but this does not prevent recurrences. There is also a marked variability in severity of disease from one patient to another. Some patients may undergo only 1 or 2 lifetime surgeries, whereas others may require >100 surgical procedures to maintain an unobstructed airway. In most patients, disease is restricted to the larynx, whereas, in a subset of patients, the trachea is also involved; a few patients have disease extending into the bronchae and lung parenchyma. Extension of disease into the lower airway is associated with a very poor prognosis. Despite the marked variability in recurrence rate and extent of disease, few indicators of severity and progression of disease exist.

It is accepted that HPV infection is controlled by the host’s immune system; this fact raises questions regard-
ing the inability of patients with RRP to prevent disease recurrence. Patients with RRP are systemically immunocompetent [5] but locally immunocompromised, with reduced levels of expression of major histocompatibility complex (MHC) class I on the cell surface [5]. This phenomenon might explain, in part, the pattern of recurrent disease. In patients with RRP, immunomodulators have been used therapeutically with only limited success [6] and without clearance of latent infection [7]. Therapeutic vaccines are being tested for HPV-16- and HPV-18–induced cervical carcinomas, and a phase 2 clinical trial for RRP is in progress. The presence of HPV E6 and E7 proteins in infected tissues has led many groups to target these proteins in vaccine development. However, E7 peptide vaccines have not elicited good cytotoxic T cell responses in vitro [8] or in phase 1 clinical trials of cervical cancer [9]. This result could reflect impairment of MHC class I antigen presentation [10], the induction of tolerance [11], or the nonspecific down-regulation of MHC class I–restricted CD8+ T cell responses [12].

TAP1 (transporter associated with antigen presentation 1) is important for MHC class I–mediated antigen presentation, and the best strategies for therapeutic vaccines have ensured that cells are able to process the viral peptide through the TAP–MHC class I pathway [13]. TAP1, a heterodimer with TAP2, is an ATP-dependent peptide transporter that imports antigenic peptides from the cytosol to the endoplasmic reticulum for assembly with MHC class I [14]. The cell surface peptide–MHC class I complex is required for recognition of CD8+ cytotoxic T cells. Deficiencies in TAP1 have been associated with many carcinomas, including prostate, renal cell, melanoma, breast, and small-cell lung cancer [15], and TAP1 gene transfer could restore immunogenicity [16]. Cervical carcinomas show losses of both TAP1 and MHC class I [17]. We have reported a statistically significant correlation (P = .0095) between TAP1 immunostaining of respiratory papillomas and clinical course [10].

TAP1 is a 748-aa protein with 3 functional domains: a transmembrane pore for transport, a peptide-binding domain, and an ATPase domain, with both the N and C termini in the cytosol [18]. The peptide-binding domain includes aa 376–487, and the ATPase domain includes aa 487–748 [18, 19]. The crystal structure of the TAP1 ATPase domain bound to ADP has been characterized; it has a rec-A domain, which contains 2 α-helices, coupled to a small α-helical domain [20].

TAP1 is highly conserved, with little genetic variability. Original reports defined only 2 polymorphic sites in the TAP1 protein [21]: one, at aa 333 (designated as 1.1,1.2), converts isoleucine to valine; the other, at aa 637 (designated 1.3, 1.4), converts aspartic acid to glycine [22]. Combinations of these 2 nucleotide substitutions define 4 TAP1 haplotypes: 1A, 1B, 1C, and 1D [22, 23]. The 1.3/1.4 polymorphism at aa 637 is located in the ATPase domain [20, 21]. Glycine is a strong inhibitor of α-helix formation [24] and could induce a large conformational change in the ATPase domain of the TAP1 1.4 polymorphism.

Although TAP1 polymorphisms have been studied extensively in autoimmune disease, the effect of antigen-presentation gene polymorphisms in chronic infectious diseases is only beginning to be identified. Although proteins from herpes simplex virus and cytomegalovirus interact with TAP1 [25–27], the site of interaction has been distant from known polymorphic sites and has probably dissuaded investigators from studying the effect of polymorphisms. The TAP1 genes are located on chromosome 6, in a cluster of antigen-presentation genes, including LMP2, LMP7, and the MHC class II genes DQ and DR [28]. TAP2 polymorphisms in patients with chronic hepatitis C virus infections [29] confer greater risk for developing cirrhosis [30], whereas a polymorphism in LMP7 can influence response to interferon [31]. TAP2 polymorphisms also occur more frequently in patients with pulmonary tuberculosis than in control subjects [32], and a TAP2 polymorphism has been implicated in the ability to respond to measles vaccine [33]. Most recently, a TAP2 polymorphism has been shown to confer resistance to HIV [34]. In addition, polymorphisms of HLA-DR2, TAP2, and, possibly, TAP1 are significant predictors of the risk of developing cervical cancer, an HPV-mediated disease [35]. Here, we report that a polymorphism in TAP1 is associated with increased severity of HPV infection in patients with RRP. We further report that other antigen-presentation genes can modulate severity of disease, resulting in genotypes that might predict clinical course.

SUBJECTS, MATERIALS, AND METHODS

Subjects and index of severity of disease. Forty-three randomly selected patients with RRP of varying severity were included in this study. Informed consent was obtained from patients or their parents, and the human-experimentation guidelines of the US Department of Health and Human Services were followed. Institutional review board approval for the study was granted. The study population included patients with both juvenile- and adult-onset disease (29 males and 14 females). The ethnic profile of this group was 70% white, 23% African American, and 7% Hispanic, which closely resembles our larger cohort of patients with RRP [3]. All patients were infected with HPV type 6 or type 11. Clinical severity is determined by extent of disease and frequency of recurrence. At each surgery, the number of disease sites, the surface area of the anatomic region involved in disease, and the degree of lumenal obstruction were summed to yield a composite score [36]. The severity of disease score is the composite score divided by the number of days elapsed since the previous surgery. Average scores of multiple surgeries defined severity scores for individual patients. A score >0.06 and 3 surgeries in the previous 12 months, or tracheal extension, was defined as severe disease, whereas a score <0.06
and no tracheal extension was defined as mild/moderate disease [5]. This scoring system has been used throughout our clinical trials. Minimal variation in patients’ scores over time was observed, except for slow, progressive improvements in scores in response to therapy. Although some patients’ scores clustered around 0.06, given a mean follow-up of 59 months and a median follow-up of 39 months, we feel confident in delineating patients as having severe or mild/moderate disease. Classification of severity of disease was based on a much larger population of patients (n>150), with normal distribution [36] (A.L.A., unpublished data). For 1 patient, photodynamic therapy (PDT) changed the patient’s severity status from severe to mild/moderate.

RNA, cDNA, polymerase chain reaction (PCR), and sequence analysis. Stored papilloma tissues or peripheral blood mononuclear cells (PBMCs) were used to prepare RNA or DNA. For TAP1 polymorphisms, RNA was extracted from snap-frozen tissue reduced to a fine powder [37] or from PBMCs isolated with ficoll hypaque density centrifugation by use of Tripure reagent (Boehringer Mannheim) or a Qiagen kit. cDNA was synthesized with oligo DT (Clontech). Primers PSF1–8, which span the polymorphism at aa 637, were used in accordance with the method of Colonna et al. [21]. Reverse-transcriptase PCR was performed in a Perkin-Elmer thermocycler at an initial cycle of 94°C for 5 min; 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, for 38 cycles, with a final extension at 72°C for 7 min. Excessive primer was removed by use of a Wizard PCR kit (Promega), and PCR products were sequenced by use of an ABI Prism (PE Biosystems) automated sequencer in both the forward and reverse directions, to confirm polymorphisms. As an additional control, 4 patients were analyzed by use of 2 separate biopsy samples obtained at different times. The identical nucleotide sequence was obtained in biopsy-sample pairs from all 4 patients, including the single homozygous patient.

Analysis of the 1.1/1.2 allelic polymorphism. A small subset of papillomas were analyzed by use of PCR with primers PSF1–4, which span the isoleucine to valine polymorphism at aa 333 [21]. If a polymorphism exists in region PSF1–8, a concurrent one exists in region PSF1–4, with glycine at aa 637 associated with valine at aa 333 in all cases [38]. Rare additional polymorphisms have been identified, with changes at aa 458 and 648 defining a new TAP1 allele, *0401 [39]. We have not sequenced the aa 458 region, but, in all 43 patients studied, no changes were identified at aa 648. Samples from 3 patients with allele 1.3/1.3 and samples from 3 patients with the 1.4 polymorphism at aa 637 were sequenced for the polymorphism at aa 333 [21]. In all cases, a polymorphism at aa 637 was linked to a polymorphism at aa 333 (GTC). This would therefore be classified as a 1B haplotype [22, 23]. Similarly, the wild-type (wt) sequence at aa 637 was linked to the wt sequence at aa 333 (ATC), which is classified as a 1A haplotype [22, 23]. Further studies determining whether the 1.2 polymorphism always segregates with the 1.4 polymorphism and whether other new polymorphisms are present in this subset of patients are in progress.

HLA-A, -B, -DRB1, and -DQB1 analysis. Given the known linkage disequilibrium between TAP1 and the class II genes DRB and DQB, we secondarily examined whether it existed in our population of patients, since most of them had been previously typed for HLA. Twenty-three patients with wt TAP1 and 10 patients with polymorphic TAP1 at aa 637 had been typed for DQB1 genotypes; 26 patients with wt TAP1 and 11 patients with polymorphic TAP1 had been typed for DRB1. Patients’ samples sequenced for the TAP polymorphism were randomly selected, and samples were not always available for HLA typing. Unfortunately, this included 3 patients with the TAP polymorphism who were lost to clinical follow-up.

Genomic DNA was isolated from PBMCs by use of a commercial extraction method (Qiagen). High-resolution typing of HLA-DRB1 was performed by group-specific PCR amplification of exon 2 [40, 41]. Seven DRB1 group–specific forward primers (specific to DRB1*01, DRB1*15/16, DRB1*03/11/13/14, DRB1*08/12, DRB1*07, DRB1*09, and DRB1*10) were used in combination with a generic reverse primer. Dideoxy sequencing of exon 2 used the Autoload solid phase sequencing kit (Amersham Pharmacia Biotech) and ALF express II automated DNA analyzer (Amersham Pharmacia). Ambiguous allele combinations were resolved by use of 3’ amplification primers specific for the dimorphic sequences at codon 86 (GTG/GGT), followed by sequence-based typing [41]. The sequence-specific primer (SSP) method was also used as a supplementary test to resolve HLA-DRB1 alleles (One Lambda). High resolution typing of HLA-DQB1 was performed by use of PCR-SSP (One Lambda), as described elsewhere [42]. HLA-A and -B loci were typed by use of PCR, followed by hybridization with sequence specific oligonucleotide probes [43] (Orchid Diagnostics).

Statistical analysis. Most variables were analyzed by use of a 2-sided Fisher’s exact test with a 95% confidence interval, as determined by use of Instat software (Graph Pad Software). For correlation to HLA type, Bonferroni’s correction was used to adjust for the number of alleles analyzed. A linear regression model with robust SEs [44] was used to estimate the effects of the DRB1*0102 allele and the time from initial surgery on the TAPI-associated severity scores measured at each surgery. The model contained an interaction term and allowed for dependent severity scores across individual subjects who had multiple surgeries (intraclass severity score correlation, 0.68), as determined by use of the Stata software package (release 8.0; StataCorp). For the purposes of statistical analysis, severity scores were transformed using a natural log scale, to more nearly satisfy a normality assumption for their distribution. Visual inspection of normal probability plots by use of transformed data (data not shown) did not indicate any further violation of this assumption.
RESULTS

Association between TAP1 polymorphism and severity of disease. Most patients treated in our center have significant RRP that affects the larynx and, in some severe cases, the trachea. Forty-three patients were included in the present study. Average severity scores, number of surgeries per patient, and average duration of follow-up are shown in table 1. Severity scores represent average scores from multiple surgeries, and, for many patients, the score represents >5 years of longitudinal data. Longitudinal data were available for all patients, with a mean follow-up period of 59 months. Minimal variation in severity score over time was observed. Patients with tracheal disease are defined as having severe disease, regardless of disease severity score.

Fourteen of the 43 patients had polymorphic TAP1, whereas the rest had wt TAP1. The presence of the polymorphism was positively correlated with severity of disease (figure 1). Thirteen of fourteen patients had disease severity scores >0.06, which was statistically significant (P = .015). One patient, with the highest severity score in the polymorphic group, was homozygous for the polymorphism. In contrast, the prevalence of the polymorphism in the patients with papillomas, as a whole, was comparable to that in the general population [21] and was not statistically significant (P = .632). Thus, the polymorphism is related to severity of disease, not to the likelihood of initial HPV infection or to the expression of clinical disease. Tracheal involvement is associated with severe disease, and association with the TAP1 polymorphism (n = 7) was almost significant, with the small number of patients with tracheal disease (n = 13) in our study (P = .077). Juvenile-onset disease also tends to be more severe, but there was no correlation between age of onset and presence of the TAP1 polymorphism.

Presence of HLA-DRB1*0102 in TAP1 polymorphism: a protective phenotype. On the basis of the results of a previous study [23], we then examined whether the TAP1 polymorphism was linked to ≥1 MHC haplotype. Among the 43 patients with polymorphic TAP1, the MHC genotype was completely determined for 34 patients and was partially determined for 3 patients (DRB in 2 patients and DQB in 1 patient). Table 2 shows the association of the TAP1 polymorphism with all the class II HLA alleles present in our patients at a reasonably high frequency. There was an association between TAP polymorphism and HLA-DRB1*0102. This association initially appeared to be statistically significant (P = .021); however, when it was corrected for multiple comparisons, significance was lost. All DRB1*0102-positive patients with the TAP polymorphism were also HLA-B14 positive. HLA-B14 and HLA-DR are usually found in association, because B14-DRB1*0102 is an ancestral haplotype [45]. The prevalence of the HLA-B14 allele was 12.5% (3/24) in the wt TAP group, compared with 50% (5/10) in the polymorphic TAP1 group, again demonstrating a trend. Only 1 of the 3 wt TAP1–HLA-B14 patients was also HLA-DRB1*0102 positive. Of interest, this patient was the only one with wt TAP1 who also was HLA-DRB1*0102 positive. In contrast, there was no linkage between the TAP1 polymorphism and other HLA-DRB1 alleles. Unlike the results of the study by Gelder et al. [46], in which HLA-DRB1*0301 was found to be both enriched and deleterious, only 2 of 34 patients in our study were HLA-DRB1*0301 positive, and both belonged to the wt TAP1 group. This may be a result of our smaller sample size.

The association between the TAP polymorphism and HLA-DRB1*0102 is shown in figure 2. Only 1 of 26 patients without the TAP polymorphism was HLA-DRB1*0102 positive, whereas 4 of 11 patients with the polymorphism were heterozygous for HLA-DRB1*0102. The association represents a trend that would probably become significant in a larger population of patients (table 2). To further analyze the relationship between TAP1-associated severity and the HLA-DRB1*0102 allele, the patients

Table 1. Demographics of patients with recurrent respiratory papillomatosis (RRP) analyzed for the TAP1 (transporter associated with antigen presentation 1) polymorphism.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No.</th>
<th>Onset of RRP; no. of patients</th>
<th>Duration of follow-up, mean (range), months</th>
<th>No. of surgeries, mean (range)</th>
<th>Disease severity scoreb, mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>43</td>
<td>17 26</td>
<td>52 (5–177) 7 (2–38)</td>
<td>0.17 (0.001–1.12)</td>
<td></td>
</tr>
<tr>
<td>Severe diseasec</td>
<td>28</td>
<td>12 16</td>
<td>54 (6–174) 13 (4–38)</td>
<td>0.25 (0.003–1.12)d</td>
<td></td>
</tr>
<tr>
<td>Mild/moderate disease</td>
<td>15</td>
<td>5 10</td>
<td>70 (5–177) 6 (2–20)</td>
<td>0.02 (0.001–0.05)</td>
<td></td>
</tr>
<tr>
<td>Tracheal disease</td>
<td>13</td>
<td>8 5</td>
<td>69 (6–174) 16 (4–38)</td>
<td>0.26 (0.003–1.01)g</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Patients both with and without adjunct therapy to surgery have been included. The no. of surgeries and length of follow-up reflect surgeries at Long Island Jewish Medical Center (New Hyde Park, NY) and do not include surgeries at other institutions.

* Defined as onset at ≤18 years of age.

* Disease severity score reflects the extent of disease at each surgery and the time between surgeries (see Subjects, Materials, and Methods for an explanation).

* Defined as a disease severity score >0.06 or tracheal extension.

* One patient had a low disease severity score but also had tracheal extension.

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Figure 1. Relationship between severity of recurrent respiratory papillomatosis and the presence of the TAP1 (transporter associated with antigen presentation 1) polymorphism. Polymorphic (PM) TAP1 has glycine at aa 637; wild-type (wt) TAP1 has aspartic acid at aa 637. Disease severity scores for 14 patients with PM TAP1 and 29 patients with wt TAP1 were calculated by the surface area of papillomas at surgery and the time elapsed since the last surgical removal. Severe disease is defined as a score >0.06 or tracheal extension. Mild/moderate disease is defined as a score <0.06 and no tracheal disease. P = .015 was considered to be statistically significant (2-sided Fisher’s exact test).

Table 2. Relationship between the TAP1 (transporter associated with antigen presentation 1) polymorphism and prevalence of HLA alleles commonly found in patients with recurrent respiratory papillomatosis (RRP).

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Allele/wild-type TAP1</th>
<th>Allele/polymorphic TAP1</th>
<th>Unadjusted P</th>
<th>Corrected P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0102</td>
<td>1/26</td>
<td>4/11</td>
<td>.021</td>
<td>.147a</td>
</tr>
<tr>
<td>B14-DRB1*0102</td>
<td>1/24</td>
<td>4/10</td>
<td>.019</td>
<td>.133a</td>
</tr>
<tr>
<td>HLA B14</td>
<td>3/24</td>
<td>5/10</td>
<td>.031</td>
<td>.217a</td>
</tr>
<tr>
<td>DRB1*0301</td>
<td>3/26</td>
<td>1/11</td>
<td>1.0a</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0701</td>
<td>6/26</td>
<td>1/11</td>
<td>.645a</td>
<td>NS</td>
</tr>
<tr>
<td>DQB1*0501</td>
<td>6/24</td>
<td>5/11</td>
<td>.263a</td>
<td>NS</td>
</tr>
<tr>
<td>DQB1*0201</td>
<td>9/24</td>
<td>3/11</td>
<td>.709a</td>
<td>NS</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>9/24</td>
<td>6/11</td>
<td>.467a</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Shown is the likelihood of certain HLA class II alleles to be in linkage disequilibrium with a TAP1 polymorphism at aa 637 in the RRP study population. Significance for each HLA allele with TAP1 was determined by a 2-sided Fisher’s exact test. P values were then adjusted by Bonferroni’s correction for multiple comparisons. NS, not significant.

a NS.

(\(P = .001\)). Of the patients with the polymorphism-positive, HLA-DRB1*0102-negative phenotype who received PDT, all reduced their severity score by one-half over the period of time studied (even if they remained in a severe category), whereas none of the patients who were PM positive and HLA-DRB1*0102 positive responded to PDT, although the numbers are too small to achieve meaningful statistics. This intriguing observation suggests that, although the TAP1 PM-positive, HLA-DRB1*0102–negative phenotype is deleterious, it may be more responsive to PDT intervention, whereas the HLA-DRB1*0102–positive phenotype may be unresponsive.

Because of the DQB genes physically exist between TAP1 and DRB on chromosome 6 [28], we examined whether there was a linkage disequilibrium between TAP1 and DQB. There was no significant association between any of the DQB alleles and TAP1 (table 2). Previously, the HLA-DQ3 phenotype was reported to be more prevalent in patients with RRP [5], and HLA-DQB1*0301 has been associated with increased risk of HPV-induced cervical cancer [47]. We did not see any association between HLA-DQB1*0301 and TAP1 in the present study.

Effect of other HLA types on severity of disease. The HLA-DRB1*0102 allele appeared to mitigate the severity of disease associated with the TAP1 polymorphism. We therefore examined whether other alleles also mitigated severity of disease (table 3). First, we addressed the effects of HLA-B14 alone and of the HLA-B14-DRB1*0102 haplotype in severe versus mild/moderate disease. Neither HLA-B14 nor the combination haplotype showed protection against severe disease when analyzed in the context of all patients. They appeared to mitigate severity of disease only in the patients with polymorphic TAP1.

Because we had not seen an increase in overall prevalence of HLA-DQB1*0301, compared with that in the population as a whole, or linkage with the TAP1 polymorphism, we examined...
whether that allele had an effect on severity of disease. The prevalence of severe disease was roughly equal in the HLA-DRB1*0301-positive and -negative groups, and the allele’s effect on severity was classified as neutral. Several other class II alleles were not associated with severity of disease, even though they appeared to be common in the patients with RRP, including HLA-DQB1*0201 and HLA-DQB1*0501, when considered as single alleles. However, a combined haplotype of HLA-B8-DRB1*03011-DQB1*0201 appeared to increase the risk of tracheal involvement, because 3 of 4 patients had tracheal disease (data not shown). Of the 25 patients without tracheal disease, only 1 had this haplotype ($P = .048$). Of interest, the 1 patient with this haplotype who did not manifest tracheal disease was also the only patient whose condition worsened after treatment with adjuvant PDT in our recent study (A.L.A. and B.M.S., unpublished data). There was no significant correlation between the HLA-B8-DRB1*03011-DQB1*0201 haplotype and the TAP1 polymorphism (data not shown), which suggests that its negative effect on disease was independent of TAP1.

**DISCUSSION**

Antigen-presentation gene polymorphisms are only beginning to be studied in chronic infectious diseases. The present study has shown that a TAP1 polymorphism correlates with severity of disease in HPV-induced RRP. We have also shown that this effect can be modulated by other HLA alleles, with the potential for both positive and negative influences in the same individual. The association between TAP1 and HLA-DRB1*0102 is a novel finding. The apparently protective phenotype of HLA-B14-DRB1*0102 in the presence of the TAP1 polymorphism at aa 637 is surprising, considering that it appears to have no relationship to severity of disease overall. Supporting this conclusion, HLA-DRB1*0101, 0102, 0104 was reduced in frequency in HPV-16-induced vulvar intraepithelial neoplasia in a previous study [48]. However, there was no analysis of TAP1 polymorphisms in that study.

All of the HLA-DRB1*0102-positive patients were white.
This allele is relatively rare, accounting for 7%–21% of the DRB locus, and is predominantly identified in African American and Hispanic subjects [49], although Reed et al. [43] have seen a reasonable prevalence of this allele in white subjects. HLA-B14 rarely segregates with HLA-DRB1*0102 in the wt TAP1 group, suggesting that this combined haplotype may have evolved as a protective mechanism to mitigate the effect of a TAP1 polymorphism on severity of disease and that the association between HLA-DRB1 and TAP1 did not occur serendipitously. The relationship between the HLA-B8-DRB1*0301-DQB1*0201 haplotype and tracheal disease is consistent with results of the recent study by Gelder et al. [46], in which the authors identified HLA-B8, HLA-DR3, and HLA-DQ2 as components of an enriched haplotype in patients with RRP. In support of our data, Rowland-Jones et al. [50] reported that an HLA-B8 haplotype failed to present an influenza virus epitope and that HLA-B8 was not associated with TAP1. The correlation of HLA-B8-DRB1*0301-DQB1*0201 haplotypes to severity of disease in RRP demonstrates the importance of studying combined haplotypes, rather than individual alleles. Other allelic combinations have also been seen to be significant in HPV-mediated diseases. HLA-DRB1*0301-DQB1*0201 has been associated with a reduced risk for both transient and persistent cervical HPV infection [51], and HLA-DRB1*0301 has been associated with a reduced risk of invasive cervical squamous cell carcinoma [47]. Given that HLA-DRB1*0301 may be deleterious in HPV-11–induced RRP but protective in high-risk HPV-induced cervical carcinomas, HPV type itself may influence the effect of HLA type on clinical disease course.

The mechanism by which the TAP1 polymorphism affects severity of disease is unknown. The TAP transporter has 2 cytosolic ATP binding cassettes/ATPases: one each on TAP1 and TAP2 [52, 53]. Deleting 1 cassette decreased transport, whereas deleting both caused complete loss of transport [53]. This domain is also important for overall folding and complex stability [54], and it contains Walker A and B sequences, which are highly conserved motifs common to many ATPases [55]. Nucleotide substitutions of the motifs generally impair ATP hydrolysis and, as a result, transporter function [54, 56]. A recently described crystal structure of TAP1 demonstrates an α-helix–rich region between Walker A and B [20]. Glycine is a strong α-helix breaker [24] that potentially could induce a large conformational change in the ATPase domain of individuals who harbor the 1.4 polymorphism, thus disrupting the TAP1 ATPase function. It is unclear how much of each form of protein is produced in a heterozygote and how functional the alternate (glycine) form is. Further studies are in progress to address this question.

Although other studies have debated a correlation between peptide selection/transport and TAP polymorphisms [57, 58], we previously showed a correlation between loss of antibody recognition of the ATPase domain of TAP1 and severity of disease [10]. We then showed that HPV 11 E7 protein binds TAP1 and inhibits ATP-dependent peptide transport [59]. Preincubation of E7 protein with microsomes containing TAP1 prevents cross-linking of 8-azido-32P-ATP, which characterizes the region of interaction in the ATPase domain (A.V., unpublished data). Studies are under way to identify whether binding of E7 is facilitated or reduced in the presence of the TAP polymorphism or whether the affinity of ATP is altered.

In conclusion, we propose that, in the future, disease prognosis and therapeutic response in any given patient with an HPV infection might be predicted by sequencing TAP1 and associated HLA class I and class II genes. Understanding the mechanisms by which the TAP1, HLA-B14-DRB1*0102, and HLA-B8-DRB1*0301-DQB1*0201 haplotypes modulate severity of disease will provide critical information needed to optimize therapeutic vaccines for HPV-associated diseases.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Prevalence overall</th>
<th>Patients with severe disease/this allele</th>
<th>Patients with severe disease/alternate alleles</th>
<th>P*</th>
<th>Effect on disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0102</td>
<td>5/37</td>
<td>3/5 (60%)</td>
<td>22/32 (69%)</td>
<td></td>
<td>Protective trend</td>
</tr>
<tr>
<td>B14-DRB1*0102</td>
<td>5/34</td>
<td>3/5 (60%)</td>
<td>20/29 (69%)</td>
<td>.87</td>
<td>Protective trend</td>
</tr>
<tr>
<td>DRB1*0301</td>
<td>4/37</td>
<td>3/4 (75%)</td>
<td>21/33 (64%)</td>
<td>1.0</td>
<td>Neutral</td>
</tr>
<tr>
<td>DQB1*0501</td>
<td>11/35</td>
<td>7/11 (64%)</td>
<td>17/24 (71%)</td>
<td>.71</td>
<td>Neutral</td>
</tr>
<tr>
<td>DQB1*0201</td>
<td>12/35</td>
<td>9/12 (75%)</td>
<td>15/23 (65%)</td>
<td>.71</td>
<td>Neutral</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>15/35</td>
<td>11/15 (73%)</td>
<td>13/20 (65%)</td>
<td>.72</td>
<td>Neutral</td>
</tr>
</tbody>
</table>

NOTE. Severity of disease was determined as described in Subjects, Materials, and Methods. Effect on disease reflects the likelihood of the allele to be associated with severe disease. Note that B14 and DRB1*0102 are not associated with overall severity of disease and only exhibit a moderate protective phenotype in patients with the TAP1 (transporter associated with antigen presentation 1) polymorphism (PM) at aa 637.

* Significance was determined by use of a 2-sided Fisher's exact test. All P values were not significant.

See figure 3.
Acknowledgments

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References


