Genetic Variants in TAP Are Associated with High-Grade Cervical Neoplasia

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Abstract

Purpose: The transporter associated with antigen processing (TAP) is essential in assembling MHC-I proteins. Human papillomavirus (HPV) evades immune recognition by decreasing class I MHC cell surface expression through down-regulation of TAP1 levels. Consistent with heterogeneity in MHC expression is the individual variability in clearing detectable HPV infections. Genetic polymorphisms in TAP genes may affect protein structure, function, and the ability to clear HPV infection.

Experimental Design: Case-control study of women with cervical intraepithelial neoplasia (CIN) II or III (n = 114) and women without high-grade CIN (n = 366). Five nonsynonymous single nucleotide polymorphisms (SNP) in *TAP1* and *TAP2* were genotyped using DNA collected in cervicovaginal lavage samples using microsphere array technology (Luminex xMAP). HPV typing was done using a PCR-based system with MY09/MY11 primers. *TAP1* and *TAP2* SNPs were validated by direct sequencing.

Results: Differences in allele distribution between women with high-grade cervical neoplasia and women without was seen for *TAP1* I333V (P = 0.02) and *TAP1* D637G (P = 0.01). The odds ratios (OR) for CIN III were significantly lower among carriers of the *TAP1* I333V polymorphism (OR, 0.28; 95% confidence interval, 0.1-0.8), and *TAP1* D637G polymorphism (OR, 0.27; 95% confidence interval, 0.1-0.7). These associations remained significant even after restricting the evaluation to women who were positive for high-risk HPV types.

Conclusions: In addition to the down-regulation of MHC-1 by oncogenic HPV, HPV pathogenesis might be facilitated by polymorphisms in the TAP proteins. Identifying *TAP* polymorphisms may potentially be used to identify women less susceptible to progression to high-grade CIN and cervical cancer.

Cervicovaginal human papillomavirus (HPV) infection is the most common sexually transmitted infection in young adults (1–3). Although genital HPV infection is extremely common, development of cervical cancer is not. Available data indicates that cervical cancer develops through progressive stages from infection to preinvasive lesions to invasive cancer. Persistent infection with oncogenic HPV increases the susceptibility of cells to malignant changes (4). However, the molecular mechanisms involved in the development of a persistent HPV infection resulting in development of cervical cancer are not well-established. Elucidating this complex pathway is difficult due to the many viral, immunologic, genetic, and epidemiologic risk factors that play a role in the natural history of pernicious papillomavirus infection.

One of the possible mechanisms why some women do not eradicate HPV is through a defect in their cell-mediated immune response. It has been shown that class I MHC (MHC-I) antigen is down-regulated in HPV 16– and HPV 18–associated cervical cancers and recurrent respiratory papillomatosis, another HPV-associated disease (5, 6). The regulation of the MHC-I is a posttranscriptionally controlled mechanism in these tumor cells (7). Therefore, one mechanism used to evade immune detection by HPV-specific cytotoxic T cells is to down-regulate MHC-I expression on HPV-infected cells.

A series of events must take place for effective antigen presentation to occur in a virus-infected cell. The transporter associated with antigen processing (TAP) is an essential component for assembling MHC-I proteins in the endoplasmic reticulum (8). TAP proteins facilitate the entry of viral peptides into the rough endoplasmic reticulum, making these peptides available to be complexed with MHC-I molecules (9). Many viruses other than HPV have been known to evade immune system recognition through interference with MHC assembly. For example, adenoviruses produce a protein that directly binds MHC-I antigen, trapping it in the endoplasmic reticulum (10). Herpes viruses produce a protein, ICP47 that blocks transport of viral peptides into the endoplasmic reticulum (4).

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Translational Relevance

The molecular mechanisms involved in the development of persistent HPV infection resulting in development of cervical cancer are not well-established. Elucidating this complex pathway is difficult due to the many viral, immunologic, genetic and epidemiologic risk factors that play a role in the natural history of persistent papillomavirus infection. One of these factors may be due to defects in the host cell-mediated immune response. A series of events must take place for effective antigen presentation to occur in a virus-infected cell. TAP is an essential component for assembling MHC-I proteins. The objective of this study was to assess TAP DNA polymorphisms in patients with CIN and evaluate the association between TAP, CIN, and high-risk HPV infection. In this study, two polymorphisms located on the *TAP1* gene were associated with development of high-grade CIN, even in women infected with high-risk HPV types. One explanation is that the mRNA product in individuals with at least one G allele at either of these polymorphisms is more resistant to degradation. Or TAP molecules in women with these polymorphisms are more effective in eliciting a more robust early cell-mediated immune response against HPV, including oncogenic (high-risk) HPV types. Identifying *TAP* polymorphisms may potentially be used to identify women susceptible to progression to high-grade CIN and cervical cancer.

There are known allelic polymorphisms in *TAP* that have been identified in different populations (11–13). *TAP* polymorphisms have also been examined in the context of cervical cancer but have not been studied in precursor cervical cancer lesions (12), cervical intraepithelial neoplasia (CIN; ref. 14). The primary objectives of this study were to classify *TAP* DNA polymorphisms in patients who participated in a case-control study of CIN and evaluate the association between *TAP*, CIN, and high-risk HPV infection.

Materials and Methods

Participant population

Four hundred and eighty women were recruited from gynecology, family planning and colposcopy clinics at the Albert Einstein College of Medicine and Montefiore Medical Center from 1992 to 1994 as previously described (15). Eligible women were over 17, not pregnant, and did not have a history of cervical cancer. All enrolled women signed a consent form approved by the local Institutional Review Board and completed a standardized questionnaire. A blood sample and cervicovaginal lavage were collected from all participants. The current analysis is restricted to participants who were HPV tested. All cases were biopsy-confirmed by two study pathologists and histopathologic grade determined by consensus.

TAP genotyping

Sample collection and processing. This study used DNA isolated from cervicovaginal lavage samples to assess genetic polymorphisms as previously described (16). Briefly, 150 μL of gravity-sedimented cellular material were removed from the 10-mL lavage sample with a disposable, sterile transfer pipette and placed in 150 μL of digestion solution [2 mmol/L EDTA, 2% Laureth-12, 100 mmol/L Tris (pH 8.5)] containing 400 mg of proteinase K (Boehringer Mannheim) per milliliter and incubated at 55°C for 2 h, followed by a 10-min incubation at 95°C (2). The processed samples were stored at -20°C before PCR.

Genotyping of two single nucleotide polymorphisms (SNP) in the *TAP1* gene (I333V and D637G) and 3 SNPs in the *TAP2* gene (I379V, A665T, and Q687STOP) was done (see Table 1 for sequences). PCR-RFLP was used to determine the genotype for the *TAP1* D637G polymorphism as previously described by Vandevyver et al. (11). For the remaining four SNPs (I333V, I379V, A665T, and Q687STOP), a multiplex PCR was done followed by a microsphere-based oligonucleotide ligation assay (OLA). The Luminex 200 flow cytometer (Luminex Corporation) was then used to determine the genotypes as described below.

Multiplex PCR and OLA. We developed a multiplexed flow cytometric assay based on the Luminex xMAP system and the FlexMAP oligonucleotide–tagged Universal Array microspheres (Luminex Corporation). The SNPs I333V, I379V, A665T, and Q687STOP were simultaneously amplified in a 25 μL multiplex PCR using primer sets previously reported (11). The OLA assays were designed as follows, using modifications described by Bruse et al.[5,6] Pairs of allele-specific probes (with the polymorphic nucleotide at the 3′ terminus) and one conserved sequence "reporter" probe (directly adjacent to the polymorphic nucleotide) for each of the four biallelic SNPs analyzed. Each allele specific probe was modified at the 5′ end by the addition of a unique 24-base FlexMAP TAG sequence (Luminex Corporation) using TAG-IT software (TM Biosciences). The reporter probes were phosphorylated at the 5′ end to allow ligation in the presence of the appropriate allele specific probe. The reporter probes were modified at the 3′ end by the addition of a 17-base sequence (GTAAAACGACGGCCAGT) that is complementary to a biotin-labeled "universal oligonucleotide."

The multiplex OLA reactions were done according to the protocol provided by Luminex Corporation. Briefly, the OLA reactions were done in a final volume of 20 μL containing 1× Taq DNA Ligase Buffer [20 mmol/L Tris-HCl buffer (pH 7.6), 25 mmol/L potassium acetate, 10 mmol/L magnesium acetate, 1 mmol/L NAD+, 10 mmol/L dithiothrietol, 0.1% Triton X-100; New England Biolabs], 5 nmol/L of each allele specific probe, 250 nmol/L of each reporter probe, 5 μL of multiplexed PCR product, and 10 units of *Taq* DNA ligase (New England Biolabs). The OLA reactions were initially heated at 96°C for 2 min, followed by 30 thermal cycles at 94°C for 15 s then 37.0°C for 1 min.

Hybridization, labeling, and detection of the OLA. The hybridization of the OLA products to the FlexMAP microspheres followed a modified protocol as described by Bruse et al.[6,7] Briefly, 50 μL of TMAC hybridization solution [3 mol/L tetramethylammonium chloride, 50 mmol/L Tris-HCl (pH 8.0), 3 mmol/L EDTA (pH 8.0), 0.10% SDS] containing beads from each FlexMAPmicrosphere set and 80 nmol/L biotinyled universal oligonucleotide (Biotin 5′-ACTGGCCGTCGTTTTAC-3′) were added directly to the 20 μL OLA reaction. The hybridization reactions were denatured at 95 °C for 90 s and hybridized at 37 °C for 20 min. After hybridization, 6 μL of TMAC hybridization solution containing 180 ng streptavidin-R-phycoerythrin (Molecular Probes-Invitrogen Corporation) were added and incubated at 37 °C for 40 min using a Luminex 200 flow cytometer (Luminex Corporation).

Quality control. Reactions were done in duplicate. Samples containing each possible allele for each SNP were confirmed by direct DNA sequencing and subsequently used as positive controls. Wells containing the OLA reaction mix without PCR template served as negative controls from which the background mean fluorescent intensity signal was determined.

Genotype assignment. Masterplex GT software (MiraiBio) was used to determine genotypes based on the fluorescence of each microsphere

[5] Unpublished data.

[6] Bruse SE, Moreau M, Azaro M, Zimmerman R, Brzustowicz L. Improvements to bead-based oligonucleotide ligation SNP genotyping assays. Biotechniques 2008;45:559–71.

[7] Unpublished data.

Table 1. Oligonucleotide probe sequences (5′-3′) for *TAP* polymorphisms

SNP	Allele	Allele Specific Probes*	Reporter Probes †
TAP1 I333V	A	tag65-TCACCATGGTCACCCTGA	TCACCCTGCCTCTGCTTTT
	G	tag1-CACCATGGTCACCCTGG	
TAP2 I379V	G	tag37-AACCGCCTTGTACCTGCTCG	TAAGGAGGGTAAGATACCAGA
	A	tag30-AACGCGCCTTGTACCTGCTCA	
TAP1 A655T	G	tag18-TTGCTCACAGGCTGCAGG	CAGTTCAGCGCGCCCA
	A	tag12-ATTGCTCACAGGCTGCAGA	
TAP2 Q687STOP	T	tag72-AGAAGCTTGCCCAGCTCT	AGGAGGGACAGGACCTC
	C	tag69-GAAGCTTGCCCAGCTCC	

*TAG-IT software (TM Biosciences) was used to determine the TAG assignments for the allele specific probes.
†Reporter probes were phosphorylated on the 5′ end and modified on the 3′ end with a 17 bp sequence that is complementary to a biotinylated "universal oligonucleotide" sequence used for detection.

set measured by the Luminex 200 flow cytometer (Luminex Corporation). The background signal determined by the negative control was subtracted from the mean fluorescent intensity for each microsphere set. Any sample, which did not generate an mean fluorescent intensity of at least 200, for 1 of the 2 alleles, was considered invalid and excluded from the data set.

Statistical analysis

Hardy Weinberg equilibrium in controls was determined by goodness-of-fit test. Differences in allele and genotype distributions between cases of high-grade CIN and controls for selected *TAP* polymorphisms were compared using Pearson's χ^2 test. Polytomous logistic regression and corresponding odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the relative risks of CIN I, CIN II, and CIN III under both dominant and recessive models of inheritance. Statistical tests for trend were done by multinomial regression assuming an ordinal outcome scale with multivariable adjustment for confounders. Effect of confounding was investigated using a change in point estimate criterion. Subset analyses were also done for women who tested positive for high-risk HPV types.

Results

To evaluate the role of variability in the *TAP1* and *TAP2* genes in the MHC class II region in the association of cervicovaginal HPV and cervix neoplasia, three nonsynonomous SNPs in *TAP2* and two in *TAP1* were tested. The observed distribution of alleles among women with no evidence of CIN was consistent with Hardy Weinberg equilibrium for the five SNPs examined (data not shown).

To assess the association between *TAP* polymorphisms and prevalence of cervical neoplasia, cases were defined as women

Table 2.

A. Allele distribution of *TAP* polymorphisms in women with and without high-grade cervical dysplasia

SNP	Allele	Control (*n*)*	Case (*n*)*	χ^2	*P* †
TAP1 I333V	A	261	119	5.40	0.02
	G	79	19		
TAP1 D637D	A	339	151	7.46	0.01
	G	95	21		
TAP2 I1379V	G	350	149	2.68	0.10
	A	70	19		
TAP2 A665T	A	335	133	0.27	0.60
	G	38	37		
TAP2 Q687STOP	T	285	120	0.18	0.67
	C	99	38		

B. Allele distribution of *TAP* polymorphisms in high-risk HPV positive women with and without high-grade cervical dysplasia

TAP1 I333V	A	138	96	4.75	0.03
	G	50	18		
TAP1 D637D	A	185	126	8.99	0.00
	G	67	20		
TAP2 I1379V	G	209	125	0.22	0.64
	A	33	17		
TAP2 A665T	A	200	115	0.07	0.79
	G	54	29		
TAP2 Q687STOP	T	172	103	0.08	0.77
	C	48	31		

*Cases include women with CIN II and CIN III, controls include women with CIN I or no CIN.
†*P* by Pearson's χ^2 test.

Table 3.

A. Multivariable association between cervical dysplasia and *TAP* genotypes

SNP	Genotype	CINI		CINII		CINIII		P_{trend} †
		OR*	(95% CI)	OR*	(95% CI)	OR*	(95% CI)	
TAP1 I333V	AA vs AG/GG	0.87	(0.4-1.8)	0.50	(0.2-1.3)	0.28	(0.1-0.8)	0.01
TAP1 D637G	AA vs AG/GG	0.76	(0.4-1.4)	0.42	(0.2-1.0)	0.27	(0.1-0.7)	0.00
TAP2 I379V	GG vs AG/AA	0.57	(0.3-1.1)	0.31	(0.1-0.8)	0.47	(0.2-1.2)	0.02
TAP2 A665T	AA vs AG/GG	1.04	(0.6-1.9)	1.45	(0.7-3.1)	0.77	(0.3-1.8)	0.97
TAP2 Q687STOP	TT vs TC/CC	0.76	(0.4-1.4)	1.06	(0.5-2.4)	0.70	(0.3-1.6)	0.62
B. Multivariable association between cervical dysplasia and *TAP* genotypes in women with high-risk HPV								
TAP1 I333V	AA vs AG/GG	0.91	(0.3-2.4)	0.53	(0.2-1.7)	0.28	(0.1-1.0)	0.03
TAP1 D637G	AA vs AG/GG	0.40	(0.2-1.0)	0.21	(0.1-0.6)	0.14	(0.0-0.4)	0.00
TAP2 I379V	GG vs AG/AA	0.42	(0.2-1.1)	0.31	(0.1-1.0)	0.51	(0.2-1.7)	0.20
TAP2 A665T	AA vs AG/GG	0.88	(0.4-2.2)	1.29	(0.5-3.6)	0.84	(0.3-2.5)	0.82
TAP2 Q687STOP	TT vs TC/CC	0.77	(0.3-2.0)	1.25	(0.4-3.6)	0.98	(0.3-2.9)	0.58

*ORs and 95% CIs by polytomous logistic regression compared with women with no CIN, adjusted for number of Pap smears, age and race.
† *P* for trend by ordinal logistic regression model.

with high-grade disease (CIN II or CIN III), whereas controls were defined as women without high-grade disease (no CIN or CIN I). CIN I is not a true cancer precursor, but a manifestation of active HPV infection (14), and CIN I is not clinically relevant as a treatable disease state, so CIN I was defined in the control group. The distribution of alleles was significantly different between cases and controls at two of the polymorphic sites investigated, *TAP1* I333V ($P = 0.02$) and *TAP1* D637G ($P = 0.01$). This difference in allele distribution between cases and controls remained significant among the subset of individuals who were positive for high-risk HPV types at both *TAP1* I333V ($P = 0.03$) and *TAP1* D637G ($P < 0.005$). No difference in allele distribution was observed at the other three polymorphic sites examined on *TAP2* between cases and controls, even when limiting the analysis to those who were positive for high-risk HPV types (Table 2A and B). Differences in genotype distribution between cases ($n = 114$) and controls ($n = 366$) paralleled differences in allele distribution (data not shown).

Multivariable polytomous logistic regression models for genotype were derived to examine the association between each of the five polymorphisms and the risk of developing CIN adjusting for history (number) of Pap smears, age, and race (Table 3). A protective association was observed with increasing grade of dysplasia for the *TAP1* D637G and I333V variant alleles, which seemed to be stronger for homozygote carriers of either allele, although the associations were not significant due to small samples. Assuming a dominant model of inheritance, a significant trend in association with grade of CIN was observed for both *TAP1* I333V ($P = 0.01$) and *TAP1* D637G ($P = 0.003$). The risk for developing high-grade CIN (CIN II or CIN III) was significantly reduced for carriers of at least one G allele at *TAP1* D637G (OR, 0.42; 95% CI, 0.2-1.0; and OR, 0.27; 95% CI, 0.1-0.7, respectively). Similarly, the risk for developing CIN III was significantly reduced for heterozygote and homozygote carriers of the G allele at *TAP1* I333V (OR, 0.28; 95% CI, 0.1-0.8) when the same model of inheritance was assumed (Table 3A). These associations remained significant after restricting the analyses to women who were positive for high-risk HPV types (Table 3B).

For *TAP2* gene alleles, a protective association with dysplasia was observed only for heterozygote and homozygote carriers of the variant *TAP2* I379V (G) allele, albeit with borderline significance (Table 3A). Furthermore, the magnitude of association was consistent for increasing grades of dysplasia, particularly when restricted to women with high-risk HPV, with similar ORs observed for CIN I, II, and III in a nondose-dependent manner (Table 3B). No association was found for the other *TAP2* polymorphisms tested (i.e., *TAP2* A665T or Q687STOP, or for women with high-risk HPV infections).

Discussion

In this study population, two polymorphisms located on the *TAP1* gene were associated with development of high-grade CIN. There were significant differences in both allele and genotype distribution between cases and controls for two of the examined polymorphisms of *TAP1*: *TAP1* I333V and *TAP1* D637G. When limiting analyses to women infected with high-risk HPV types, genotype and allele distribution between cases and controls remained significantly different for *TAP1* I333V and *TAP1* D637G. Additionally, the odds of developing high-grade CIN (II and III) was reduced at least 2-fold among participants with at least one G allele at *TAP1* I333V compared with wild-type homozygous (AA) carriers. The OR was also reduced 2.3-fold and more among participants with at least one G allele at *TAP1* D637G. This protective effect against development of high-grade CIN remained significant for these two polymorphisms even among participants infected with high-risk HPV types. This suggests that the role of *TAP1* I333V and *TAP1* D637G products are similar in protecting against immune evasion regardless of the oncogenicity of HPV type. One feasible explanation is that the mRNA product generated from individuals with at least one G allele at either of these polymorphisms is more resistant to degradation. Or the TAP molecules generated in women with these polymorphisms are more effective in eliciting a robust early cell-mediated immune response against HPV, including oncogenic HPV types.

Cytotoxic T cells are largely responsible for the elimination of virally infected and transformed cells. For effective elimination of an affected cell, a multistep process must occur, which requires both loading and presentation of peptide on MHC class I molecule and eventual expression of MHC class I molecule on the cell surface. Once proteins derived from virally infected tumor cells are degraded, the peptide products are transported from the cytosol to the endoplasmic reticulum by products of *TAP1* and *TAP2*. Only after the peptide-MHC class I complex is formed, can the MHC class I molecule be expressed on the cell surface.

MHC class I down-regulation in virally infected and transformed cells is acknowledged as a form of immune evasion. Down-regulation of MHC class I expression has been noted in many diseases, in particular those associated with HPV such as recurrent respiratory papillomatosis, condyloma acuminatum, and cervical cancer (5, 17–19). In a number of studies, down-regulation of MHC class I correlated with down-regulation of TAP1, suggesting a causal link between altered expression of TAP1 and MHC-1 (6, 18–20). Knowledge regarding the molecular mechanism culminating in the down-regulation of TAP1 is limited, however, the corollary relationship suggests a posttranscriptional mechanism. More recently, Tao et al. (19) found a significant decrease in TAP1 mRNA among specimen collected from women with condyloma acuminatum compared with those collected from healthy women.

Reported race was adjusted for in our multivariable analyses. However, when comparing allele frequencies by race to those already reported among Black and White racial groups, our study population had similar distributions compared with those reported. In this study, cases and controls were recruited from the same geographic locale. Cases and controls were also limited to individuals who tested positive for HPV. This allowed for investigation of genes whose products may shed light on proposed evasion mechanisms of virally infected cells that are allowed to persist and ultimately become transformed.

Results found in this study parallel those of a study conducted by Gostout et al. (21), in which a specific combination of the residues located at *TAP1* 333 and *TAP1* 637 was significantly associated with a protective effect for development of cervical cancer. In our analysis, there was no significant difference in allele or genotype distribution at the three polymorphisms examined on *TAP2* between cases and controls. Although risk for HPV-related cancers has been associated with certain polymorphisms located on *TAP2* in previous studies, there was no significant difference in OR for high-grade CIN III between cases and controls in dominant and recessive models created for the three examined polymorphisms located on *TAP2* (21–24). Correlations of these polymorphisms with immune responses may also prove to be helpful in determining ways to improve local immune responses in women with persistent HPV infection. We do not expect *TAP* polymorphisms to influence the efficacy of the HPV vaccine because it is thought to operate through antibody-mediated mechanisms. Nevertheless, the role of *TAP1* polymorphisms are likely to be more important with therapeutic vaccine development and should be considered as a translational correlate of interest.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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