

Different Profiles of Allelic Losses in Cervical Carcinoma Cases in Surinam and the Netherlands

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BACKGROUND. Cervical carcinoma is the second most common malignancy among women worldwide. The highest incidence rates are observed in developing countries. The increased susceptibility to cervical carcinoma in high incidence populations may result from several factors including human papillomavirus exposure and both inherited and acquired genetic traits. Using comparative molecular analysis of cervical carcinomas from Surinam, a high incidence area, and the Netherlands, a low incidence area, distinct molecular genetic profiles were studied in two populations with contrasting risk for the disease.

METHODS. In the two populations, the authors compared allelic loss as a marker for the involvement of putative tumor suppressor genes in 40 and 67 carcinoma specimens from Surinam and the Netherlands, respectively. Loss of heterozygosity (LOH) analysis was performed using polymorphic microsatellite markers at sites of known tumor suppressor genes (17p [p53], 13q [Rb, BRCA2], 16q [E-cadherin], and 17q [BRCA1]) and at chromosomes 3p, 6p, 6q, and 11q, which frequently are lost in cervical carcinoma.

RESULTS. Remarkable differences in LOH were found between both populations. The most prominent observation was the extremely high frequency of LOH, up to 72%, in the region of the major histocompatibility complex on chromosome 6p in specimens from Surinam. In the group of specimens from the Netherlands, only 45% of LOH was observed at this locus. In addition, LOH was detected significantly more frequently at 6q and 13q in the cases from Surinam whereas LOH was found more frequently at 17p in cases from the Netherlands.

CONCLUSIONS. The results of the current study show that heterogeneity exists in tumor-associated somatic genetic alterations between these two populations that may be indicative of the existence of multiple genetic pathways in cervical tumorigenesis. *Cancer* 1999;86:997-1004. © 1999 American Cancer Society.

KEYWORDS: cervical neoplasms, comparative study, ethnic groups, loss of heterozygosity, chromosomes, heterogeneity, Surinam, the Netherlands.

The incidence of invasive cervical carcinoma varies widely by geographic area. The highest rates have been reported from Latin American countries.^{1,2} Population-based studies in countries with contrasting risks of cervical carcinoma revealed results that were too conflicting to support a role for human papillomavirus (HPV) as a determinant of the variability in the rate of incidence of this disease worldwide.³ Moreover, because only a minority of premalignant lesions of the cervix develop to invasive lesions,⁴ additional genetic factors are suggested to be necessary for the neoplastic progression of HPV-infected cells.⁵⁻⁷ The inactivation of tumor suppressor genes appears to play an important role in cervical carcinogenesis according to analysis of loss of heterozygosity (LOH). Frequent allelic losses have been reported at 3p, 6p, 11q, and 17p, suggesting candidate

tumor suppressor genes on these loci are involved in the development of cervical carcinoma.⁸⁻¹¹

In contrast to the vast amount of information regarding HPV prevalence and genotype distribution worldwide,¹²⁻¹⁵ limited information exists regarding the additional molecular changes in cervical carcinoma from patients of different ethnic origins and/or geographic regions. Interpopulation variation in genetic alterations has been described for malignancies at other sites. In regions with contrasting risks for oral carcinoma, esophageal carcinoma, and liver carcinoma, differences in the prevalence and spectrum of mutations, amplifications, and LOH of oncogenes and tumor suppressor genes have been described.¹⁶⁻¹⁹ These studies suggest a variation in the etiology and pathways of malignant transformation in distinct areas and ethnic groups. The complex interaction between several factors, both exogenous and endogenous, may contribute to these differences.

The incidence of cervical carcinoma in Surinam, a former Dutch colony in South America, far exceeds that of the Netherlands.²⁰ To determine whether similar genetic mechanisms are involved in the development of cervical carcinoma in Surinam and the Netherlands, countries with a nearly fivefold difference in rates of incidence,²⁰ genetic alterations were evaluated in cervical carcinoma specimens from patients from Surinam and compared with data from the Netherlands. In addition to HPV detection, we examined LOH at sites of known tumor suppressor genes (17p [p53], 13q [Rb, BRCA2], 16q [E-cadherin], and 17q [BRCA1]) and at chromosomes 3p, 6p, 6q, and 11q, which frequently are lost in cervical carcinoma.⁸⁻¹¹ The results may provide a better understanding of the sequence of events that lead to cervical carcinoma and may help to explain the geographic distribution of the disease.

MATERIALS AND METHODS

Tissue Samples

Formaldehyde fixed, paraffin embedded cervical carcinoma tissues were obtained from 40 Surinamese patients who were referred to the Leiden University Medical Center between 1989-1995 for radical hysterectomy and lymphadenectomy and 67 Dutch cervical carcinoma patients primarily referred to the Leiden University Medical Center for similar surgical treatment between 1984-1995. Hematoxylin and eosin-stained sections were reevaluated to confirm the diagnosis. Only patients with clinical Stage I-II disease, as determined by one gynecology team on the basis of the International Federation of Gynecology and Obstetrics (FIGO) system,²¹ without any other form of treatment prior to radical surgery were included in the

study. The age of the patients in the Surinamese population ranged from 28-67 years with a mean age of 45 years and ranged from 22-76 years with a mean age of 45 years in the Dutch population.

DNA Isolation and LOH Analysis

The tumor fraction was collected from formaldehyde fixed, paraffin embedded tissue. When tumors contained <50% tumor cells, tumor cells were isolated by microdissection. Constitutive genomic DNA was isolated from tissue that was obtained from the uterine wall that did not contain tumor cells to serve as a "normal" DNA control. DNA extraction was performed using standard proteinase K digestion and phenol-chloroform extraction according to the protocol described by Kersemaekers et al.¹¹ Polymerase chain reaction (PCR) was performed as described by Weber and May.²² The PCR products were denatured in formamide, electrophoresed on a 6% denaturing polyacrylamide gel, and visualized by autoradiography for 14-18 hours at room temperature. All gels were scored by two independent individuals. Normal and tumor lanes were compared and tumors were designated as homozygous (noninformative) or heterozygous. Results of heterozygous cases were quantified on a phosphor imager (Phosphorimager 445 SI; Molecular Dynamics, Sunnyvale, CA). Using Molecular Dynamics Image Quant Software, allelic imbalance factors were calculated by the quotient of the ratio of the normal and ratio of the tumor allele intensities ($N1:N2/T1:T2$ or $1/[N1:N2/T1:T2]$ when an allele ratio revealed a number of <1). An imbalance factor <1.8 was interpreted as retention, whereas an imbalance factor of ≥ 1.8 was considered as LOH.²³ Cases in which the allelic imbalance was confirmed in subsequent analysis were considered to show LOH. Cases that failed to produce PCR products after repeated attempts were considered noninformative for these polymorphic markers.

Polymorphic DNA Markers

To evaluate LOH, paired normal-tumor DNAs were analyzed for LOH using 19 primer sets flanking microsatellite dinucleotide and tetranucleotide repeat polymorphisms located on the following chromosomal arms: 3p, 6p, 6q, 11q, 13q, 16q, 17p, and 17q. Primers were chosen from the Genome Data Base on the basis of their location, heterozygosity percentage, and allele length. A more extensive selection of markers located at chromosome 6 was made because of the high percentage of allelic loss on chromosome 6. The microsatellite markers and their map positions are depicted in Table 1.

TABLE 1
Frequency of LOH with 19 Microsatellite Markers in 40 Cervical Carcinoma Specimens from Surinamese Patients Compared with 67 Cervical Carcinoma Specimens from Dutch Patients

Chromosome	Map position	Marker	LOH (%) ^a		P value Surinam vs. that for the Netherlands
			Surinam	The Netherlands	
3p	3p22-14	D3S2456	9/19 (47)	18/44 (41)	0.78
3p	3p22-21.3	β -catenin	3/14 (21)	13/35 (37)	0.34
6p	6p25.1-24.3	F13A1	15/21 (71)	14/43 (33)	0.007
6p	6p23	D6S89	15/29 (52)	13/39 (33)	0.14
6p	6p22.1-21.33	D6S105	14/28 (50)	12/31 (39)	0.44
6p	6p22.3-21.3	D6S265	21/29 (72)	13/29 (45)	0.06
6p	6p12-11	D6S294	16/33 (48)	4/18 (22)	0.07
6q	6q22.3-23.1	D6S87	9/20 (45)	6/31 (19)	0.06
6q	6q16.3-21	D6S251	10/17 (59)	12/46 (26)	0.02
6q	6q	D6S1010	10/17 (59)	8/25 (32)	0.12
11q	11q21-22	D11S898	7/22 (32)	19/37 (51)	0.18
11q	11q22.1	D11S876	7/18 (39)	11/23 (48)	0.75
13q	13q14.1-14.3	D13S153	4/19 (21)	2/50 (4)	0.04
13q	13q12.1	D13S289	2/9 (22)	6/62 (10)	0.58
16q	16q22.1	D16S2624	0/20 (0)	5/47 (11)	0.31
16q	16q22.1	D16S752	4/25 (16)	7/47 (15)	0.99
17p	17p13.1	TP53	2/31 (6)	9/44 (20)	0.11
17p	17p13.3	D17S513	2/14 (14)	16/42 (38)	0.18
17q	17q21	D17S855	2/22 (9)	2/41 (5)	0.61

LOH: loss of heterozygosity.

^a Number of patients with loss of heterozygosity per total number of heterozygous informative cases.

HPV Detection

HPV detection in the tumor samples was performed by PCR using the E1 consensus primers (CPI/IIG) as described previously by Tieben et al.²⁴ Samples that did not show HPV positivity using the CPI/IIG primer set also were tested with the MY09/MY11 primer set²⁵ and the GP5+/GP6+ primer set²⁶ according to the protocol described by Bauer et al.²⁷ and de Roda Husman et al.,²⁶ respectively. Direct sequence analysis was performed on HPV positive products.

Statistical Analysis

Difference in LOH frequencies at the studied loci in tissues from Surinam and the Netherlands and correlations of LOH and HPV type were analyzed using the two-tailed Fisher exact test. A *P* value of ≤ 0.05 was considered statistically significant.

RESULTS

Loss of Heterozygosity

All 40 Surinamese and 67 Dutch tumors were analyzed for the same polymorphic markers at chromosomes 3p, 6p, 6q, 11q, 13q, 16q, 17p, and 17q. Relatively more DNA samples from Surinamese patients failed to amplify. Using the chi-square test, there was a significant difference between specimens from Suriname versus

those from the Netherlands in the percentage of heterozygosity versus homozygosity within D6S294 and TP 53 with relatively more heterozygosity observed in specimens from Surinam compared with those from the Netherlands (data not shown). Table 1 shows the frequencies of LOH on the different loci in both populations.

The frequencies of LOH at 6p varied for each locus and ranged from 48% on D6S294 to a highest rate of incidence of LOH in 72% on D6S265 in the Surinamese group and from 22% on D6S294 to 45% on D6S265 in the Dutch group. At the F13A1 locus, LOH was observed in 71% of the tumors from Surinamese patients whereas only LOH was observed in 33% of the tumors from Dutch patients, a statistically significant difference. In addition, for the other markers on 6p (D6S89, D6S105, D6S265, and D6S295) LOH was observed more often in the Surinamese population than in the Dutch population although these differences were less significant. The results shown in Figure 1 indicate that in all tumors from Surinamese patients showing LOH on 6p and the F13A1 and D6S265 markers were involved when informative, which identifies two common regions of LOH encompassing 6p25.1–24.3 (F13A1) and 6p22.3–21.3 (D6S265). Figure 2 shows phosphorimager traces of representative tumors

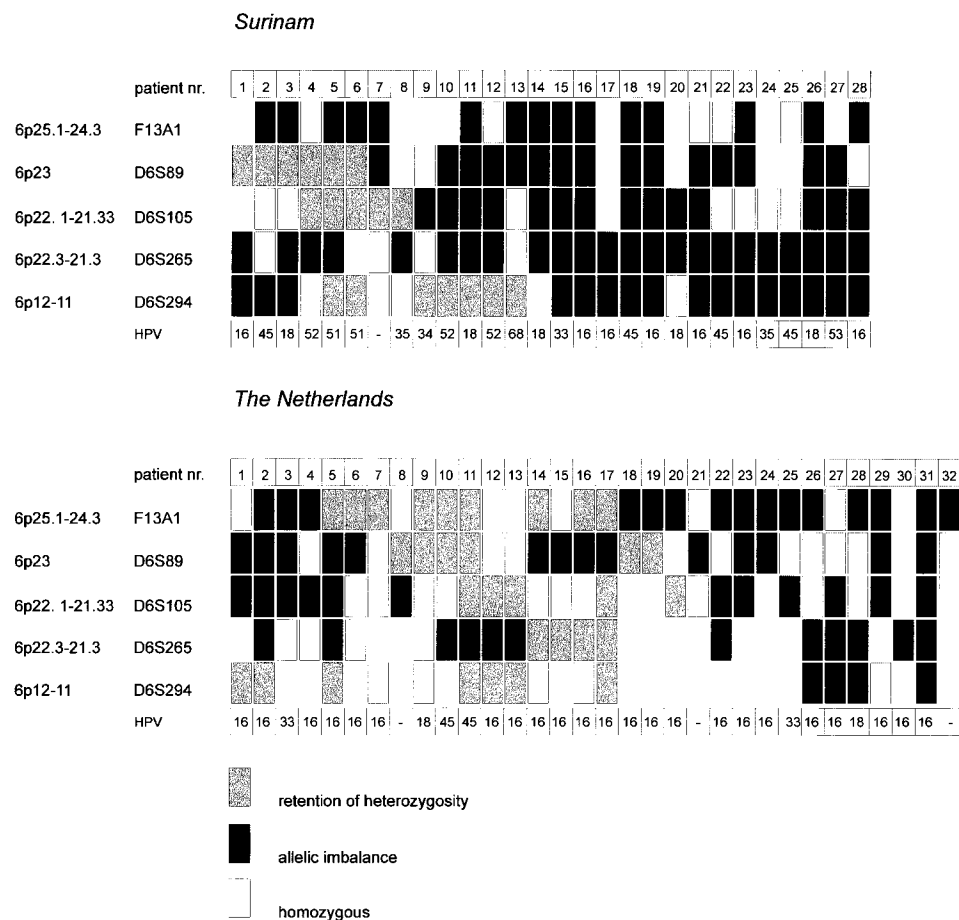


FIGURE 1. Patterns of loss of heterozygosity (LOH) at chromosome 6p of the 28 of 40 tumors from Surinam and the 32 of 67 tumors from the Netherlands showing LOH at 6p. Names and map positions of the polymorphic markers are shown on the left of the ideogram. The sample number is indicated at the top of each column. Gray rectangles indicate retention of heterozygosity, black rectangles indicate LOH, and white rectangles indicate homozygosity (non-informative cases). Open spaces indicate nonamplifiable DNA samples. HPV: human papillomavirus.

of Surinamese patients revealing LOH at chromosome 6p.

On chromosome 6q, the highest frequencies of LOH were found using marker D6S1010 in both populations (59% and 32% in specimens from Surinam and the Netherlands, respectively). However, to our knowledge the precise localization of this marker is not known. The difference in LOH rates on D6S251 was statistically significant (59% in Surinamese specimens and only 26% in specimens from the Netherlands).

At 13q, LOH rates of 21% and 22% were observed on D13S153 and D13S289, respectively, in specimens from Surinam whereas only LOH rates of 4% and 14% were observed on these loci in specimens from the Netherlands. The difference in the rate of incidence of LOH on D13S153 between specimens from Surinam and the Netherlands was statistically significant.

In contrast, LOH on 11q and 17p was shown more frequently in the specimens from the Dutch population than those from the Surinamese population although the differences were not statistically significant. There were no striking differences in LOH

patterns on the other loci studied. In both populations, a high frequency of allelic imbalance was noted on chromosome 3p, in particular at D3S2456, with LOH rates of 47% and 41% in specimens from Surinam and the Netherlands, respectively. Low rates of LOH observed on 16q and 17q were comparable in both populations.

Prevalence of HPV

HPV DNA was detected in 39 of the 40 Surinamese tumor samples (98%). HPV-16 was present in 12 samples and HPV-18 was present in 8 samples. The other cases contained HPV-33 (two cases), HPV-34 (one case), HPV-35 (two cases), HPV-45 (five cases), HPV-51 (two cases), HPV-52 (three cases), HPV-53 (one cases), HPV-59 (two cases), and HPV-68 (one case). Of the 67 Dutch tumors, 60 contained HPV DNA (90%). HPV-16 was detected in 50 samples, HPV-18 in 5 samples, HPV-33 in 3 samples, and HPV-45 in 2 samples. Figure 3 shows the distribution of HPV genotypes categorized in groups (i.e., HPV-16, HPV-18, HPV-33, HPV-45, and other HPV genotypes) as a percentage of HPV positive

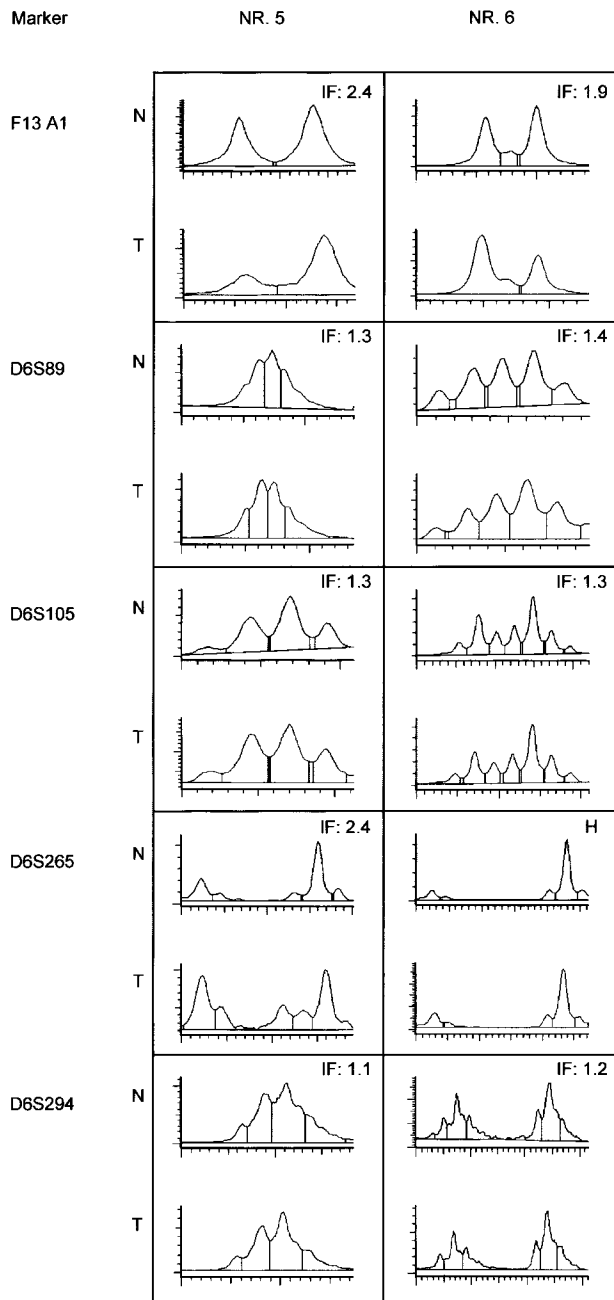


FIGURE 2. Representative phosphorimager traces of the polymerase chain reaction products of two representative tumors of Surinamese patients revealing complex loss of heterozygosity patterns at chromosome 6p. Loci designations and tumor numbers correspond to those given in Table 1 and Figure 1. N: normal constitutive cells; T: tumor cells of the same subject; IF: imbalance factor; H: constitutional homozygosity.

cases within the Surinamese and Dutch specimen populations.

In the Surinamese population specimens, no correlation was found between the presence of HPV-16 or other HPV types and LOH per marker or LOH per

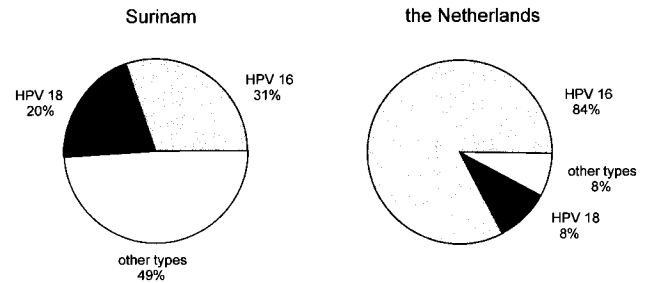


FIGURE 3. Prevalence of human papillomavirus (HPV) genotypes as a percentage of the total of HPV positive samples categorized in three groups in the Surinamese and Dutch populations, respectively. HPV types other than HPV-16 and HPV-18 were predominant in the Surinamese population whereas HPV-16 was detected in the majority of cases in the Netherlands. In the Surinamese and Dutch groups, 1 of 40 tumors and 7 of 67 tumors, respectively, were negative with all 3 primer sets.

chromosome arm. In the specimens from the Dutch population, a correlation was found between HPV-16 and LOH at D6S251 ($P = 0.03$). LOH at TP53 was associated weakly with HPV negativity or HPV types other than HPV-16 ($P = 0.04$). An association between LOH patterns and different HPV types other than HPV-16 could not be established because of the low numbers of cases per distinct HPV type in both populations.

DISCUSSION

In this study, the accumulation of genetic defects was compared in cervical carcinoma specimens from patients from Surinam, a country with a high rate of incidence, and the Netherlands, a country with a low rate of incidence of the disease.

Comparison of LOH frequencies in the two populations using polymorphic microsatellite markers at sites of several known or putative tumor suppressor genes showed significant differences in LOH rates between specimens from Surinam and the Netherlands, especially at chromosome 6. It is known from the literature that the short arm of chromosome 6 often is involved in cervical carcinoma.^{8-10,28} However, to our knowledge the high frequencies noted in the Surinamese population in this study have not been reported previously. At 6p25.1-24.3 (F13A1), tumors from Surinamese patients showed LOH in 71% of cases. Significantly fewer cases of LOH (33%) at this locus were observed in the specimens from the Dutch population. This observation suggests the presence of one or more tumor suppressor genes in the telomeric region of the D6S89 marker that play a more important role in the tumorigenesis in cervical carcinoma patients in Surinam than in those in the Netherlands.

This region may include 6p23 depending on the exact location of the D6S89 marker. Further mapping will be required to identify candidate genes. LOH at 6p22.3–21.3 (D6S265) was shown in 72% of the tumors in the Surinamese group of specimens, which is substantially higher than the LOH rate of 45% observed at this locus in the Dutch group of specimens. Candidate genes in the 6p22.3–21.3 region may include the WAF-1/CIP-1 gene because of its described role in the cell cycle.²⁹ Other candidate genes in this region include those that play a role in the immune response such as the major histocompatibility complex genes, TNF- α , and TAP1. Loss of these loci may result in tumor growth advantage by the tumor escaping immune surveillance and in this context these genes may act as tumor suppressor genes.

Overall imbalance at 6q was identified at a lower frequency than at 6p, but with a significantly higher frequency of loss in the Surinamese population with the polymorphic marker D6S251. Thus, putative tumor suppressor gene(s) within this region may be more important in the development of cervical carcinoma in the Surinamese population. Conversely, the frequency of imbalance at 6q also may be a bystander effect with loss of the whole chromosome in the process of increasing genomic instability.

In addition, at chromosome 13q14.1–14.3 substantial differences in LOH frequencies were found. More frequent LOH was found at this locus in specimens from Surinam. Rb1 is a likely candidate tumor suppressor gene at 13q14. In addition to inactivation of the Rb protein by forming a complex with the E7 protein of HPV, deletion of the wild-type allele, leaving the cell with a mutated allele, may be important in a subset of the Surinamese patient group.

In contrast, we found higher frequencies of LOH in specimens from the Netherlands on 11q and 17p (including the p53 locus), which suggests a higher selection pressure on the inactivation of the putative tumor suppressor genes by allelic loss at these sites in cases from the Netherlands compared with cases from Surinam.

We found comparable high frequencies of LOH on 3p. Putative tumor suppressor genes in this region appear to play a similar role in both populations. The loss on chromosome 3p has been reported to be a common genetic alteration in cervical carcinoma and is suggested to be an early event in its development and a potential marker of risk for progressive disease in patients with premalignant lesions.^{8,9,30}

Low frequency of LOH on 13q12.1 (BRCA-2), 16q (E-cadherin), and 17q (BRCA-1) in both populations is consistent with the supposed limited role attributed to

allelic loss at these loci in the development of cervical carcinoma.^{8–10,31}

Although the percentage of heterozygosity varied between cases from Surinam and the Netherlands for some markers, there was only a significant difference in the percentage of heterozygosity versus homozygosity within D6S294 and TP53. Within these two markers more heterozygosity was observed in cases from Surinam than those from the Netherlands. This finding did not appear to influence the observed variation in the prevalence of LOH on the different loci analyzed. However, it should be stated that with low numbers of informative samples among certain markers (e.g., D13S289 in the Surinamese population), incidental cases of LOH will have a strong influence on the observed frequency of LOH.

The HPV positivity rates of 98% and 90% in the cases from the Surinamese and Dutch populations, respectively, is in agreement with previously reported findings indicating that the association between HPV and cervical carcinoma is consistent worldwide.^{12–15} The distribution of the genotypes differed between the two populations. Although HPV-16 was found in the majority of the carcinoma specimens from Dutch patients, HPV types other than HPV-16 and HPV-18 were more prevalent in the carcinoma specimens from Surinamese patients. These results confirm previously described differences in HPV type distribution by geographic region.^{12,14} The HPV type distribution in patients with cervical carcinoma in Surinam may reflect the prevalence of the different genotypes in the general population of Surinam, or may be the result of a distinct oncogenic potential of diverse HPV genotypes that are present more frequently in non-Western European countries. It may be speculated that the apparent difference in the occurrence of allelic loss between patients in Surinam and the Netherlands may be due to infection with different HPV types. In the Dutch population LOH for marker D6S251 was correlated with HPV-16 whereas LOH for marker TP53 was associated with HPV types other than HPV-16 and HPV negativity. We may have missed a correlation between LOH and HPV type in the Surinamese population because of the relatively small number of cases and the wide variation in HPV types.

Thus, this study reveals differences in HPV genotype distribution and LOH frequencies at various loci in cervical carcinoma specimens from Surinam and the Netherlands. One explanation for the more extensive LOH in the Surinamese group is that the higher LOH frequencies reflect increased genomic instability associated with an overall more progressive stage of disease in this population, considering that advanced stage of disease may be both the cause or the result of

increased genomic instability. However, patients in both groups initially were selected with the same stage of disease (FIGO Stages I and II). Moreover, the finding that at other loci similar or even higher LOH rates were observed in the specimens from the Dutch population supports the hypothesis that the observed differences reflect true genetic tumor heterogeneity. Apparently, the two ethnic groups experience both similar and dissimilar allelic losses that result in a distinct accumulation of genetic defects, which has contributed to tumor development. It is assumed that carcinogens differ from each other in terms of the spectrum of mutations they produce (e.g., deletions, recombinations, and translocation).¹⁹ The spectra of allelic losses in tumors from Surinam and the Netherlands may be due to a difference in exposure to environmental agents, including infectious agents such as HPV. In analogy with the synergistic interaction between hepatitis B virus and aflatoxin, which has been suggested to cause high rates of liver carcinoma in Africa and Asia,^{32,33} cervical carcinoma may be due to an interaction between distinct HPV genotypes with environmental carcinogens. However, in the Surinamese population, detailed information regarding factors in addition to HPV infection that might be of influence (e.g., dietary factors, smoking habits, and sexual behavior) was lacking in the patient charts. In addition, among the biologic factors that might explain a differential risk of cervical carcinoma in certain groups are variations in inherited genetic traits, such as genetic polymorphisms. These genetic characteristics may involve a differential potential to increase susceptibility to specific carcinogenic factors.

In the current study we observed genetic heterogeneity between two populations, patients from Surinam and patients from the Netherlands, that may reflect various carcinogenic pathways. This genetic heterogeneity may result from a combination of exposure to HPV and possible other exogenous factors, and variable susceptibility factors in the different populations. Future investigations to confirm these findings may help in the further identification of factors that are responsible for the wide variations in the incidence rate of cervical carcinoma worldwide. Exploring the genetic alterations with more markers, in particular on chromosome 6p, may elucidate the suggested presence of putative tumor suppressor genes further, especially in the high incidence area of Surinam.

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