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Cervical intraepithelial neoplasia: prognosis by combined LOH analysis of multiple loci

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Abstract

Objective. Cervical intraepithelial neoplasias (CIN) show markedly variable clinical behavior. Clinically, it is important to distinguish CIN lesions with different behaviors and identify those likely to persist and progress. The purpose of this study is to explore whether CIN lesions with different clinical behaviors can be stratified by analysis of loss of heterozygosity (LOH) at multiple loci.

Methods. One hundred sixty-four cases of CIN (54 CIN1, 59 CIN2 and 51 CIN3) were screened for LOH at 12 microsatellite markers including 10 from 3p14, 3p21–22, 6p21 and 11q23. LOH was correlated with clinical follow-up data and high-risk HPV infection.

Results. In a pilot study of 71 cases of CIN, screening of 12 microsatellite markers identified four (D3S1300, D3S1260, D11S35, and D11S528) at which LOH was significantly associated with disease persistence/progression. These four markers were further investigated in a larger cohort, which brought the total number of cases examined to 164. Combined analysis of LOH at the above four loci permitted the identification of 22–47% of CIN lesions depending on the histological grade, which showed disease persistence/progression. LOH at these loci was significantly associated with HPV16 infection. Bioinformatic analysis identified several candidate genes including the fragile histidine triad gene and progesterone receptor gene that may be the target of deletions.

Conclusions. LOH at D3S1300, D3S1260, D11S35 and D11S528 was significantly associated with CINs that showed persistence/progression, and combined LOH analyses at these loci could be used to identify such cases.

Keywords: CIN; LOH; Prognosis; HPV

Introduction

Most cervical carcinomas are derived from pre-neoplastic epithelial lesions known as cervical intraepithelial neoplasia (CIN). CIN is classified in a three-tier system (CIN1, 2 and 3) according to histological features. In addition, a separate category describing more minor cell changes than CIN1 is used both in the United Kingdom (borderline changes) and North America (atypical squamous cells of undetermined significance, ASCUS). Clinically, CIN lesions show markedly variable behaviors despite morphological homogeneity within each subgroup. Approximately 50% of CIN will persist or progress in the absence of treatment [1] and around 5% show disease recurrence despite treatment [2,3].

A big challenge for clinical management of patients with CIN is to identify those that likely persist and progress despite treatment. The prognostic value of HPV typing has been extensively studied. Persistent infection of high-risk HPVs has been shown to be associated with persistence and progression of CIN lesions [8] and continuous monitoring HPV infection every 6 months may help to identify CIN lesions with high risk of progression. However, HPV typing at single points alone does not provide a reliable prognostic indication as high-risk HPVs are frequently present in normal cervical smears (2–10%) [4–6], borderline (10–50%) and CIN1 (20–80%) lesions [4,6,7]. In addition, HPV infection may be transient [8].

There is strong evidence that HPV infection alone is insufficient to cause malignant transformation. Growing evidence indicates that genetic changes play a critical role in the transformation of HPV-infected cells and may account for the irreversible clinical behavior of CIN. Many chromosomal regions including 3p14.1–p22, 4p16, 4q21–35,

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5p13–15, 6p21.3–22, 6q21–25, 11p15, 11q23, 13q12.3–q13, 17p13.3 and 18q12.2–22 show loss of heterozygosity (LOH) in cervical cancer [9,10]. LOH at 3p14.2, 3p21–22, 6p21 and 11q23 frequently occurs in cervical cancer [11–22] and CIN, more commonly seen in CIN2, CIN3 and invasive cancer than in CIN1 [19,21]. In addition, LOH at 3p21 positively correlates with mitotic activity of tumor cells [11]. Deletion at 6p21 is significantly associated with poor overall and disease-free survival in patients with cervical cancer [20]. Finally, functional studies have shown that chromosome 11 carries genes that suppress tumorigenic properties of human cervical cancer cell lines [23]. LOH analysis of cervical cancer and study of in vitro immortalization of human keratinocytes by HPV16 indicate that the tumor suppresser genes located at 11q23 [22,24].

In view of the above evidence, allelic deletion at 3p14.2, 3p21–22, 6p21 and 11q23 may be critical for development of CIN lesions and thus influence their clinical behavior and bear prognostic value. However, whether LOH at these loci can be used for CIN prognosis is largely unknown. To test this, we screened 12 microsatellite markers including 10 from the above chromosomal regions for LOH in 164 cases of CIN and evaluated their value as prognostic markers.

Materials and methods

Patient materials

One hundred sixty four cases of CINs (54 CIN1, 59 CIN2 and 51 CIN3) were retrieved from the Department of Histopathology, University College London Hospital (UCLH). They were chosen purely according to the availability of diagnostic specimens and clinical follow-up data except in the case of Pap smear where those with abundant clumps of dyskaryotic cells were selected for convenience of microdissection. The follow-up period ranged from 6 to 387 months, with an average of 34.8 months. The diagnosis of CIN was based on histology. All patients were treated according to the same protocol at UCLH. The study was approved by the joint UCL and UCLH Committees on the Ethics of Human Research.

Microdissection and DNA preparation

The morphology of Pap smears and biopsies in all cases was reviewed by two cytopathologists (AE and GK) before microdissection. For Pap smears, dyskaryotic cells were identified and marked with a diamond pen on the reverse side of the slide. After removing the coverslip, both dyskaryotic and normal cells were separately microdissected [25]. For cervical biopsies, neoplastic and normal cells were microdissected from freshly prepared hematoxylin stained sections [25].

The microdissected cells were digested with 100 μg/ml proteinase K in 1× PCR buffer at 56°C for 16–20 h in a volume of 50–100 μl. The digests were heated at 95°C for 10 min to inactivate proteinase K and the resulting supernatant was used for PCR [25].

Detection of LOH by PCR

Twelve microsatellite markers including 10 from 3p14, 3p21–22, 6p21 and 11q23, at which LOH frequently occurs in cervical cancer [11,12,14–22], were studied (Table 1). Primers were designed to immediately flank the tandem repeats, thus the fragment to be amplified is small, allowing amplification from DNA samples prepared from archival fixed specimens. One of the paired primers was fluorescently labeled and PCR was carried out in a thermal cycler using a

Table 1
Sequences of PCR primers used for LOH analysis of microsatellite loci and HPV typing

Target	Chromosomal location	Forward primers	Reverse primers	Estimated size (bp)
D3S1566	3p13	5′ TAATTGAGAAGTCATGCCCTG3′	5′ GCTCAAAGAAATGTCCTGGA3′	170
D3S1285	3p14.1	5′ CACCCTACTGTGCTATCAAACA3′	5′ AGTATCCAGAGGCTGGGAAG3′	153
D3S1300	3p14.2	5′ GCTCACATTCTAGTCAGCCTG3′	5′ TGTCACAGAATAGTCTTTCCCA3′	165
D3S1289	3p21.1	5′ GCAACTTGTAAGAGAGCATTCA3′	5′ ACATTACAGAAATGTGATGCA3′	259
D3S1260	3p22.2	5′ GCTACCAGGGAAGCACTGTA3′	5′ GCTAAACTGAAGACCCTGCA3′	
D3S1611	3p22.3	5′ TAATCCCAGCACTTCGAGAG3′	5′ AGACTACAGGCATTTGCCAC3′	117
D5S406	5p15.32	5′ TGCCAATACTTCAAGAAAAACA3′	5′ ACTTGGGATGCTAACTGCTG3′	188
D6S105	6p21.1	5′ CCAAAGTGCTGGGATTACAG3′	5′ TCAAGGAAGAGAGACCATGC3′	145
D6S265	6p21.1	5′ ATCACCCCCCTCACACAC3′	5′ TCTAATCGAGGTAAACAGCAGA3′	100
D6S277	6p24.3	5′ AGCTGAATAACACGAGGTG3′	5′ TACATTTTTGATGACAATGGAA3′	101
D11S35	11q22.1	5′ GAGGAAAGTCATGAACGCAG3′	5′ ATCGATTAACCAACTTCACACA3′	100
D11S528	11q23.3	5′ GCCTAACTAATGGTGTCCCC3′	5′ GACCCCAGTGTGAGATGAAT3′	150
HPV16	NA	5′ CAGGACCCACAGGAGCGACC3′	5′ CGACCGGTCCACCGACCCCT3′	398
HPV18	NA	5′ CCGAGCACGACAGGAACGACT3′	5′ TCGTTTTCTTCCTCTGAGTCGCTT3′	174
HPV33	NA	5′ AACGCCATGAGAGGACACAAG3′	5′ ACACATAAACGAACTGTGGTG3′	212
HPV45	NA	5′ TTGGAATTTTGGTGTCCCTC3′	5′ TCCTGCTTTTCTGGAGGTGT3′	114
HPV56	NA	5′ TGATGCACGAAAAATTAATC3′	5′ CGGGGATAACCCAATATTCC3′	160

NA: not applicable.

"hot-start touch-down" program [25]. The specificity of PCR products was verified on agarose gels and products analyzed on an ABI377 DNA sequencer using the GeneScan software (Version 3.0). Dyskaryotic/neoplastic and normal cells from the same cases were analyzed in parallel. Allelic loss was identified by a computer program when the peak height ratio of tumor to normal alleles was 0.25 or less as described previously [26], then confirmed by visual inspection in each case. The molecular data were generated without the knowledge of clinical follow-up details.

Detection of high-risk HPVs

High-risk HPVs including 16, 18, 33, 45 and 56 were detected by PCR separately using primers established in previous studies (Table 1) [27–29]. DNA samples prepared from microdissected dyskaryotic or neoplastic cells were used for HPV detection in each case. Strict laboratory procedures including separate set-up areas for DNA preparation, PCR and post-PCR analysis were followed and serial controls at various stages of DNA preparations and PCR set-up were included in each set of experiments to avoid and detect potential cross contamination.

Data analysis

To correlate LOH with disease behavior and identify those that may bear prognostic value, we divided patients into two groups according to the results of cytological and histological examination of follow-up specimens: (1) disease-free group, those that became disease-free within 6 month after the first treatment and (2) disease persistence/progression, those that showed disease persistence at the same or a higher CIN grade 6 months after the initial treatment. Student t test was used for comparison of age, follow-up time, HPV status and treatment methods between CINs showing different clinical behaviors, that is, disease-free vs. disease persistence/progression. χ^2 was used to analyze the relationship between LOH and clinical outcome, HPV status and treatment methods.

Results

Identification of loci at which LOH is potentially valuable in CIN prognosis

Primers for microsatellite analysis were designed to amplify the minimum fragment containing the tandem repeat, thus suitable for amplification of DNA samples prepared from archival specimens. PCR was successful for all DNA samples prepared from Pap smears and 95% of those from biopsies. For cases that were failed for PCR in the first instance, repeat PCRs with various amounts of template DNA were carried out and those without successful PCR amplification were excluded from the analysis.

To identify the loci at which LOH was associated with disease behavior and may thus bear prognostic value, we first screened 12 microsatellite markers and correlated LOH with clinical outcome (disease-free vs. disease persistence/progression) in 71 cases of CIN (15 CIN1, 22 CIN2 and 34 CIN3) using diagnostic Pap smears. Within the disease persistence/progression group, the average time showing the disease recurrence was 26 months, ranging from 6 to 90 months. As resection margin could not be reliably evaluated in each case, no attempt was made to correlate the extent of tumor involvement with the outcome of treatments.

As the number of cases examined from each CIN group was relatively small, we correlated LOH with clinical outcome irrespective of CIN grade in the initial analysis. Among the 12 markers investigated, D3S1300 (3p14.2), D3S1260 (3p22.2) and D11S528 (11q23.3) showed LOH at significantly higher frequencies in the disease persistence/progression group than the disease-free group ($P < 0.01$, Fig. 1A). D11S35 (11q22.1) exhibited the next highest statistical difference between the two groups although not at a significant level. Using a stepwise statistical analysis testing various combination of the 12 markers, the above four markers collectively gave the highest statistical significance ($P < 0.02$) between the two groups and were selected for further analysis. As the value of using these loci alone as prognostic markers was limited, we tested the four makers together (Fig. 1B). By stepwise examination of the sensitivity and specificity of the combined four LOH markers, that is, one or two or three or all of the four loci showing LOH, the best cut-off point was when two of the four loci showing LOH was applied. At this threshold, around 45–58% of CIN lesions in the disease persistence/progression group but none in the disease-free group were identified.

Prognostic value of LOH at D3S1300 (3p14.2), D3S1260 (3p22.2), D11S35 (11q22.1) and D11S528 (11q23.3) in CIN

To further confirm the prognostic value of LOH at the above four markers in CIN, we examined a further series of 93 cases using diagnostic biopsies since it was much easier to microdissect neoplastic cells from biopsies than Pap smears. LOH was correlated with clinical follow-up as above. The data from these additional cases were compatible to those obtained from the Pap smears in the pilot study although the LOH frequency in CIN1 and CIN2 of the disease persistence/progression group was higher in the Pap smears than biopsies (Figs. 1B and 2C). This most likely reflected the selection of Pap smears with abundant clumps of dyskaryotic cells, which may bias selection of those with "bulky" disease, and the number of the cases examined in the pilot study was small. No statistical differences were found in the age, HPV status, treatment modalities and follow-up time between Pap smears and biopsies.

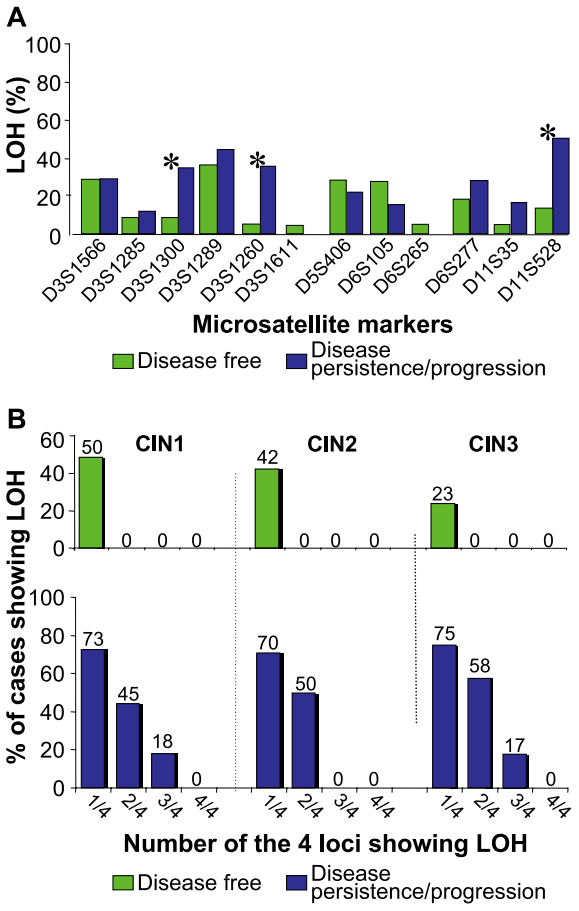

Fig. 1. Identification of LOH loci that are potentially valuable in CIN prognosis. (A) Frequencies of LOH at 12 microsatellite markers between CIN lesions showing disease-free or disease persistence/progression after initial treatment. *The incidence of LOH at D3S1300 (3p14.2), D3S1260 (3p22.2) and D11S528 (11q23.3) is significantly higher in the disease persistence/progression group than the disease-free group ($P < 0.05$ in each). D11S35 (11q22.1) shows the next highest statistical difference between the two groups, although not at a significant level. Using a stepwise statistical analysis testing various combination of the 12 markers, the above four markers collectively gave the highest statistical significance ($P < 0.02$) between the two groups and were further analyzed as detailed in B. (B) Prognostic value of combined LOH analysis at D3S1300 (3p14.2), D3S1260 (3p22.2), D11S528 (11q23.3) and D11S35 (11q22.1). By stepwise testing the sensitivity and specificity of the combined four LOH markers in CIN prognosis, that is, one or two or three or four of the four loci showing LOH, the best cut-off point is LOH at two of the four loci. At this threshold, between 45% and 58% of CIN lesions in the disease persistence/progression group but none in the disease-free group show LOH.

In total, we examined 164 cases of CIN (54 CIN1, 59 CIN2 and 51 CIN3). The frequency of LOH at D3S1300, D3S1260 and D11S35 positively correlated with CIN grade, while the incidence of LOH at D11S528 was similar among different CIN groups (Fig. 2A). As expected, the frequency of LOH at these loci was significantly higher in the disease persistence/progression group than the disease-free group ($P < 0.0005$, Fig. 2B). By combining the four markers together as above, 22% of CIN1, 28% of CIN2 and 47% of CIN3 of the disease persistence/progression group but none of the disease-free group showed LOH at two or more of the four loci examined (Fig. 2C). Thus, LOH analysis at these loci could identify 22–47% of CIN lesions that showed disease persistence despite treatment.

The percentage of infection of high-risk HPVs was significantly higher in the disease persistence/progression group (90%) than in the disease-free group (43%) ($P < 0.0005$). Among different high-risk HPVs, HPV16 infection was significantly associated with the disease persis-

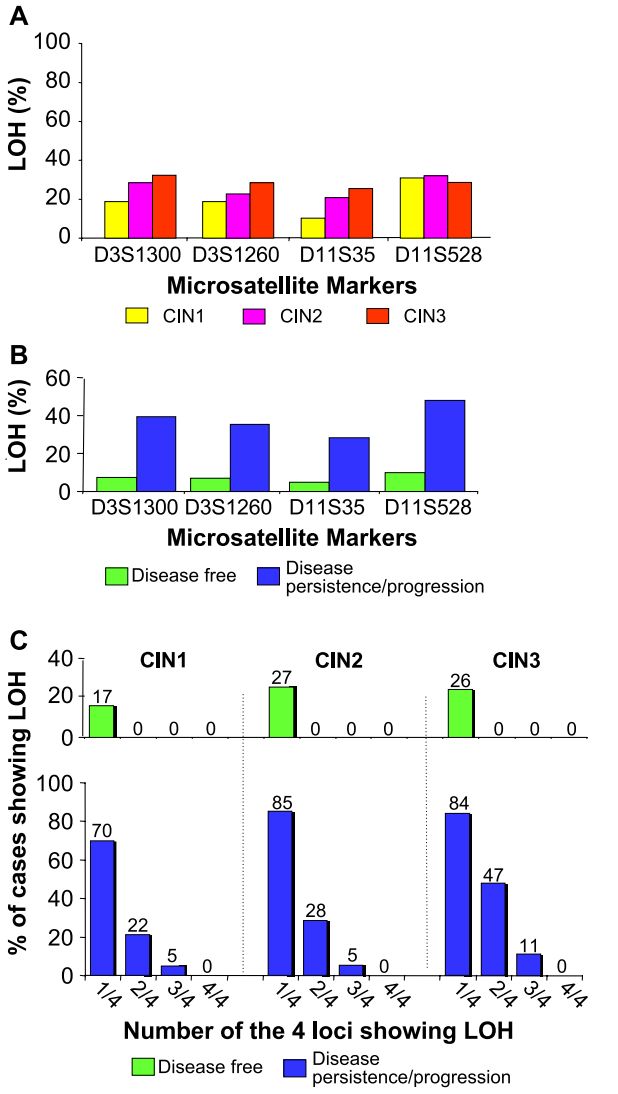

Fig. 2. Prognostic value of LOH at D3S1300 (3p14.2), D3S1260 (3p22.2), D11S35 (11q22.1) and D11S528 (11q23.3). (A) Correlation of LOH with CIN grade. LOH at D3S1300, D3S1260 and D11S35 correlates positively with the grade, while the incidence of LOH at D11S528 is similar among different CIN lesions. (B) Comparison of LOH between CIN lesions showing disease-free or disease persistence. (C) By stepwise testing the sensitivity and specificity of the combined four LOH markers in CIN prognosis, that is, one or two or three or four of the four loci showing LOH, the best cut-off point is LOH at two of the four loci. At this threshold, between 22% and 47% CIN lesions of the disease persistence/progression group can be identified with 100% specificity.

tence/progression group ($P < 0.001$) despite we may have underestimated its incidence in samples prepared from paraffin-embedded tissue specimens, since the fragment to be amplified is relatively large. Interestingly, only HPV16 was significantly associated with LOH at each of the four loci examined ($P < 0.01$), particularly D3S1300 ($P < 0.0005$). All patients were treated, but accurate treatment information was available in 102 cases including 47 and 55 from the disease-free and disease persistence/progression group, respectively. Laser ablation was more frequently used in the disease-free group than the disease persistence/progression group ($P < 0.02$), whereas large loop excision of the transformation zone (LLETZ) was opposite, applied more often to the disease persistence/progression group (62%) than the disease-free group (21%) ($P < 0.005$) (Table 2). There were no significant differences in other treatment modalities between the two groups. Thus, at least in general, patients in disease persistent/progression group were not under-treated as compare to those in disease-free group.

To further evaluate the association of LOH with clinical outcome of CIN, we examined both diagnostic and follow-up biopsies in 22 cases from the disease persistence/progression group. Of these cases, five (numbers 1–5) became disease-free after the first follow-up biopsy (7–15 months) and none of them showed accumulation of LOH in the follow-up biopsy (Table 3). The remaining 17 cases showed disease persistence at the same (eight cases) or higher CIN grade (nine cases) during follow-up, and 13 (numbers 9–21) displayed LOH at additional loci (Fig. 3). The average time to gain LOH at an additional locus was 30 months, ranging from 5 to 93 months. Except case 22, the LOH observed in the diagnostic sample was always seen in the follow-up biopsy (Table 3). In case 22, LOH was seen at D3S1260 and D11S35 in the diagnostic biopsy but not in the follow-up biopsy 3 years later, suggesting that the two CIN lesions may not be clonally related.

In six cases (numbers 12–14, 16–18), the diagnostic biopsy showed no LOH or LOH at only one of the four loci, but the follow-up biopsy displayed LOH at two or more of the four loci, reaching the threshold of prognostic significance as detailed above. Interestingly, two of these cases (numbers 17 and 18) also showed infection by an additional high-risk HPV.

Putative genes targeted for deletion at D3S1300 (3p14.2), D3S1260 (3p22.2), D11S35 (11q22.1) and D11S528 (11q23.3)

Bioinformatic analysis of the genomic regions at D3S1300, D3S1260, D11S35 and D11S528 identified putative genes that may be target of the deletions. LOC152071 (an EST clone), *FHIT* (fragile histidine triad gene) and *NPCR* (nasopharyngeal carcinoma related protein) are at or in the vicinity of D3S1300. Since *FHIT* is frequently deleted in a variety of human cancers including cervical cancer [30–32], it is a likely target of the deletion. Among the genes in the vicinity of D3S1260 including

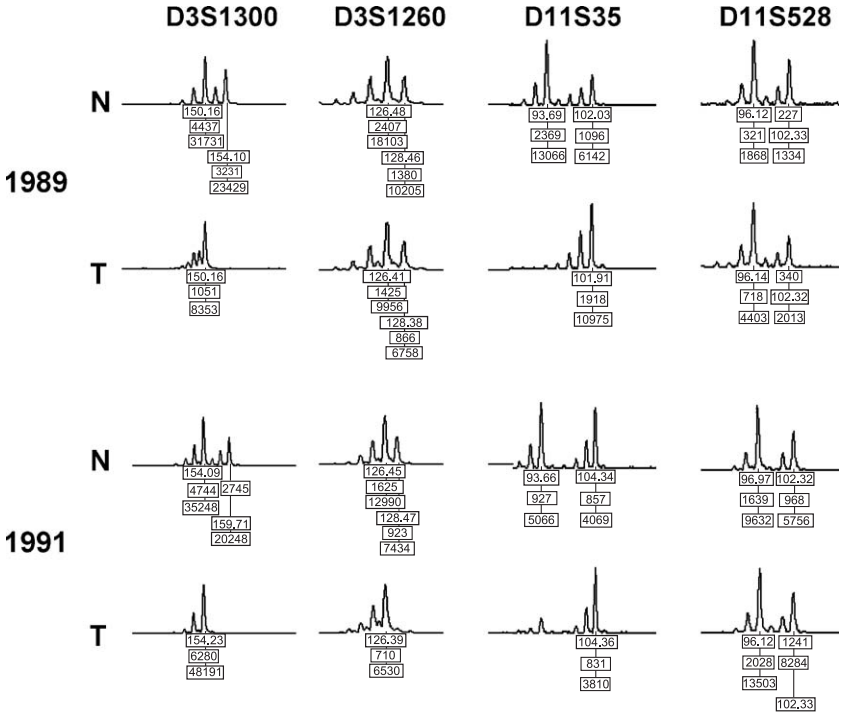

Fig. 3. Example of LOH analysis. The diagnostic biopsy (CIN3) of case 19 shows LOH at D3S1300 and D11S35. Despite treatment, the patient continually presented with CIN3. The follow-up biopsy 17 months after diagnosis displays additional LOH at D3S1260. N, normal cells; T, tumor cells.

Table 2
Clinical features of CIN lesions between the disease-free and the disease persistence/progression group

	Disease-free		Disease persistence/ progression	
Number of cases	75		89	
Age (year)				
Mean	44		36	
Range	31–66		23–56	
High-risk HPV infection				
Number of cases with high-risk HPV*	32	43%	80	90%
HPV16*	1	1%	26	29%
HPV18	9	12%	18	20%
HPV33	15	20%	18	20%
HPV45	5	7%	9	10%
HPV56	1	1%	2	2%
Double virus	1	1%	7	8%
Treatment				
Number of cases with available data	47		55	
Cold coagulation	1	2%	0	–
Laser ablation*	15	32%	3	6%
Cone biopsy	21	45%	18	32%
LLETZ*	10	21%	34	62%
Follow up				
Mean	34		36	
Range	16–376		6–387	

LLETZ: large loop excision of the transformation zone.
* Significant difference between disease-free and disease persistence/progression groups.

ORCTL (organic cationic transporter-like 4) and XYLB (Xylukokinase homologue), DLEC1 (deleted in lung and esophageal cancer 1) may be relevant as it is associated with cancer. AD031, PR (progesterone receptor) and TRPC6 (transient receptor potential cation channel, superfamily C, member 6) are at or in the vicinity of D11S35. PR appears to be the most relevant given its role in inhibition of human endometrial cancer. Finally, TRIM29 (tripartite motif-containing 29) and several hypothetical genes are at D11S528. TRIM29 has multiple zinc finger motifs and a leucine zipper motif and may act as a transcriptional regulatory factor.

Discussion

We retrospectively investigated the prognostic value of LOH at 12 microsatellite markers from chromosomal regions including 3p14.2, 3p21–22, 6p21 and 11q23 in CIN and showed that combined LOH analysis at D3S1300, D3S1260, D11S35 and D11S528 could identify, with 100% specificity, 22–47% of CIN lesions depending on histological grade, which showed disease persistence/progression. The association of LOH at these loci with clinical behavior of CIN was further reinforced by analysis of follow-up biopsies in cases from the disease persistence/progression group. None of the five cases that became disease-free after the first follow-up biopsy exhibited accumulation of LOH in the follow-up specimens. In contrast, 13 of the 17 cases that had disease persistence or progression showed accumulation of LOH at additional loci in the follow-up specimens and in six cases the prognostic value of LOH reached the threshold of significance. The results strongly indicate that LOH analysis of follow-up specimens could further segregate CIN lesions with different clinical outcome. It remains to be determined when is the best interval to implement such analysis although our study showed that an average of 30 months was required for acquisition of an additional LOH at the four loci examined.

Infection of high-risk HPVs is significantly associated with increased risk of cervical cancer development. In support of this, our results showed that the percentage of infection of high-risk HPVs, particularly HPV16 and double viruses, was higher in the disease persistence/progression group than the disease-free group. Furthermore, HPV16 infection significantly correlated with LOH at each of the four markers examined, particularly D3S1300. HPV16 integrations in cervical cancers preferentially target common fragile sites including FRA3B (3p14.2) where the *FHIT* gene and D3S1300 are located, and are accompanied by deletion of cellular genes [33,34]. Thus, LOH at these loci may be directly associated with HPV16 infection in some cases.

The significant association of LOH at D3S1300 (3p14.2), D3S1260 (3p22.2), D11S35 (11q22.1) and D11S528 (11q23.3) with persistence of dysplasia 6 months after treatment strongly suggests that these markers may be at or in the vicinity of tumor suppresser genes. Bioinformatic analysis of the genomic region at these loci identified several genes including two known genes *FHIT* and *PR*, which could be the target of deletions.

FHIT, spanning the FRA3B fragile site and the breakpoint of *t*(3;8) of familial renal carcinoma, has been proposed as a tumor suppresser gene [35]. Deletion of the *FHIT* gene occurs in a wide range of human cancers including cervical cancer [30–32,36,37]. In CIN, LOH of the *FHIT* gene is associated with reduced expression of the protein and positively correlates with the histological grade [36,38,39]. Our results further indicate that deletion of the gene has prognostic value.

PR, encodes two isoforms, PR-A and PR-B, which have different transcription activation properties and play distinctive roles in different tissues [40]. PR-A functions as a transcriptional repressor and is critical for progesterone dependent reproductive responses in the uterus and ovary [40]. PR-A represses the activity of oestrogen receptor alpha [40], which may underlie the mechanisms of progesterone mediated antiproliferative effect in endometrial cancer. Deletion of the *PR* gene and loss of its expression are associated with aggressive endometrial cancer and epithelial ovarian tumors [41–43]. In cervical cancer, reduced PR expression

Table 3
Comparison of LOH and HPV status between diagnostic and follow-up specimens

Case no.[a]	Diagnosis	Time (year-month)	Treatment	HPV	D3S1300	D3S1260	D11S35	D11S528
1	CIN3	1995-6	cone biopsy	33	+	H	–	H
	CIN3	1996-3	cone biopsy	33	+	H	–	H
2	CIN2	1999-5	LLETZ	45	–	H	–	–
	CIN2	1999-12	NA	45	–	H	–	–
3	CIN1	1998-9	No	18	–	–	–	+
	CIN1	1999-12	NA	18	–	–	–	+
4	CIN1	1989-8	No	33	–	+	–	–
	CIN2	1990-6	LLETZ	33	–	+	–	–
5	CIN1	1997-10	LLETZ	33	–	–	H	–
	CIN1	1998-6	LLETZ	NA	–	–	H	–
6	CIN2	1994-11	LLETZ	33	–	–	+	–
	CIN2	1995-5	NA	33	–	–	+	–
7	CIN2	1998-11	No	16	–	+	–	–
	CIN3	2000-2	LLETZ	16	–	+	–	–
8	CIN1	1994-7	LLETZ	18	+	–	H	–
	CIN1	1995-3	NA	18	+	–	H	–
9	CIN2	1992-2	cone biopsy	18	–	H	–	–
	CIN2	1998-11	NA	18	–	H	–	+
10	CIN1	1997-9	NA	18	–	–	H	–
	CIN2	1999-4	NA	18	–	–	H	+
11	CIN1	1997-4	LLETZ	33	–	H	–	–
	CIN3	2000-9	cone biopsy	33	–	H	–	+
12	CIN1	1991-10	No	16	–	–	+	–
	CIN3	1995-1	LLETZ	16	+	–	+	–
13	CIN2	1995-7	No	16	+	–	–	–
	CIN3	1998-4	LLETZ	16	+	–	–	+
14	CIN1	1995-3	No	NA	–	–	–	–
	CIN1	1996-10	No	NA	–	+	+	+
15	CIN1	1989-4	No	16	+	–	–	+
	CIN3	1992-8	LLETZ	33	+	–	+	+
16	CIN2	1988-11	cone biopsy	16	–	–	–	+
	CIN3	1992-2	cone biopsy	16	–	+	+	+
17	CIN3	1991-5	cone biopsy	18	–	–	+	–
	CIN3	1994-11	cone biopsy	18+16	–	+	+	–
18	CIN2	1991-10	cone biopsy	16	+	H	–	–
	CIN3	1997-10	LLETZ	16+45	+	H	+	–
19	CIN3	1989-11	cone biopsy	16	+	–	+	–
	CIN3	1991-4	NA	16+18	+	+	+	–
20	CIN1	1989-6	NA	18	+	–	–	+
	CIN1	1989-12	LLETZ	18	+	–	+	+
21	CIN1	1990-4	NA	16	+	–	+	+
	CIN2	1994-4	NA	16	+	–	+	+
	CIN3	1994-9	NA	16	+	+	+	+
22	CIN3	1990-1	NA	33	–	+	+	–
	CIN3	1993-1	NA	33	NA	–	–	–

NA: not available; +: LOH positive; –: LOH negative; H: homozygous.
[a] Cases 1–5 became disease-free after the first follow-up biopsy; cases 7–21 continually showed disease persistence or progression after the first follow-up biopsy (data not included because of space limitation).

has been found in cervical carcinoma in comparison with normal cervix [44,45]. The finding of LOH at the vicinity of the PR gene in the present study warrants further study to investigate whether loss of PR function is involved in the development of cervical cancer.

In summary, we have identified four markers at which LOH is significantly associated with the clinical behavior of CIN. Combined analysis of LOH at these loci could identify at least a third of CIN lesions that persist despite treatment. In view of the enhanced power of combined LOH analysis of multiple loci, addition of other molecular markers of prognostic significance should further improve the sensitivity of the current assay and eventually allow development of a prognostic test for CIN. This will undoubtedly lead to improved management of patients with CIN lesions.

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