Type- and age-specific distribution of human papillomavirus in women attending cervical cancer screening in Finland

M K Leinonen*[,1], A Anttila[2], N Malila[1,3], J Dillner[4,5], O Forslund[4] and P Nieminen[6]

[1]Finnish Cancer Registry, Pieni Roobertinkatu 9, FI-00130 Helsinki, Finland; [2]Mass Screening Registry, Finnish Cancer Registry, Pieni Roobertinkatu 9, FI-00130 Helsinki, Finland; [3]Tampere School of Health Sciences, University of Tampere, Medisiinarinkatu 3, FI-33014 Tampere, Finland; [4]Department of Medical Microbiology, Lund University, Jan Waldenströms gata 59, 205 02 Malmö, Sweden; [5]Departments of Laboratory Medicine, Medical Epidemiology and Biostatistics, Karolinska Institute, SE-17177 Stockholm, Sweden and [6]Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Turuntie 150, FI-02740 Espoo, Finland

Background: Large-scale data on type-specific HPV prevalences and disease burden are needed to monitor the impact of HPV vaccination and to plan for HPV-based cervical screening.

Methods: 33 043 women (aged 25–65) were screened for HPV by a Hybrid Capture 2 (HC2) in a population-based programme. HPV-positive women ($n = 2574$) were triaged by cytology and HPV genotyped using PCR-Luminex. Type-specific prevalence of HPV infection and its correlation to findings in cytology triage and histology as well as Population Attributable Fractions for a referral to colposcopy and findings in histology were calculated.

Results: Among HC2-positive women, 61.5% had normal, 23.1% had ASC-US and 15.5% had LSIL or more severe (LSIL+) results in cytology. Out of HC2-positive samples, 57% contained the 13 Group 1/2A HPV types, which were targeted by the HC2, 15% contained Group 2B types, 8.5% Group 3 types and 30% were found to be negative in HPV genotyping. The proportion of samples positive for HPV by the HC2, but negative in HPV genotyping increased with age and decreased with increasing cytological abnormality. The most frequent types were HPV 16 (0.9% of screened women and 12.1% of the HC2-positive women), HPV 31 (0.7% and 8.9%, respectively) and HPV 52 (0.5% and 6.3%, respectively). The prevalence of Group 1/2A HPV types increased with increasing CIN grade and attributed 78.3% (95% CI 53.4–89.9) of the CIN 3+ lesions, while HPV 16 attributed 55.8% (40.0–67.5) of them.

Conclusion: The type-specific prevalence of HPV were slightly lower than the average in international meta-analyses. Genotyping for HPV 16 better identified women with CIN 3+ than cytology triage at the threshold of LSIL+. The high proportion of women that were HC2-positive but HPV-negative in genotyping suggests that HPV genotyping may be useful also for validation of results in HPV screening. The large-scale HPV genotyping data were found to be directly useful for planning further preventive efforts for cervical cancer.

Persistent infection with some types of human papillomavirus (HPV) is established as carcinogenic to human (Munoz *et al*, 2003; IARC, 2012). There are currently 12 HPV types classified as Group 1 carcinogens to human and one type that is probably carcinogenic (Group 2A carcinogen) (IARC, 2012). All of these belong to the same evolutionary branch in the phylogenetic tree of papillomaviruses, suggesting that the carcinogenicity reflects viral evolution (Schiffman *et al*, 2005). With increasing age, a larger proportion

of screen-detected HPV infections represent persistent HPV infections. However, it should be noted that some HPV types will never cause neoplastic progression, even if they do persist (Schiffman *et al*, 2005; Rodriguez *et al*, 2010).

HPV DNA testing has emerged as a highly sensitive screening test that can detect cervical precancerous lesions earlier than cytology (Naucler *et al*, 2007; Sargent *et al*, 2010; Rijkaart *et al*, 2012a). However, a low specificity of an HPV DNA test warrants a triage test, and a Pap smear following a positive HPV result seems to be the most attractive screening strategy in countries where cytology is of good quality (Cuzick *et al*, 2008; Kotaniemi-Talonen *et al*, 2008; Leinonen *et al*, 2009; Naucler *et al*, 2009; Rijkaart *et al*, 2012b). On the other hand, the interpretation of Pap smears is subjective, and there is an increasing interest towards more objective molecular tests. HPV genotyping has been suggested as a possible alternative option to cytology in triaging HPV-positive women. This approach allows stratifying women to an appropriate management depending on the specific carcinogenic HPV type present and its associated risk for development of cervical intraepithelial neoplasia grade 3 (CIN 3) or cancer (Sherman *et al*, 2003; Khan *et al*, 2005; Schiffman *et al*, 2005; Naucler *et al*, 2007; Kjaer *et al*, 2010; Guan *et al*, 2012).

HPV 16 and 18 are the most common types of HPVs found in ICC. Whereas the proportion of HPV 16 and 18-associated cancers appears rather constant worldwide, the relative importance of the other carcinogenic HPV types differs slightly by geographical region (de Sanjose *et al*, 2010; Li *et al*, 2011; Guan *et al*, 2012). The HPV type distribution in high-grade cervical lesions, that is, CIN 2 and 3, is not entirely representative of those in cervical cancer. HPV 16, 18 and 45 are more common in ICC than in the high-grade cervical lesions, but the other carcinogenic HPV types are more common in CIN 2 and 3 compared with ICC (Smith *et al*, 2007; Guan *et al*, 2012).

Data on the type-specific prevalence of carcinogenic HPV types are needed to monitor the impact of HPV vaccinations and to plan HPV-based cervical screening programmes. The aim of the study was to determine the type-specific prevalence of HPV infection and its correlation to findings in cytology triage and histology. The study also provides useful information on the performance and clinical validity of HPV screening using a Hybrid Capture 2 test (HC2, Qiagen Inc., Gaithersburg, MD, USA), in relation to the HPV genotypes present.

MATERIALS AND METHODS

In all, 108 327 women aged 25–65 years were invited for organised cervical screening in nine Finnish municipalities between 1 January 2003 and 31 December 2005. They were individually randomly assigned (1:1, using computer-generated random numbers) to either the primary HPV DNA testing group, followed by cytology triage ($n = 54 140$) or to the primary conventional cytological screening group ($n = 54 187$). Only women allocated to primary HPV screening were included in the present study. Women who had been randomised but died, emigrated or were diagnosed with invasive cervical cancer before the invitation ($n = 104$), were excluded. Women who did not attend ($n = 18 270$) were also excluded. The exact dates of the screening visits were not recorded in the screening register and the 1st calendar day of the visit month was used as an approximation.

At the screening visit, a cytological smear was taken with an Ayre's spatula, and cervical cells were collected with the sampler brush included in the HC2 test kit (instead of a regular cytobrush). The brush was then placed into a tube containing HC2 transport medium, which was subsequently processed with the supplies and reagents of the HC2 assay in two separate laboratories. The probe

mixture to detect 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) was used according to the manufacturer's instructions.

Cytological slides were stained and interpreted in a conventional manner, but cytology triage was used only for samples positive for the HPV DNA in the HC2 test, that is, when the ratio of relative light units (rlu ratio) was 1.00 or higher. Cytological diagnosis LSIL + (a low-grade squamous intraepithelial lesion or more severe) corresponding to earlier used Papanicolaou classes III–V resulted in a referral to colposcopy. ASC-US (atypical squamous cells of undetermined significance) and AGC-NOS (atypical glandular cells, not otherwise specified) findings (earlier Papanicolaou class II) or a normal result obtained in cytology triage resulted in an invitation to re-screening after 12 to 24 months.

After the screening visit, the cells were stored within the Standard Transport Medium (STM) buffer at $-20 \,^\circ C$. In the spring of 2010, all HC2-positive samples were retrieved from the archives and HPV genotyped. From each sample, 200 µl (STM) was treated overnight with proteinase K (20 µg/ml, Roche Diagnostics GmbH, Mannheim, Germany) at $37 \,^\circ C$. The DNA was then extracted with the Total NA-kit (Roche) by the use of MagNA Pure LC (200 µl input and 100 µl output). Sample adequacy was assessed by testing 5 µl of the sample for the human betaglobin gene with a real-time PCR (Sturegard *et al*, 2013). For the simultaneous identification of 38 genital HPV types , 5 µl of the extracted material was added to a total volume of 25 µl for Modified General Primer (MGP) PCR amplification and subsequent genotyping with Luminex (Schmitt *et al*, 2006; Soderlund-Strand *et al*, 2009). The Luminex assay included probes for HPV types 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91 and 114.

The classification of human carcinogens by the International Agency for Research on Cancer (IARC) was used by combining IARC Group 1 (including carcinogenic HPV types of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and Group 2A (probably carcinogenic HPV 68) as Group 1/2A for the current study. Possibly carcinogenic Group 2B are HPV types that have close phylogeny to the carcinogenic types, but have limited epidemiological evidence for carcinogenicity to humans. HPV 73 has an uncertain carcinogenicity and is not targeted by the HC2 assay, for which it was included in Group 2B in the current study. HPV types in Group 3 included those in IARC Group 3 or those, apart from HPV 73, that have not yet been classified (Doorbar *et al*, 2012; IARC, 2012).

The following databases were used to obtain data on cervical lesions: the Mass Screening Registry (MSR), the Finnish Cancer Registry (FCR) and the Care Registers for Social Welfare and Health Care (HDR in this paper). The highest grade cervical intraepithelial neoplasia recorded within 1 year after a screening visit that resulted in a referral to colposcopy was considered to be screen detected and was therefore included in the current study. This follows recommendation by the European guidelines for cervical cancer screening (Arbyn *et al*, 2008). The same diagnostic window was also allowed for women who were HPV-positive and had cytology ASC-US or normal at the screening visit. The women were individually linked to the three health-care registries using their personal forms of identification as previously described in detail (Leinonen *et al*, 2012). The diagnosis of invasive cervical cancer (ICC) was taken from the FCR only. Diagnoses of dysplasia gravis, carcinoma *in situ* (including adenocarcinoma in situ and epithelial neoplasm NOS in situ) or CIN 3 were grouped together as CIN 3 or AIS. CIN 3 or AIS diagnoses were available from all three registers, whereas CIN 1 (equal to dysplasia levis) and CIN 2 (equal to dysplasia moderata) were available from both the MSR or HDR.

Statistical analysis. Type-specific prevalence of HPV infection and their exact binomial 95% confidence intervals (CI) were calculated for women overall and stratified by age as well as by cytologic and histologic findings. Type-specific HPV prevalence included the presence of a given HPV type either alone or with other concomitant types. It was calculated as a proportion of women positive for a given type out of all HPV-tested women, including women testing negative for HPV in the HC2 assay. Poisson regression analyses were performed to evaluate the association between type-specific infection and a referral to colposcopy as well as findings in histology through relative rates with 95% CIs. Women not infected with a particular HPV type (i.e. women with other HPV infections or without any HPV type) were used as the referent group. Relative rate estimates were adjusted by including age as a continuous variable and type-specific HPV data as single variables in a multivariate regression model. Population attributable fractions (PAFs) were calculated as $p*(RR-1)/RR$ where p is the prevalence among cases and RR is the type-specific adjusted relative rate. 95% CIs for PAFs were defined from the variance of the log-transformed complement of PAFs [log $(1 - PAF)$] (Rothman et al, 2008). All analyses were conducted on a per protocol basis, and they were performed by using Stata (version 12.0, StataCorp, College Station, TX, USA).

Ethics. This randomised trial on public health policy is registered as an International Standard Randomised Controlled Trial (ISRCTN23885553). The National Authority for Medicolegal Affairs decided that written informed consent from the women was not required as the practice of the trial was considered routine and it also involved a very large number of women. The screening protocol and data collection were approved by the Ethical Committee of Obstetrics and Gynaecology in Hospital District of Helsinki and Uusimaa and the Health Boards of the committed municipalities. A separate permission for the trial extension was obtained from The National Authority for Medicolegal Affairs. This extension was used to analyse the formerly collected cervical screening samples for research purposes. The extended register-based study was also given approval by the ethical committee of the same local hospital district.

RESULTS

Overall, 35 766 women attended HPV screening in Finland during 2003–2005. In all, 33 043 (92.4%) of them were screened by using the HC2 test and are included in this study. The mean age of the study population was 45.2 years (ranging between 25 and 65 years). Eleven samples that were initially positive for HPV in the HC2 assay (HC2-positive) were not found in the archives and could not be genotyped. Of the 2611 samples that were genotyped, there were 80 (3.1%) samples with undetectable levels of human β-globin DNA, although 43 of the samples contained viral DNA. Thirty-seven samples with no amplification of either β-globin or HPV DNA were excluded (Figure 1).

In all, 2574 women (7.8%) were HPV-positive in the HC2 test. They underwent triage testing by cytology and HPV genotyping and were thus eligible for the final analyses. Of the HC2-positive women, 61.5% had normal, 23.1% had ASC-US and 15.5% had LSIL or more severe (LSIL +) result in cytology triage (Figure 1 and Table 1).

Almost one-third of the women who were HC2-positive tested negative in the HPV genotyping. The proportion of samples positive in the HPV screening but negative in the HPV genotyping tended to increase with age, ranging from 25% among 25 years old to over 50% among 65 years old (Table 2). Among samples HPV-positive in the HC2 test, 57.1% contained HPV genotypes belonging to carcinogenic Group 1/2A, that is, types targeted by

the HC2 test. Further, 15.3% of HC2-positive samples contained possibly carcinogenic HPV types (Group 2B) and 8.5% low-risk types (Group 3), although these types were not tested for by the HC2. When simultaneous infections with HPV types from a more carcinogenic group were excluded, prevalence of Group 2B and Group 3 HPV types were 10.3% and 2.6%, respectively. Among HC2-positive women, a second peak in prevalence within Group 2B and Group 3 of HPV types could be detected in middle-aged and older women (Table 2).

The most common HPV type was HPV 16, with a prevalence of 0.9% (95% CI: 0.8–1.1) among the Finnish screening population and 12.2% (10.9–13.5) among HC2-positive samples. HPV 31 was the next frequent type with a prevalence of 0.7% (95% CI: 0.6–0.8) and 8.9% (7.9–10.1), respectively. The third most common HPV type was the HPV 52 with a prevalence of 0.5% (95% CI: 0.4–0.6) among the screened women and 6.3% (5.4–7.3) in HC2 positives. A large majority of the Group 1/2A HPV types each accounted for between 4 and 5% of all HC2-positive samples. HPV 35, 39 and 59 made an exception to this rule, as these were less frequently detected. HPV 16 and related HPV types in the HPV species α9 accounted for 38.4% of HC2-positive samples and 54.9% of samples positive in HPV genotyping. The age-specific prevalence of HPV types among the screened women is shown in Figure 2.

Less than 10% of the HC2-positive women who also had clearly abnormal cytology were found to be negative by the HPV genotyping, whereas among the HC2-positive women who had normal cytology close to 40% (Figure 1) were negative in HPV genotyping. The prevalence of Group 1/2A HPV types increased with increasing cytology, from around 50% of the HC2-positive women with normal cytology to almost 80% of HC2-positive women with abnormal cytology, triggering a referral to colposcopy. When samples with concomitant infection with Group 1/2A HPV types were excluded, infections with Group 2B HPV types were most frequent among women with ASC-US cytology and Group 3 HPV types (in the absence of group 1/2A/ 2B types) were most often associated with normal cytology (Table 3). In general, there was a slight difference in type-specific prevalence of carcinogenic HPV types among HC2-positive women with normal compared with ASC-US cytology. The prevalence of HPV 16 and other types of the α9 species were the highest among women with LSIL + cytology. The only exceptions were HPV 52 and 58, which were more frequent among women with ASC-US (Table 3).

The likelihood of CIN 1+ was 54.5% among women with LSIL + cytology and 0.7% among women who attended the screening. This included 21 patients diagnosed with CIN within 12 months following screening after either ASC-US or a normal result in cytology triage. The proportion of infections with Group 1/2A HPV types increased with increasing CIN grade. Indeed, 90.5% of the women with CIN 3 or cancer lesions were found to be positive for Group 1/2A HPV types. When considered as the most severe infections detected, the IARC Group 2B types were over-represented among women with CIN 1 or no lesion in colposcopy and the proportion of infections with only Group 3 HPV types remained stable regardless of the severity of lesion. In general, there was no difference in type-specific HPV prevalences among women with CIN 1 lesion or a negative result in colposcopy (Table 4).

The prevalence of both HPV 16 and 52 increased with increasing CIN grade (60.3% in CIN 3+ vs 15.1% in CIN 1 and 7.9% vs 1.4% for HPV 16 and HPV 52, respectively), whereas the opposite was true for HPV 51 (1.5% in CIN 3+ vs 18.3% in CIN 1). There were three cancers detected, two adenocarcinomas and one microinvasive squamous cell carcinoma. Two of the cancers were positive for HPV 16 and the third was positive for HPV 45.
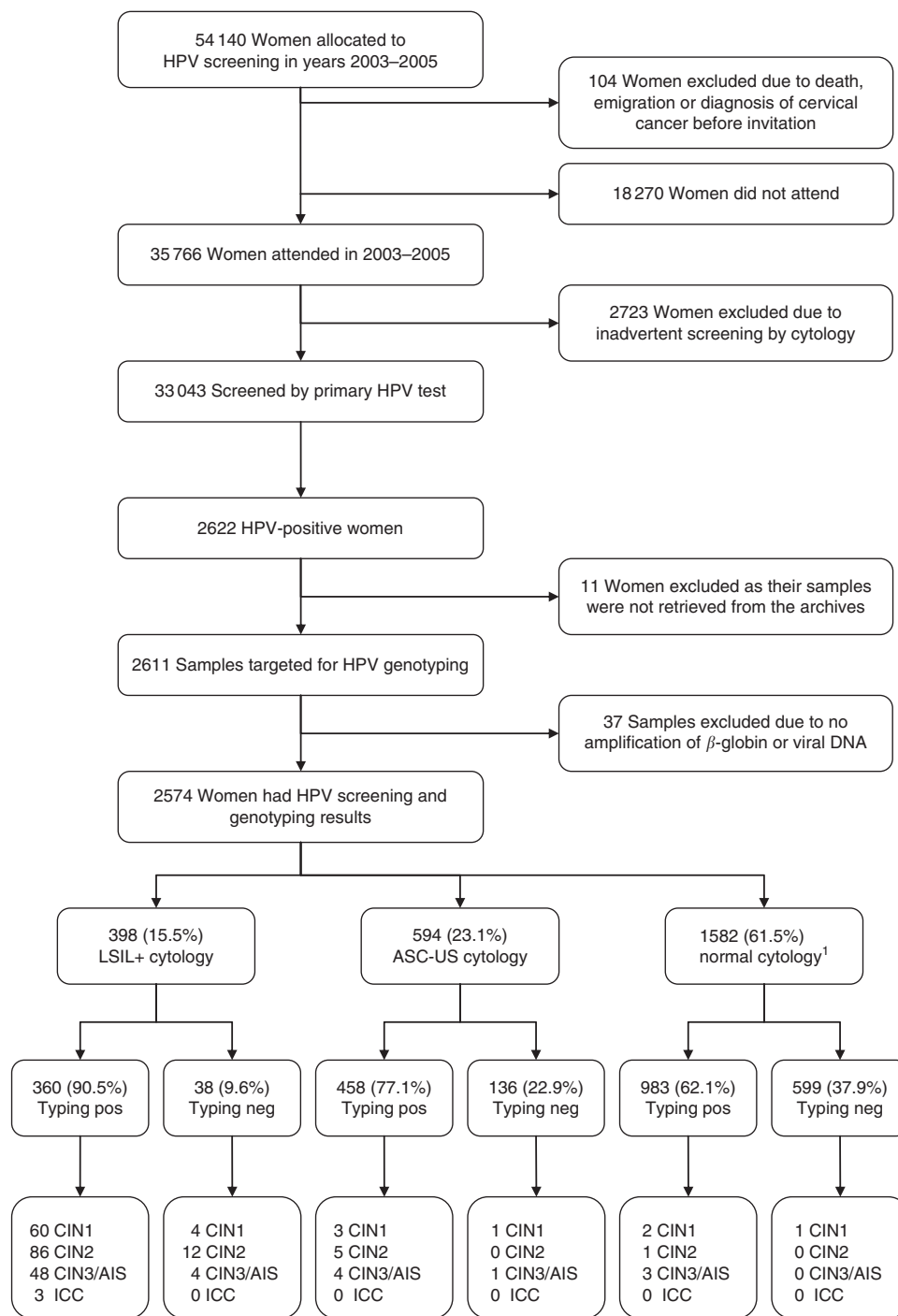
Figure 1. Flowchart of the women invited for HPV screening in 2003–2005. Results of HPV screening and genotyping, cytology triage and histology at the baseline screening visit. [1]Including seven women whose cytology triage test was missing or unsatisfactory. AIS = adenocarcinoma in situ; ASC-US = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia; HC2 = Hybrid Capture 2; HPV = human papillomavirus; ICC = invasive cervical cancer; LSIL + = low-grade squamous intraepithelial lesion or worse.

Group 1/2A HPV types attributed 78.3% of all CIN 3 + cases. Among these, HPV 16 was the most important single type attributing 55.8% of all CIN 3 + cases and 19.5% of all colposcopy referrals. This was followed by HPV 31 (attributing 6.5% vs 6.6%, respectively). HPV 52 had no significant role in the burden of colposcopy referrals but was the third most important HPV type in CIN 3 + cases with a PAF of 3.8%. HPV types of Group 2B attributed about 6% of colposcopy referrals but were not attributing any high-grade cervical lesions. Group 3 HPV types had no significant role either in colposcopy referrals or in cervical lesions (Table 5).

DISCUSSION

The present study has provided a large-scale determination of the type-specific HPV prevalences in Finland and their relative contribution to the cervical disease burden. The three most

Table 1. Age-specific results of primary HPV screening with the HC2 test and cytology triage test among eligible women who attended HPV screening trial in years 2003–2005

| | HPV screening | | | | Cytology triage[a] | | | | | |
| | Total | Negative | | Positive | | Normal | | ASC-US[b] | | LSIL+[c] | |
Age group (y)	n	n	%	n	%	n	% of HC2+	n	% of HC2+	n	% of HC2+
25	1 724	1 307	75.8	417	24.2	258	61.7	97	23.2	62	14.8
30	3 724	3 174	85.2	550	14.8	321	58.3	132	24.0	97	17.6
35	3 900	3 531	90.5	369	9.5	194	52.3	99	26.7	76	20.5
40	4 649	4 347	93.5	302	6.5	167	55.1	74	24.4	61	20.1
45	4 335	4 112	94.9	223	5.1	145	64.7	52	23.2	26	11.6
50	4 575	4 342	94.9	233	5.1	154	65.8	58	24.8	21	9.0
55	4 885	4 629	94.8	256	5.2	182	70.8	46	17.9	28	10.9
60	3 988	3 798	95.2	190	4.8	139	72.0	28	14.5	23	11.9
65	1 215	1 181	97.2	34	2.8	22	64.7	8	23.5	4	11.8
Total	32 995	30 421	92.3	2574	7.8	1582	61.5	594	23.1	398	15.5

Abbreviations: HC2 = Hybrid Capture 2; HC2+ = positive result in the HC2 test.
[a]Cytology triage missing or smear unsatisfactory for seven women who were included and cytology result was assumed to be normal.
[b]Papanicolaou class II.
[c]Papanicolaou class III-V, small proportion of II.

Table 2. Age-specific results of HPV genotyping by HPV carcinogenicity categories among women positive in HPV screening

| | PCR test | | | | | HPV classification[a] | | | | | |
| | Total | Negative | | Any HPV | | Group 1/2A[b] | | Group 2B[c] | | Group 3[d] | |
Age group (y)	n	n	% of HC2+	n	% of HC2+	n	% of HC2+	n	% of HC2+	n	% of HC2+
25	417	105	25.2	312	74.8	273	65.3	27	6.5	12	2.9
30	550	136	24.7	414	75.3	361	65.5	44	8.0	9	1.6
35	369	104	28.1	265	71.8	220	59.3	37	10.0	8	2.2
40	302	66	21.9	236	78.1	190	62.7	42	13.9	4	1.3
45	223	84	37.7	139	62.3	106	47.3	31	13.8	2	0.9
50	233	76	32.6	157	67.4	118	50.4	32	13.7	7	3.0
55	256	104	40.6	152	59.4	109	42.4	28	10.9	15	5.9
60	190	80	42.1	110	57.9	80	41.5	21	10.9	9	4.7
65	34	18	52.9	16	47.1	14	41.2	2	5.9	0	—
Total	2 574	773	30.0	1 801	70.0	1 471	57.1	264	10.3	66	2.6

[a]On the basis of the classification of carcinogens to humans by the International Agency for Research on Cancer (IARC), Group 2B including HPV 73 in the current study.
[b]Infection with one ore more Group1/2A type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with HPV types of Group 2B and / or Group 3 allowed.
[c]Infection with one or more Group 2B HPV type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with HPV types of Group 3 allowed.
[d]Infection with one or more Group 3 HPV type (6, 7, 9, 11, 40, 42, 43, 61, 74, 81, 83, 86, 87, 89, 90, 91 and 114).

prevalent carcinogenic HPV types in the Finnish screening population were HPV 16 (0.9%), HPV 31 (0.7%) and HPV 52 (0.5%). The prevalence of HPV 16 in our study was slightly lower than reported in the meta-analysis of women with normal cytology in Northern and Western European countries (de Sanjose et al, 2007). HPV 16 and its related α9 types accounted for 38% of all HPV infections among HC2-positive samples and almost 55% of the high-risk HPV infections detected by HPV genotyping. A similar HPV type distribution was reported from Greece using genotyping with restriction fragment length polymorpishm-PCR of HC2-positive samples (Agorastos et al, 2009). HPV 52 occurrence among women with normal cytology ranks second in Africa and third in Asia and worldwide. It ranks third also in Denmark but is not among the most frequent genotypes for most of Europe (de Sanjose et al, 2007; Kjaer et al, 2008; Bruni et al, 2010; Guan et al, 2012). HPV 18 is the second most common type in Europe, but showed a somewhat lower prevalence in our study. Similar findings have been reported from countries of Eastern Europe (Bardin et al, 2008; Shipitsina et al, 2011; Ucakar et al, 2012), suggesting that the HPV type distribution found in Finland is consistent with the regional HPV distribution in the world.
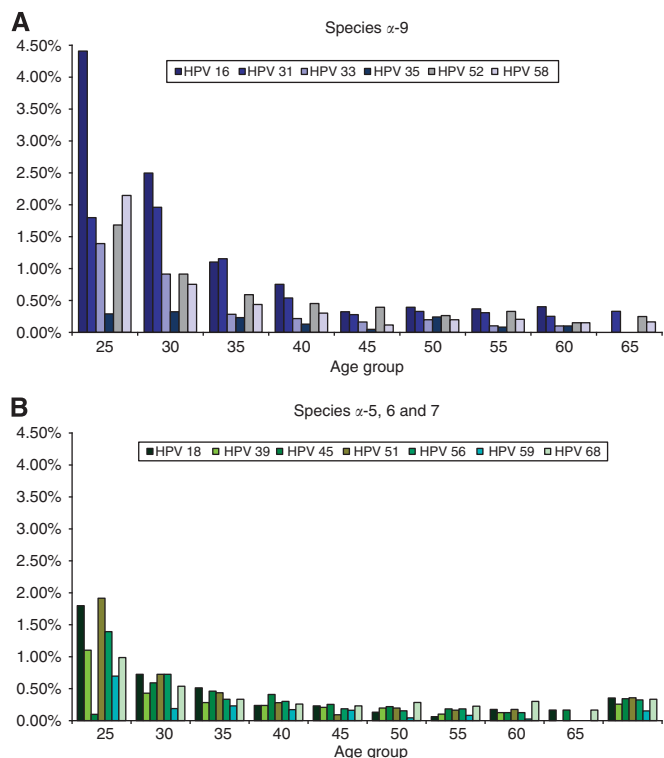
Figure 2. (A,B) Age-specific distribution of the human papillomavirus types in women attending cervical cancer screening in Finland in 2003–2005. HPV = human papillomavirus.

The HPV prevalence in ICC has been reported to be 89.9% worldwide, and it has increased significantly over time because of increased sensitivity of the detection methods (Li *et al*, 2011). HPV 16 and HPV 18 account for more than 70% of invasive cervical cancers worldwide, and the next most common types (HPV 31, 33, 35, 45, 52 and 58) combined together account for about 20% of the global cervical cancer burden (de Sanjose *et al*, 2010; Li *et al*, 2011). In Europe, the five most commonly found HPV types in women with ICC are 16, 18, 31, 33 and 45. Among patients with CIN 2 and 3, HPV 16, 31, 33, 18, and 58 are the most common (Smith *et al*, 2007; Li *et al*, 2011; Guan *et al*, 2012). In Finland, the most prevalent HPV types and their proportions in CIN 3 + lesions were similar to those in worldwide meta-analyses except for a rather low prevalence of HPV 18 and 45.

A Swedish study on HPV triaging of women with low-grade cervical cytology found no increased risk for CIN 3 among women positive for HPV 18 and HPV 45 in comparison with no HPV triaging at all (Soderlund-Strand *et al*, 2011). However, compared with normal cytology, there is a clear association between HPV 18, 45 and HSIL cytology (Arbyn *et al*, 2009). Also, a worldwide study of 10 575 cases of invasive cervical cancer concluded that HPV-DNA-based screening should focus on HPV types 16, 18 and 45 (de Sanjose *et al*, 2010). The most recent meta-analysis of 115 789 HPV-positive women suggested that studies using CIN 3 as the principal outcome underestimate the relative carcinogenic potential of HPV 18 and 45 (Guan *et al*, 2012). HPV 18 is the second most common HPV type in ICC and preferentially found in adenocarcinoma (de Sanjose *et al*, 2010). New data indicate that HPV 16 and 18 are about equally important in ADC (de Sanjose *et al*, 2010; Li *et al*, 2011).

Table 3. Type-specific HPV prevalences with exact binomial 95% confidence intervals (CI) by cytology triage test result

	Cytology triage							
	Normal (*n* = 1582)		ASC-US (*n* = 594)		LSIL + (*n* = 398)		Total (*n* = 2574)	
Type(s)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
HPV 16	143	9.0 (7.7–10.6)	58	9.8 (7.5–12.4)	112	28.1 (23.8–32.8)	313	12.2 (10.9–13.5)
HPV 18	64	4.0 (3.1–5.1)	29	4.9 (3.3–6.9)	24	6.0 (3.9–8.8)	117	4.5 (3.8–5.4)
HPV 31	117	7.4 (6.2–8.8)	60	10.1 (7.8–12.8)	53	13.3 (10.1–17.1)	230	8.9 (7.9–10.1)
HPV 33	52	3.3 (2.5–4.3)	23	3.9 (2.5–5.8)	29	7.3 (4.9–10.3)	104	4.0 (3.3–4.9)
HPV 35	24	1.5 (1.0–2.2)	18	3.0 (1.8–4.7)	11	2.8 (1.4–4.9)	53	2.1 (1.5–2.7)
HPV 39	45	2.8 (2.1–3.8)	21	3.5 (2.2–5.4)	19	4.8 (2.9–7.4)	85	3.3 (2.6–4.1)
HPV 45	68	4.3 (3.4–5.4)	30	5.1 (3.4–7.1)	15	3.8 (2.1–6.1)	113	4.4 (3.6–5.2)
HPV 51	51	3.2 (2.4–4.2)	31	5.2 (3.6–7.3)	36	9.0 (6.4–12.3)	118	4.6 (3.8–5.5)
HPV 52	96	6.1 (4.9–7.4)	44	7.4 (5.4–9.8)	21	5.3 (3.3–8.0)	161	6.3 (5.4–7.3)
HPV 56	49	3.1 (2.3–4.1)	31	5.2 (3.6–7.3)	27	6.8 (4.5–9.7)	107	4.2 (3.4–5.0)
HPV 58	66	4.1 (3.2–5.2)	41	6.9 (5.0–9.2)	21	5.3 (3.3–8.0)	128	5.0 (4.2–5.9)
HPV 59	30	1.9 (1.3–2.7)	13	2.2 (1.2–3.7)	7	1.8 (0.7–3.6)	50	1.9 (1.4–2.6)
HPV 68	75	4.7 (3.7–5.9)	25	4.2 (2.7–6.2)	10	2.5 (1.2–4.6)	110	4.3 (3.5–5.1)
Group 1/2A[a]	777	49.1 (46.6–51.6)	376	63.3 (59.3–67.2)	318	79.9 (75.6–83.7)	1471	57.1 (55.2–59.1)
Group 2B[b]	153	9.7 (8.3–11.2)	74	12.5 (9.9–15.4)	37	9.3 (6.6–12.6)	264	10.3 (9.1–11.5)
Group 3[c]	53	3.4 (2.5–4.4)	8	1.3 (0.6–2.6)	5	1.3 (0.4–2.9)	66	2.6 (2.0–3.3)
Any HPV	983	62.1 (59.7–64.5)	458	77.1 (73.5–80.4)	360	90.5 (87.1–93.2)	1801	70.0 (68.2–71.7)

[a]Infection with one or more Group 1/2A HPV type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with HPV types of Group 2B and/or Group 3 allowed.
[b]Infection with one or more Group 2B HPV type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with HPV types of Group 3 allowed.
[c]Infection with one or more Group 3 HPV type (6, 7, 9, 11, 40, 42, 43, 61, 74, 81, 83, 86, 87, 89, 90, 91 and 114).

Table 4. Type-specific HPV prevalences with exact binomial 95% confidence intervals (CI) by histologically confirmed cervical lesion

	Histological diagnosis							
	No lesion (n = 181)		CIN 1 (n = 71)		CIN 2 (n = 104)		CIN 3 + (n = 63)	
Type(s)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
HPV 16	28	15.5 (10.5–21.6)	11	15.5 (8.0–26.0)	40	38.5 (29.1–48.5)	38	60.3 (47.2–72.4)
HPV 18	12	6.6 (3.5–11.3)	4	5.6 (1.6–13.8)	6	5.8 (2.1–12.1)	2	3.2 (0.4–11.0)
HPV 31	20	11.0 (6.9–16.5)	4	5.6 (1.6–13.8)	24	23.1 (15.4–32.4)	8	12.7 (5.6–23.5)
HPV 33	13	7.2 (3.9–12.0)	5	7.0 (2.3–15.7)	6	5.8 (2.1–12.1)	5	7.9 (2.6–17.6)
HPV 35	4	2.2 (0.6–5.6)	2	2.8 (0.3–9.8)	4	3.8 (1.1–9.6)	1	1.6 (0.04–8.5)
HPV 39	11	6.1 (3.1–10.6)	3	4.2 (0.9–11.9)	4	3.8 (1.1–9.6)	1	1.6 (0.04–8.5)
HPV 45	8	4.4 (1.9–8.5)	3	4.2 (0.9–11.9)	3	2.9 (0.6–8.2)	1	1.6 (0.04–8.5)
HPV 51	19	10.5 (6.4–15.9)	13	18.3 (10.1–29.3)	4	3.8 (1.1–9.6)	1	1.6 (0.04–8.5)
HPV 52	10	5.5 (2.7–9.9)	1	1.4 (0.04–7.6)	7	6.7 (2.7–13.4)	5	7.9 (2.6–17.6)
HPV 56	18	9.9 (6.0–15.3)	5	7.0 (2.3–15.7)	2	1.9 (0.2–6.8)	2	3.2 (0.4–11.0)
HPV 58	11	6.1 (3.1–10.6)	4	5.6 (1.6–13.8)	7	6.7 (2.7–13.4)	3	4.8 (1.0–13.3)
Group 1/2 A[a]	136	75.1 (68.2–81.3)	51	71.8 (59.9–81.9)	88	84.6 (76.2–90.9)	57	90.5 (80.4–96.4)
Group 2B[b]	23	12.7 (8.2–18.5)	13	18.3 (10.1–29.3)	4	3.8 (1.1–9.6)	1	1.6 (0.04–8.5)
Any HPV	163	90.1 (84.7–94.0)	65	91.5 (82.5–96.8)	92	88.5 (80.7–93.9)	58	92.1 (82.4–97.4)

[a]Infection with one or more Group 1/2A HPV type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with HPV types of Group 2B and/or Group 3 allowed.
[b]Infection with one or more Group 2B HPV type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with HPV types of Group 3 allowed.

Table 5. Type-specific relative rates (RRs) and Population Attributable Fractions (PAFs) of referral to colposcopy and histologically confirmed cervical lesions by the most important HPV types

	Colposcopy indicated		CIN 2 +		CIN 3 +	
Type(s)	RR[a] (95% CI)	PAF (95% CI)	RR[a] (95% CI)	PAF (95% CI)	RR[a] (95% CI)	PAF
HPV 16	3.27 (2.59–4.11)	19.5% (14.4–24.3)	7.27 (5.23–10.10)	40.3% (31.1–48.2)	13.5 (7.75–23.4)	55.8% (40.0–67.5)
HPV 18	1.66 (1.09–2.53)	2.4% (0–4.8)	1.51 (0.73–3.12)	1.6% (0–4.9%)	1.26 (0.30–5.25)	0.7% (0–5.0)
HPV 31	1.98 (1.47–2.66)	6.6% (2.9–10.1)	3.02 (2.03–4.50)	12.8% (6.1–19.1)	2.04 (0.96–4.36)	6.5% (0–14.9)
HPV 52	1.03 (0.66–1.60)	0.1% (0–2.4%)	1.47 (0.80–2.68)	2.3% (0–6.4)	1.92 (0.74–4.96)	3.8% (0–10.6)
Group 1/2A[b]	3.15 (2.44–4.05)	54.5% (44.5–62.7)	4.71 (2.98–7.44)	68.4% (53.2–78.7)	7.45 (3.17–17.5)	78.3% (53.4–89.9)
Group 2B[c]	1.50 (1.15–1.96)	5.9% (1.5–10.1)	0.86 (0.51–1.44)	NA	0.75 (0.30–1.90)	NA
Group 3[d]	1.15 (0.82–1.61)	1.3% (0–4.5)	0.43 (0.20–0.93)	NA	NA	NA
Any HPV[e]	3.95 (2.82–5.53)	67.6% (56.1–76.0)	3.60 (2.18–5.96)	64.9% (44.8–77.6)	5.11% (2.04–12.8)	74.0% (39.7–88.8)

Abbreviation: NA = not applicable.
[a]Association between type-specific infection and outcome by Poisson regression. Type-specific RRs adjusted for age and for coinfection with all single Group 1/2A HPV types and pooled Group 2B and Group 3 HPV types, unless otherwise specified.
[b]Infection with one or more Group 1/2A HPV type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with HPV types of Group 2B and / or Group 3 allowed. Adjusted for age and Group 2B and Group 3 HPV types.
[c]Infection with one or more Group 2B HPV type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with HPV types of Group 3 allowed.
[d]Infection with one or more Group 3 HPV type (6, 7, 9, 11, 40, 42, 43, 61, 74, 81, 83, 86, 87, 89, 90, 91 and 114).
[e]Infection with one or more HPV of any type. Adjusted for age.

The population attributable fraction (PAF) is the proportional reduction in incidence of a disease that would occur if exposure to a risk factor, for example, particular HPV type, was eliminated from the population. HPV-associated PAFs in Finland have previously been estimated using HPV serology. The study reported that HPV 16 was responsible for 61% of SCCs but only 6% of CIN 3 lesions (Laukkanen et al, 2010). We estimated HPV 16-associated PAF to be 55.8% in CIN 3 +, but could not estimate the PAF for cervical cancer because of too few cases. Our PAF estimate for the role of HPV 16 in CIN 3 + is similar to the serology-based PAF estimate for the role of HPV 16 in SCC. The difference in PAF

estimates for HPV 16 in CIN 3 is most likely because of the different methods used to assess the HPV exposure (serology vs HPV genotyping).

There is an inverse relationship between HPV prevalence and age with a peak at younger ages (< 25 years of age) and then a monotonic decline at older ages. However, a second prevalence peak from 45 years onwards has been reported in Latin America, the Caribbean and in most countries across Africa (Bruni et al, 2010). In our study, HPV prevalences for most of the single types declined monotonously with age. However, the peak prevalence of Group 2B HPV types was seen among women aged between 40

and 50 years. There was also a second peak of Group 3 genotypes from age 50 onwards among HC2-positive women. The reasons for a bimodal curve are unclear, but it has been speculated that Pap smear taking may induce cell-mediated immunity, which could protect against subsequent HPV infections (Passmore *et al*, 2007). Sexual behaviour, viral characteristics and host susceptibility may be other contributing factors to the second peak (Bruni *et al*, 2010). A large cohort study found that HPV positivity at older ages does not result in cytologic abnormalities in the same way as it does at younger ages (Kovacic *et al*, 2006). Our finding in relation to HC2-positive women supports this notion.

Similar to other studies, the prevalence of HPV 16 and Group 1/2A HPV types increased with increasing CIN grade (Kjaer *et al*, 2008; Guan *et al*, 2012). Low-grade cervical lesions are merely a manifestation of viral infection and can also be caused by low-risk HPV types. We also found that none of the women with a high-grade cervical lesion had only low-risk (Group 3) HPV types. Only five CIN 2 + lesions were detected with possibly carcinogenic HPV types (Group 2B). Our prevalence of low-risk types was similar among women with normal cytology but slightly lower in mild cervical atypia and CIN 1 than reported in the large population-based study conducted in Denmark (Kjaer *et al*, 2008).

An important issue in HPV screening is how to triage HPV-positive women with normal cytology. These women have a risk of CIN 3 or cancer over 5–10 years that is even comparable to women with high-grade cytology at the screening visit (Sherman *et al*, 2003; Dillner *et al*, 2008; Katki *et al*, 2011). For HPV-positive women with normal cytology, HPV genotyping for the most carcinogenic HPV types such as HPV 16 and 18 could offer a useful way to triage women for colposcopy. Alternatively, women who are HPV 16 and 18-positive could be subjected to a more intensive follow-up (6 to 12 months). All others could be managed with repeated HPV testing (Meijer *et al*, 2006; Huh *et al*, 2010). Interestingly, cancers that are diagnosed following HPV + /cytology − results often have a glandular component and are diagnosed at an early stage when the disease is still potentially curable. This suggests that a screening algorithm of HPV + /cytology − women should therefore also include, for example, an endocervical curettage (ECC) to search for ADC (Kinney *et al*, 2011). We need longitudinal data to compare the risk assessment ability of HPV screening with cytology triage to that of HPV screening with triaging by genotyping.

When the vaccinated young birth cohorts will enter into screening programmes in the future, the prevalence of cervical abnormalities will decrease, which certainly leads to a decline in performance of subjective Pap tests because of the monotony of reading negative smears. However, HPV screening that is followed by Pap triaging will increase the proportion of smears that have a high probability of containing relevant abnormalities, and thus seems to be an attractive screening strategy also during the vaccine era (Huh *et al*, 2010). HPV 16 is a major driver of sensitivity and a positive predictive value of the HC2 test for high-grade cervical lesions. Further, the cumulative incidence of CIN 3 + related to HPV types other than HPV16 and 18 is only 3.0% among HC2-positive women (Khan *et al*, 2005). It has been shown that the positive predictive values of cytology and HPV testing decrease when HPV16 and 18 vaccinated cohorts enter into the screening programmes, and they might warrant tailored screening algorithms (Coupe *et al*, 2008).

We found a high proportion of samples that were HPV-positive in the HC2 test but not in HPV genotyping, particularly among women with normal cytology. These could be false positives or may represent cross-reactions with non-carcinogenic HPV types. This drawback of the HC2 test has also been reported by others (Castle *et al*, 2008; Sargent *et al*, 2010). Two studies have reported that by increasing the cutoff level from the FDA-approved of 1.0 to 2 or 3 relative light units (RLU/Co ratio) greatly decreases the HPV prevalence among women with normal or mild cytological

abnormalities and reduces false positive results (Hesselink *et al*, 2006; Sargent *et al*, 2010).

Strenghts and limitations. The current study is an extension of the randomised Finnish HPV screening trial, which has been executed within the national organised cervical cancer screening programme. We previously reported that the women attending HPV screening were comparable to women attending conventional cytology screening in terms of age, place of residence, screening laboratory, marital status and parity (Leinonen *et al*, 2009). However, there are also limitations. We did not genotype HC2-negative samples. We do not expect that typing also the over 30 000 samples negative for HPV by the FDA-approved HC2 test would have markedly affected the HPV type-specific prevalence estimates. Because the HC2 test used for screening targets only HPV types in Group 1/2A, our detection of HPV genotypes in Group 2B and Group 3 may represent cross-reactivity of the HC2 test. Thus, our data on Group 2B and 3 HPV types should not be used for estimating type-specific prevalences in the general screening population.

In ~3% of the samples, a human β-globin DNA could not be amplified. We did not do HPV genotyping directly after HPV screening, but the samples were first stored in the STM buffer at − 20 °C for 5–7 years. Although long-term storage may affect the integrity of nucleic acids (Holland *et al*, 2003; Anchordoquy, Molina, 2007), the proportion of inadequate samples was comparable to results when testing freshly taken samples (Forslund *et al*, 2002). Also, a pilot study for a small subset of samples conducted 2 years earlier found similar results

The role of multiple infections was not assessed in the current study. The regression model estimates were adjusted for all the HPV types, and women not infected with a particular HPV type (i.e. women with other HPV infections or without any HPV type) were used as the referent group. This is a suitable approach for estimating the PAFs as eliminating one HPV type, for example, by vaccination, does not imply that there would be no HPV infections at all in the population. However, a role of multiple infections is evident in the PAFs, as the PAF of CIN 3 + for any HPV type (the regression model adjusted only for age) was lower than that for Group 1/2A HPV types (adjusted for age, Group 2B and Group 3 HPV types).

CONCLUSIONS

HPV 16 and related α9 types accounted for 38% of samples positive in HPV screening and more than 50% of the types detected by HPV genotyping. Prevalence of Group 1/2A HPV types increased with increasing CIN grade and attributed to almost 80% of all CIN 3 + lesions, mainly because of HPV 16. Genotyping for HPV 16, as compared with cytology triage at the threshold of LSIL +, works better to identify women at risk for CIN 3 +. The high proportion of HC2-positive results that could not be confirmed by HPV genotyping, particularly among women with normal cytology, suggest that HPV genotyping can be used to evaluate performance and clinical validity of HPV screening tests. To conclude, this study provides a substantial amount of new information directly useful for planning preventive efforts for cervical cancer.

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REFERENCES

Agorastos T, Lambropoulos AF, Sotiriadis A, Mikos T, Togaridou E, Emmanouilides CJ (2009) Prevalence and distribution of high-risk human papillomavirus in Greece. *Eur J Cancer Prev* **18**: 504–509.

Anchordoquy TJ, Molina MC (2007) Preservation of DNA. *Cell Preserv Technol* **5**: 180–188.

Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, Wiener HG, Herbert A, Daniel J, von Karsa L (eds) (2008) European guidelines for quality assurance in cervical cancer screening 2nd edn. 1–291. European Communities: Belgium.

Arbyn M, Benoy I, Simoens C, Bogers J, Beutels P, Depuydt C (2009) Prevaccination distribution of human papillomavirus types in women attending at cervical cancer screening in Belgium. *Cancer Epidemiol Biomarkers Prev* **18**: 321–330.

Bardin A, Vaccarella S, Clifford GM, Lissowska J, Rekosz M, Bobkiewicz P, Kupryjanczyk J, Krynicki R, Jonska-Gmyrek J, Danska-Bidzinska A, Snijders PJ, Meijer CJ, Zatonski W, Franceschi S (2008) Human papillomavirus infection in women with and without cervical cancer in Warsaw, Poland. *Eur J Cancer* **44**: 557–564.

Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S (2010) Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* **202**: 1789–1799.

Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S, Schiffman M (2008) Human papillomavirus genotype specificity of hybrid capture 2. *J Clin Microbiol* **46**: 2595–2604.

Coupe VM, Berkhof J, Bulkmans NW, Snijders PJ, Meijer CJ (2008) Age-dependent prevalence of 14 high-risk HPV types in the Netherlands: implications for prophylactic vaccination and screening. *Br J Cancer* **98**: 646–651.

Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH, Dillner J, Meijer CJ (2008) Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine* **26**(Suppl 10): K29–K41.

de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, Bosch FX (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis* **7**: 453–459.

de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menendez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andujar M, Castellsague X, Sanchez GI, Nowakowski AM, Bornstein J, Munoz N, Bosch FX (2010) Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* **11**: 1048–1056.

Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C, de Sanjose S, Naucler P, Lloveras B, Kjaer S, Cuzick J, van Ballegooijen M, Clavel C, Iftner T (2008) Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. *BMJ* **337**: a1754.

Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA (2012) The biology and life-cycle of human papillomaviruses. *Vaccine* **30**(Suppl 5): F55–F70.

Forslund O, Antonsson A, Edlund K, van den Brule AJ, Hansson BG, Meijer CJ, Ryd W, Rylander E, Strand A, Wadell G, Dillner J, Johansson B (2002) Population-based type-specific prevalence of high-risk human papillomavirus infection in middle-aged Swedish women. *J Med Virol* **66**: 535–541.

Guan P, Howell-Jones R, Bruni L, de Sanjose S, Franceschi S, Clifford GM (2012) Human papillomavirus types in 115,789 HPV-positive women:

A meta-analysis from cervical infection to cancer. *Int J Cancer* **131**: 2349–2359.

Hesselink AT, Bulkmans NW, Berkhof J, Lorincz AT, Meijer CJ, Snijders PJ (2006) Cross-sectional comparison of an automated hybrid capture 2 assay and the consensus GP5 + /6 + PCR method in a population-based cervical screening program. *J Clin Microbiol* **44**: 3680–3685.

Holland NT, Smith MT, Eskenazi B, Bastaki M (2003) Biological sample collection and processing for molecular epidemiological studies. *Mutat Res* **543**: 217–234.

Huh W, Einstein MH, Herzog TJ, Franco EL (2010) What is the role of HPV typing in the United States now and in the next five years in a vaccinated population? *Gynecol Oncol* **117**: 481–485.

IARC (2012) Biological agents. *IARC Monogr Eval Carcinog Risks Hum* **100B**: 1–498. IARC Press: Lyon.

Katki HA, Kinney WK, Fetterman B, Lorey T, Poitras NE, Cheung L, Demuth F, Schiffman M, Wacholder S, Castle PE (2011) Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. *Lancet Oncol* **12**: 663–672.

Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, Rush BB, Glass AG, Schiffman M (2005) The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* **97**: 1072–1079.

Kinney WB, Fetterman B, Cox JT, Lorey T, Flanagan T, Castle PE (2011) Characteristics of 44 cervical cancers diagnosed following Pap-negative, high risk HPV-positive screening in routine clinical practice. *Gynecol Oncol* **121**: 309–313.

Kjaer SK, Breugelmans G, Munk C, Junge J, Watson M, Iftner T (2008) Population-based prevalence, type- and age-specific distribution of HPV in women before introduction of an HPV-vaccination program in Denmark. *Int J Cancer* **123**: 1864–1870.

Kjaer SK, Frederiksen K, Munk C, Iftner T (2010) Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst* **102**: 1478–1488.

Kotaniemi-Talonen L, Anttila A, Malila N, Tarkkanen J, Laurila P, Hakama M, Nieminen P (2008) Screening with a primary human papillomavirus test does not increase detection of cervical cancer and intraepithelial neoplasia 3. *Eur J Cancer* **44**: 565–571.

Kovacic MB, Castle PE, Herrero R, Schiffman M, Sherman ME, Wacholder S, Rodriguez AC, Hutchinson ML, Bratti MC, Hildesheim A, Morales J, Alfaro M, Burk RD (2006) Relationships of human papillomavirus type, qualitative viral load, and age with cytologic abnormality. *Cancer Res* **66**: 10112–10119.

Laukkanen P, Laara E, Koskela P, Pukkala E, Virkkunen H, Lehtinen M (2010) Population fraction of cervical neoplasia attributable to high-risk human papillomaviruses. *Future Oncol* **6**: 709–716.

Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P, Anttila A (2009) Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. *J Natl Cancer Inst* **101**: 1612–1623.

Leinonen MK, Nieminen P, Lonnberg S, Malila N, Hakama M, Pokhrel A, Laurila P, Tarkkanen J, Anttila A (2012) Detection rates of precancerous and cancerous cervical lesions within one screening round of primary human papillomavirus DNA testing: prospective randomised trial in Finland. *BMJ* **345**: e7789.

Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM (2011) Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer* **128**: 927–935.

Meijer CJ, Snijders PJ, Castle PE (2006) Clinical utility of HPV genotyping. *Gynecol Oncol* **103**: 12–17.

Munoz N, Bosch X, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJF, Meijer CJLM (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* **348**: 518–527.

Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, Radberg T, Strander B, Forslund O, Hansson BG, Hagmar B, Johansson B, Rylander E, Dillner J (2009) Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J Natl Cancer Inst* **101**: 88–99.

Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Hansson BG, Rylander E, Dillner J (2007) HPV type-specific risks of high-grade CIN during 4 years of follow-up: a population-based prospective study. *Br J Cancer* **97**: 129–132.

Passmore JA, Morroni C, Shapiro S, Williamson AL, Hoffman M (2007) Papanicolaou smears and cervical inflammatory cytokine responses. *J Inflamm* **4**: 8.

Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NW, Heideman DA, Kenter GG, Cuzick J, Snijders PJ, Meijer CJ (2012a) Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. *Lancet Oncol* **13**: 78–88.

Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Hesselink AT, Rozendaal L, Heideman DA, Verheijen RH, Bulk S, Verweij WM, Snijders PJ, Meijer CJ (2012b) Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. *Int J Cancer* **130**: 602–610.

Rodriguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, Solomon D, Guillen D, Alfaro M, Morales J, Hutchinson M, Katki H, Cheung L, Wacholder S, Burk RD (2010) Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst* **102**: 315–324.

Rothman KJ, Greenland S, Lash TL (2008) *Modern Epidemiology*. Lippincot Williams & Wilkins: Philadelphia, PA, USA.

Sargent A, Bailey A, Turner A, Almonte M, Gilham C, Baysson H, Peto J, Roberts C, Thomson C, Desai M, Mather J, Kitchener H (2010) Optimal threshold for a positive hybrid capture 2 test for detection of human papillomavirus: data from the ARTISTIC trial. *J Clin Microbiol* **48**: 554–558.

Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC, Bratti MC, Sherman ME, Morales J, Guillen D, Alfaro M, Hutchinson M, Wright TC, Solomon D, Chen Z, Schussler J, Castle PE, Burk RD (2005) The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* **337**: 76–84.

Schmitt M, Bravo IG, Snijders PJ, Gissmann L, Pawlita M, Waterboer T (2006) Bead-based multiplex genotyping of human papillomaviruses. *J Clin Microbiol* **44**: 504–512.

Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, Mielzynska-Lohnas I, Rush BB, Schiffman M (2003) Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst* **95**: 46–52.

Shipitsyna E, Zolotoverkhaya E, Kuevda D, Nasonova V, Romanyuk T, Khachaturyan A, Orlova O, Abashova E, Kostyuchek I, Shipulina O, Anttila A, Savicheva A (2011) Prevalence of high-risk human papillomavirus types and cervical squamous intraepithelial lesions in women over 30 years of age in St. Petersburg, Russia. *Cancer Epidemiol* **35**: 160–164.

Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* **121**: 621–632.

Soderlund-Strand A, Carlson J, Dillner J (2009) Modified general primer PCR system for sensitive detection of multiple types of oncogenic human papillomavirus. *J Clin Microbiol* **47**: 541–546.

Soderlund-Strand A, Eklund C, Kemetli L, Grillner L, Tornberg S, Dillner J (2011) Genotyping of human papillomavirus in triaging of low-grade cervical cytology. *Am J Obstet Gynecol* **205**(145): e1–e6.

Sturegard E, Johansson H, Ekstrom J, Hansson BG, Johnsson A, Gustafsson E, Dillner J, Forslund O (2013) Human papillomavirus typing in reporting of condyloma. *Sex Transm Dis* **40**: 123–129.

Ucakar V, Poljak M, Klavs I (2012) Pre-vaccination prevalence and distribution of high-risk human papillomavirus (HPV) types in Slovenian women: a cervical cancer screening based study. *Vaccine* **30**: 116–120.