EUROPEAN UROLOGY 62 (2012) 1063–1070

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Infections

Editorial by Guido Barbagli, Salvatore Sansalone and Massimo Lazzeri on pp. 1071–1073 of this issue

Oral Mucosa as a Reservoir of Human Papillomavirus: Point Prevalence, Genotype Distribution, and Incident Infections Among Males in a 7-year Prospective Study

Katja Kero [a,*], *Jaana Rautava* [b], *Kari Syrjänen* [c,d], *Seija Grenman* [a], *Stina Syrjänen* [b]

[a] *Department of Obstetrics and Gynaecology, Turku University Hospital, University of Turku, Turku, Finland;* [b] *MediCity Research Laboratory and Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Department of Pathology, Turku University Hospital, Turku, Finland;* [c] *Department of Oncology and Radiotherapy, Turku University Hospital, Turku, Finland;* [d] *Teaching and Research Institute, Barretos Cancer Hospital, Barretos-SP, Brazil*

Article info

Article history:
Accepted June 20, 2012
Published online ahead of print on June 28, 2012

Keywords:
Oral
Human papillomavirus
Mouth
Frequency
Male
Prevalence
Incidence
Longitudinal study

EU ACME
www.eu-acme.org/europeanurology

Please visit www.eu-acme.org/europeanurology to read and answer questions on-line. The EU-ACME credits will then be attributed automatically.

Abstract

Background: In addition to the anogenital malignancies, human papillomavirus (HPV) has been linked to oropharyngeal cancer as an important risk factor in both men and women. Knowledge of oral HPV infection among males is needed to elucidate the transmission routes and potential for prevention.
Objective: To assess the prevalence, genotype distribution, and incidence of oral HPV infections among healthy Finnish men followed for 7 yr.
Design, setting, and participants: Oral scrapings for HPV testing were taken from 131 fathers-to-be (mean age: 28.9 yr) at baseline and at 2-mo, 6-mo, 12-mo, 24-mo, 36-mo, and 7-yr follow-up visits to detect prevalent and incident HPV infections. Purified DNA extracted from scrapings was used for HPV genotyping, with the Multimetrix kit (Progen Biotechnik, Heidelberg, Germany) detecting 24 genotypes.
Outcome measurements and statistical analysis: Point prevalence, genotype distribution, and incident rates of oral HPV infections. Demographic data were collected using structured questionnaires, and covariates of incident oral HPV infections were analysed using uni- and multivariate Poisson regression (for panel data).
Results and limitations: The point prevalence of oral HPV infection fluctuated from 15.1% to 31.1% during the follow-up period. In total, 17 different HPV genotypes were found. At baseline, the single most frequent genotype among the HPV-positive samples was HPV16 (33.3%; 8 of 24), followed by HPV33 (12.5%) and HPV82 (12.5%). Multiple-type infections comprised 16.7% (4 of 24), HPV16 being involved in all combinations. For baseline-negative men, the mean time to the first incident infection ranged from 3.9 mo (HPV82) to 25.7 mo (HPV56). None of the demographic factors was a significant independent predictor of incident oral HPV infections in multivariate models.
Conclusions: Detection of oral HPV DNA carriage in men is common, HPV16 being the most prevalent genotype. Oral mucosa may play a significant role in HPV transmission.
© 2012 European Association of Urology. Published by Elsevier B.V. All rights reserved.

* Corresponding author. Obstetrics and Gynaecology, Turku University Hospital, PO Box 52, 20521 Turku, Finland. Tel. +358 503 465 542; Fax: +358 233 38 399.
E-mail address: katja.kero@utu.fi (K. Kero).

1. Introduction

Human papillomavirus (HPV) has been linked with oropharyngeal cancer as an important causal factor in both genders, but the natural history of oral HPV is not well established [1–5]. Miller et al. (1996) were the first to address the gender aspect of oral HPV [6]. Recent data from the United States, Mexico, and Brazil have indicated that

0302-2838/$ – see back matter © 2012 European Association of Urology. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.eururo.2012.06.045

oral HPV infections in healthy individuals are more common among males than women, ranging from 4.0% [7,8] to 6.9% [9,10]. From the published data, no firm conclusions can be drawn concerning the HPV genotype distribution in oral mucosa [11].

Apart from the prospective Finnish Family HPV (FFHPV) study, few studies are reporting incident and prevalence for oral HPV infections among males. We have previously published the 2-yr follow-up results of 131 men [12,13], showing a cumulative incidence of new high-risk HPV infections that is identical in both spouses, but with virus clearance among men starting earlier than in their wives. Among 68 human immunodeficiency virus (HIV)–infected men with high-risk, low-risk, or undetermined genotype at baseline, 75% had persistent oral HPV infections [14]. Although immunodeficiency, smoking, and oral sex seem to increase the risk of oral (and other) HPV infections [9,15], HPV in the anogenital tract does not seem to predispose patients to oral HPV, even with a history of oral sex [1,16–18]. As in the genital tract, oral HPV infections in both genders seem to follow a bimodal pattern with respect to age, with a peak prevalence at 30–34 and 60–64 yr of age [10].

This study is the first to provide genotype-specific point prevalence, incidence times and rates, and the predictors of these incident infections in a 7-yr follow-up of healthy young men.

2. Materials and methods

The FFHPV study is a longitudinal cohort study, conducted at the Department of Gynaecology and Obstetrics, Turku University Hospital, and Department of Oral Pathology, University of Turku. Pregnant women ($n = 329$) in their third trimester were enrolled, together with their offspring ($n = 331$) and spouses ($n = 131$), as previously detailed [12,13,19]. The study protocol and its amendments (#2/1998 and #2/2006) have been approved by the Research Ethics Committee of Turku University Hospital. The subjects of the present analysis consist of 131 fathers (mean age: 28.7 yr; range: 19–46) followed for 7 yr (Fig. 1). In all, 58 fathers were compliant with the 7-yr follow-up visit. Demographic data were collected using structured questionnaires at baseline and at the 7-yr follow-up visit.

Oral scrapings for HPV testing were taken at baseline and at the 2-, 6-, 12-, 24-, 36-, and 84-mo follow-up visits using a brush (Cytobrush, CooperSurgical, Trumbull, CT, USA), from the buccal mucosa of both cheeks as well as from the upper and lower vestibular areas. The brush was immersed in 80% ethanol, frozen, and stored at −70 °C until used. At the 7-yr follow-up visit, a thorough clinical examination of the oral mucosa was performed for 55 compliant men.

HPV DNA was extracted from the oral scrapings using the high-salt method [20,21]. HPV testing was done with a nested polymerase chain reaction (PCR) using MY09 and MY11 as external primers and GP05$^+$/GP06$^+$ as internal primers [21]. The PCR products were first hybridised with a digoxigenin-labelled high-risk HPV oligoprobe cocktail (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, and 58) to determine whether the samples were high-risk HPV positive or negative [22].

The PCR products available from previous high-risk HPV positive or negative testing were used for HPV genotyping with the Multimetrix kit (Progen Biotechnik, Heidelberg, Germany). The assay detects the following HPV types: low-risk HPV-types 6, 11, 42, 43, 44, and 70 and high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 68, 73, and 82. The test was performed as described in the protocol, except that we reamplified the earlier nested PCR product (described above) for biotinylation with GP05$^+$ and bioGP06$^+$ primers. The hybrids were analysed using a Luminex LX100 analyser (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, CA, USA). A median fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cut-off value for each run and HPV type was 1.5 times background MFI (negative control) plus 5 MFI. All HPV16-positive samples were retested starting from the original sample using nested PCR and a bead-based HPV16 genotyping assay. This test was performed to identify possible contamination with HPV16 during the many amplifications [23].

All statistical analyses were run using SPSS v.19.0.1 (IBM Corp., Armonk, NY, USA) and Stata v.12 software (Stata Corp., College Station, TX, USA). Times (months) to incident infections were calculated from the baseline visit to the first incident event separately for actuarial and crude

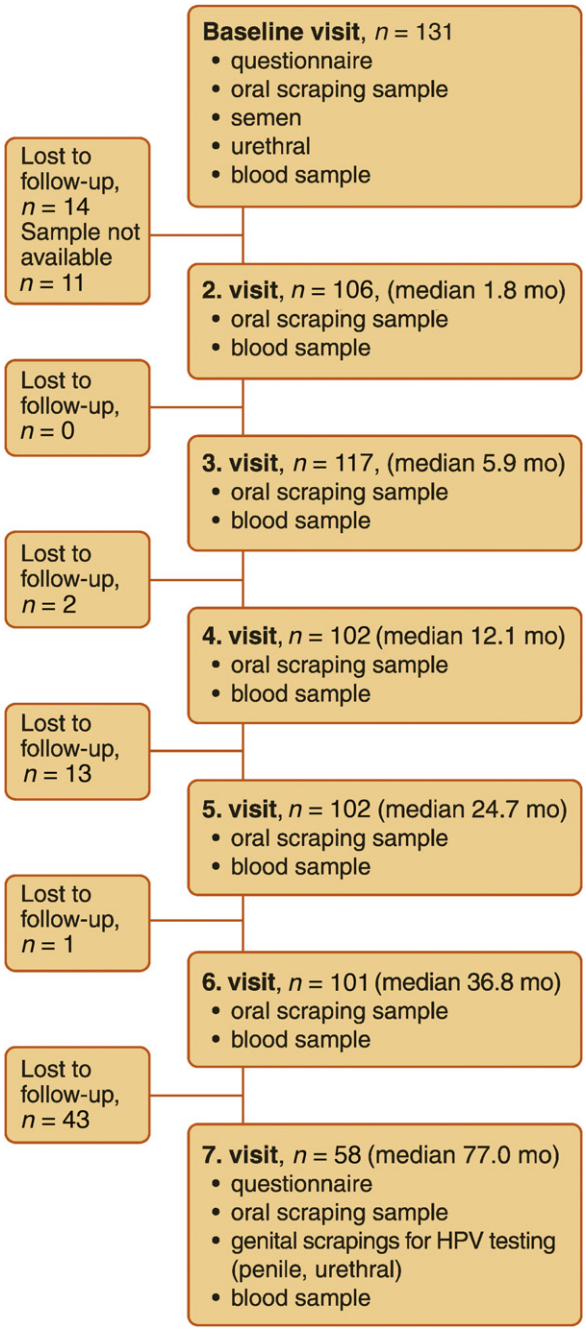

Fig. 1 – Flowchart of the study.

Table 1 – Demographic data of the fathers-to-be at baseline

Characteristic	Patients, no. (%)
Age, yr (*n* = 127)	28.9 (median: 28.0)
<20	2 (1.6)
21–30	88 (69.3)
31–40	34 (26.8)
>40	3 (2.4)
Marital status (*n* = 122):	
Single	1 (0.8)
Stable relationship	120 (98.3)
Divorced	1 (0.8)
Education (*n* = 121):	
Compulsory school	8 (6.6)
Vocational training	50 (41.3)
Upper secondary school graduate	33 (27.3)
College graduate	13 (10.7)
Academic degree	17 (14.0)
Employment status (*n* = 120):	
Employed	103 (85.8)
Student	10 (8.3)
Unemployed	7 (5.8)
Allergic symptoms (*n* = 118):	
No	71 (60.2)
Yes	47 (39.8)
Atopic symptoms (*n* = 118):	
No	110 (93.2)
Yes	8 (6.8)
No. of children at lifetime	1.3 (1–4)
sexual relationships (*n* = 115):	
1	65 (56.5)
2	30 (26.1)
3	12 (10.4)
4	5 (4.3)
5	2 (1.7)
6	1 (0.9)
Age at first intercourse, yr (*n* = 121):	
<13	3 (2.5)
14–16	49 (40.5)
17–19	58 (47.9)
>20	11 (9.1)
No. of lifetime sexual partners (*n* = 121):	
0–2	16 (13.2)
3–5	27 (22.3)
6–10	26 (21.5)
>10	52 (43.0)
No. of lifetime sexual partners <20 yr of age (*n* = 120):	
0–2	49 (40.8)
3–5	40 (33.3)
6–10	19 (15.8)
>10	12 (10.0)
No. of sexual partners during this pregnancy (*n* = 118):	
1	115 (97.5)
2–5	2 (1.7)
>5	1 (0.8)
No. of intercourse episodes per month (*n* = 119):	
0–1	1 (0.8)
2–4	42 (35.3)
5–10	57 (47.9)
>10	19 (16.0)
Practices oral sex (*n* = 120):	
Never	14 (11.7)
Occasionally	76 (63.3)
Regularly	30 (25.0)
Practices oral sex: father (*n* = 120):	
Yes	106 (88.3)
No	14 (11.7)
Practices anal sex (*n* = 120):	
Never	94 (78.3)
Occasionally	26 (21.7)
Smoking (*n* = 119):	
Never	75 (63.0)
1–10 cigarettes per day	16 (13.4)
11–20 cigarettes per day	18 (15.1)
>20 cigarettes per day	10 (8.4)
Age at onset of smoking, yr (*n* = 49):	
10–13	12 (24.5)
14–17	29 (59.2)
18–21	6 (12.2)
22–25	1 (2.0)
>25	1 (2.0)
Use of snuff (*n* = 112):	
Never	106 (94.6)
1 can per month	3 (2.7)
2 cans per week	3 (2.7)
Use of alcohol (*n* = 120):	
Never	3 (2.5)
1 dose per day	4 (3.3)
1 dose 2–3 times per week	43 (35.8)
1 dose per week	44 (36.7)
1 dose per month	26 (21.7)
History of STD (*n* = 44):	
None	20 (45.5)
Chlamydia trachomatis	16 (36.4)
Genital HSV	3 (6.8)
Multiple STDs	5 (11.4)
History of genital warts (*n* = 113):	
No	94 (83.2)
Yes	19 (16.8)
Age at diagnosis of genital warts, yr (*n* = 93):	
Never	74 (79.6)
<20	4 (4.3)
20–24	10 (10.8)
>25	5 (5.4)
Treatment of genital warts (*n* = 23):	
No treatment	4 (17.4)
Topical treatment	15 (65.2)
Laser therapy	1 (4.3)
Several treatments	3 (13.0)
History of oral warts (*n* = 112):	
Never	104 (92.9)
Yes, no treatment	7 (6.3)
Yes, surgical treatment	1 (0.9)
Skin warts (*n* = 115):	
Never	63 (54.8)
Hands	30 (26.1)
Feet	14 (12.2)
Multiple sites	8 (7.0)

STD = sexually transmitted disease; HSV = herpes simplex virus.

times. In the former, all baseline HPV-negative men (*n* = 107) were included in Kaplan-Meier analysis, with cases censored at the first incident event or at the end of follow-up (those with no incident event). To calculate the genotype-specific (crude) incidence times, only those 47 men with an incident event were included. Both actuarial and crude genotype-specific incidence rates were calculated (expressed per 1000 person-months at risk [pmr]), following the same principle, that is, the number of incident events divided by the total pmr (2778 mo for all baseline HPV-negative men and 436 mo for the 47 men with incident events). We analysed the predictors of species-specific incident infections (species 5, 6, 7, and 9) with the Poisson regression models for panel data, using a population-averaged model [24,25]. The follow-up visit was the time variable in the panel settings, and incident HPV infection (count variable) was the dependent variable, with the Poisson log link function, independent within-group correlation structure, and robust variance estimator (of 95% confidence interval [CI]) to account for within-subject correlation. All covariates previously implicated as potential risk factors of HPV in this cohort [12] were first tested in univariate Poisson models, followed by the final multivariate model, with

all significant (or borderline significant) univariates entered. All statistical tests were two sided and declared significant at a p value <0.05.

3. Results

The demographic data of the cohort at baseline are presented in Table 1. At baseline, 18.6% ($n = 24$ of 131) of the oral samples tested HPV positive. In these asymptomatic males, oral HPV infection was used to denote HPV carriage, unless otherwise defined. The single most frequent genotype among the HPV-positive samples was HPV16 (33.3%; 8 of 24), followed by HPV33 (12.5%; 3 of 24) and HPV82 (12.5%; 3 of 24). Multiple-type infections comprised 16.7% ($n = 4$ of 24) of all HPV-positive cases, with HPV16 invariably involved in them.

HPV prevalence fluctuated during the follow-up period. At the second visit (median: 1.8 mo), HPV prevalence increased to 31.1% (33 of 106), then declined to 24.8% (29 of 117) at the third visit (median: 5.9 mo) and to 18.8% (22 of 115) at the fourth visit (median: 12.1 mo), with a further increase to 23.1% (21 of 102) at the fifth visit (median: 24.0 mo). At 36 mo (median: 36.8), the prevalence declined to 15.8% ($n = 16$), with HPV16 covering 68.8% (11 of 16) of the cases. After 7 yr (median: 77.0 mo), HPV prevalence was unchanged at 15.1% (14 of 58), with HPV16 now comprising 71.5% (10 of 14) of these cases. There were no multiple-type infections at the 7-yr follow-up visit. HPV6 was common at the 2-mo ($n = 6$; 18.2%), 6-mo ($n = 4$; 13.8%), and 12-mo ($n = 5$; 22.7%) visits, the prevalence rate decreasing afterwards. Only

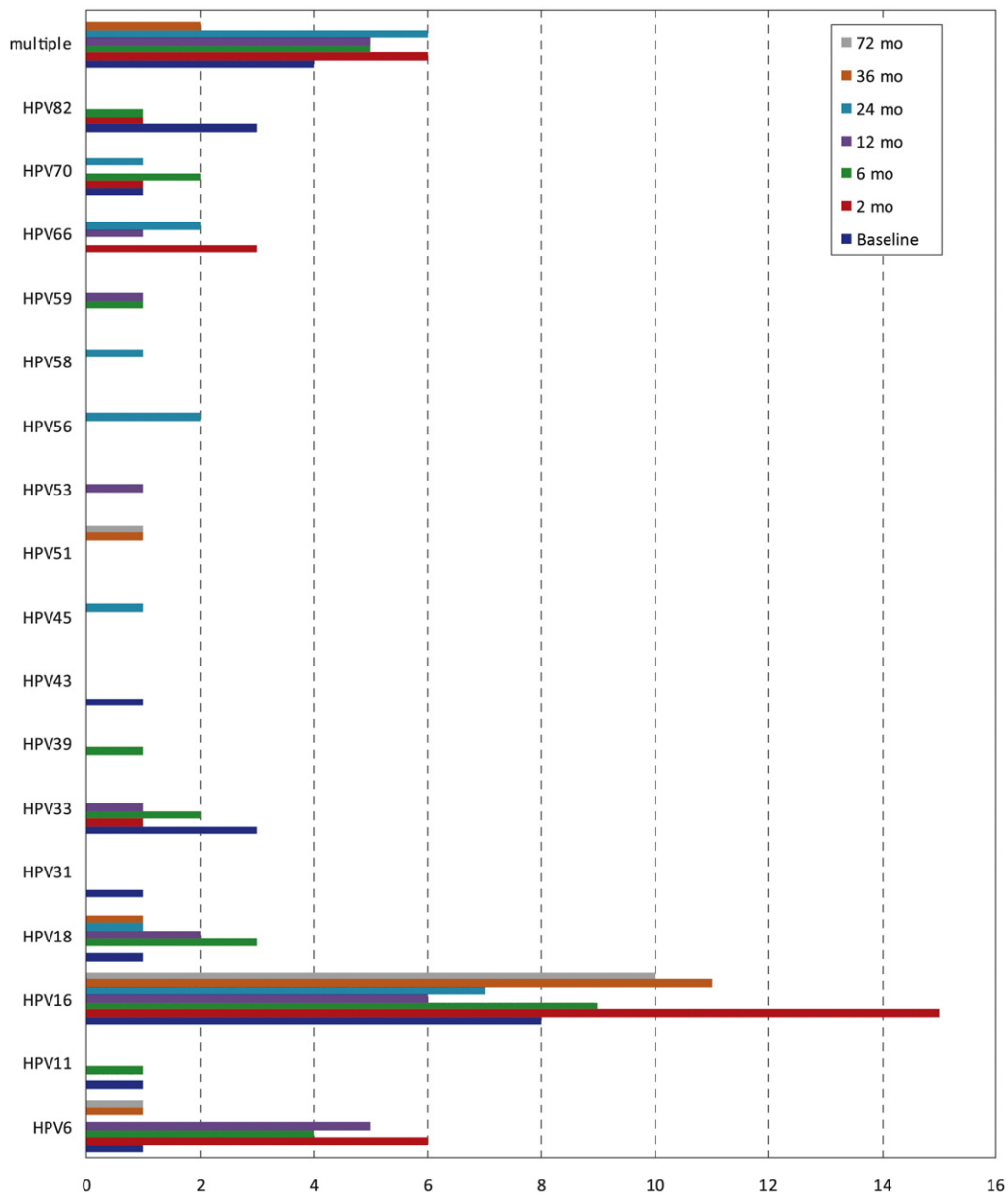

Fig. 2 – Genotype distribution of oral human papillomavirus (HPV) infections during the follow-up. The individual colours represent different follow-up points (months). Different HPV genotypes are marked on the *y*-axis, while the number of cases is presented on the *x*-axis.
HPV = human papillomavirus.

Table 2 – Point prevalence of oral human papillomavirus genotypes during the follow-up

	Baseline		2 mo		6 mo		12 mo		24 mo		36 mo		72 mo	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HPV+	24	18.3	33	31.1	29	24.8	22	18.8	21	23.1	16	15.8	14	15.1
HPV−	107	81.7	73	69.9	88	75.2	93	81.2	81	76.9	85	84.2	44	84.9
Genotype (% of HPV$^+$ cases)														
HPV6	1	4.2	6	18.2	4	13.8	5	22.7	–	–	1	6.3	1	7.1
HPV11	1	4.2	–	–	1	3.4	–	–	–	–	–	–	–	–
HPV16	8	33.3	15	45.5	9	31.0	6	27.3	7	33.3	11	68.8	10	71.4
HPV18	1	4.2	–	–	3	10.3	2	9.0	1	4.8	1	6.3	–	–
HPV31	1	4.2	–	–	–	–	–	–	–	–	–	–	–	–
HPV33	3	12.5	1	3.0	2	6.9	1	4.5	–	–	–	–	–	–
HPV39	–	–	–	–	1	3.4	–	–	–	–	–	–	–	–
HPV43	1	4.2	–	–	–	–	–	–	–	–	–	–	–	–
HPV45	–	–	–	–	–	–	–	–	1	4.8	–	–	–	–
HPV51	–	–	–	–	–	–	–	–	–	–	1	6.3	1	7.1
HPV53	–	–	–	–	–	–	1	4.5	–	–	–	–	–	–
HPV56	–	–	–	–	–	–	–	–	2	9.5	–	–	–	–
HPV58	–	–	–	–	–	–	–	–	1	4.8	–	–	–	–
HPV59	–	–	–	–	1	3.4	1	4.5	–	–	–	–	–	–
HPV66	–	–	3	9.0	–	–	1	4.5	2	9.5	–	–	–	–
HPV70	1	4.2	1	3.0	2	6.9	–	–	1	4.8	–	–	–	–
HPV82	3	12.5	1	3.0	1	3.4	–	–	–	–	–	–	–	–
Multiple HPV types	4	16.7	6	–	5	–	5	–	6	–	2	12.5	–	–
Distribution of HPV species (% of HPV+ cases)														
Species 5 (HPV26, 51, 69, 82)	5	–	1	–	–	–	1	–	–	–	2	–	3	–
Species 6 (HPV30, 53, 56, 66)	–	–	5	–	1	–	2	–	6	–	1	–	–	–
Species 7 (HPV18, 39, 45, 59, 68, 70, 85)	2	–	4	–	8	–	5	–	9	–	1	–	1	–
Species 8 (HPV7, 40, 43, 91)	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Species 9 (HPV16, 31, 33, 35, 52, 58, 67)	17	–	23	–	16	–	14	–	12	–	13	–	12	–
Species 10 (HPV6, 11, 13, 44, 55, 74)	3	–	6	–	8	–	5	–	–	–	1	–	1	–

HPV = human papillomavirus.

two cases of HPV11 were detected during the follow-up period (Fig. 2; Table 2).

Among the baseline HPV-negative men, the mean time to the first incident infection ranged from 3.9 mo (HPV82) to 25.7 mo (HPV56) (Table 3). HPV16 was present in 29.8% of incident events, seconded by HPV6 (12.6%). Incident infections by multiple types were found in 19.1% of the events. In univariate and multivariate panel Poisson regression models, none of the questionnaire-recorded covariates was significantly associated with incident high-risk HPV infections (Table 4).

4. Discussion

The present study is the first to provide the genotype-specific point prevalence and incidence of oral HPV infections among young, sexually active, healthy men. Despite the fact that all men were living in a stable heterosexual relationship, the point prevalence of oral HPV infection was considerably high, fluctuating from 15.1% to 31.1% during follow-up. These figures are substantially higher than those reported in the recent studies from the United States, with prevalence ranging between 4% and 10.1% [8–10]. There are several potential reasons for these disparities, including the different study designs, composition of the cohorts, and length of follow-up, as well as major differences in the sampling and HPV testing methods. These potential reasons are addressed in detail in our recent review [3]. For optimal results, we used scraping samples from nonkeratinised buccal mucosa combined with highly purified DNA for nested PCR, including reamplification of the nested PCR product for the highly sensitive Multimetrix HPV genotyping assay. In genital samples, this approach increases HPV detection rate two-fold [17].

The data on HPV genotype distribution in oral mucosa are scanty and in part inconclusive. Thus, Beachler et al. did not disclose any dominant genotype in oral samples of 547 men [15]. In their cohort of older males, two cases of HPV58 and three cases of HPV62 were found, while all other high-risk HPV genotypes and low-risk HPV genotypes were present as single infections [4]. According to the study by Kreimer et al, oral HPV16 was rare among young healthy men [8]. In another recent study, the prevalence of HPV16 was significantly higher in men than in women (1.6% vs 0.3%; $p < 0.00.1$), but no detailed genotype-specific data were presented among males [10].

In the present cohort, the acquisition time of incident oral HPV varied from 3.9 (HPV82) to 25.7 (HPV56) mo of follow-up. Interestingly, most of the incident infections occurred during the first 6 mo of follow-up. The present cohort is unique, consisting of families enrolled in the third trimester of pregnancy. Pregnancy constitutes an immunologically depressive state, with profound changes in the hormonal status of the mother. As recently discussed, it also inevitably changes the sexual functions of the couples, with potential impact on the genotype-specific incidence rates [26]. We have previously reported collective (non–genotype-specific) data on oral high-risk HPV infections in this same

Table 3 – Times to incident oral infections and incidence rates for individual human papillomavirus genotypes and species

	Incident infection		Mean time to first incident infection		IR per 1000 pmr			
	No.	%	Mean		Actuarial[1] IR (95% CI)	Events/ pmr	Crude[2] IR (95% CI)	Events/ pmr
HPV genotype:								
HPV6	6	12.8	6.2	2.2–10.1	2.15 (0.43–3.88)	6/2.778	162.1 (43.4–280.9)	6/37.1
HPV11	1	2.1	6.2	–	0.36 (-0.03 to 1.06)	1/2.778	161.2 (−131.0 to 464.8)	1/6.2
HPV16	14	29.8	6.3	0.4–12.1	5.04 (2.41–7.67)	14/2.778	159.1 (82.6–235.5)	14/88
HPV18	4	3.7	8.6	3.6–13.6	1.43 (0.02–2.84)	4/2.778	117.6 (9.3–225.9)	4/34.3
HPV33	2	4.3	9.0	1.9–16.0	0.72 (−0.02 to 1.71)	2/2.778	111.1 (−34.0 to 256.3)	2/18
HPV39	1	2.1	4.8	–	0.36 (−0.03 to 1.06)	1/2.778	208.3 (−150.9 to 550.6)	1/4.8
HPV53	1	2.1	8.5	–	0.36 (−0.03 to 1.06)	1/2.778	117.6 (−0.94 to 316.4)	1/8.5
HPV56	1	2.1	25.7	–	0.36 (−0.03 to 1.06)	1/2.778	38.4 (−3.5 to 112.4)	1/25.7
HPV59	1	2.1	6.3	–	0.36 (−0.03 to 1.06)	1/2.778	158.7 (−131.5 to 464.9)	1/6.3
HPV66	4	8.5	13.5	0.1–27.9	1.43 (0.02–2.84	4/2.778	74.1 (4.2–143.9)	4/54
HPV70	2	4.3	13.5	0.1–35.4	0.72 (−0.02 to 1.71)	2/2.778	74.1 (−24.7 to 172.8)	2/27
HPV82	1	2.1	3.9	–	0.36 (−0.03 to 1.06)	1/2.778	256.4 (−174.4 to 674.3)	1/3.9
Multiple types	9	19.1	13.6	4.7–22.4	3.23 (1.12–5.35)	9/2.778	73.8 (27.4–120.1)	9/122
HPV species:								
Species 10 (6, 11, 13, 44, 55, 74)	7	18.4	5.4	2.6–8.1	2.51 (0.65–4.38)	7/2.778	162.1 (52.4–273.1)	7/43.2
Species 9 (16, 31, 33, 35, 52, 58, 67)	16	42.1	6.6	1.4–11.8	5.75 (2.94–8.57)	16/2.778	150.9 (82.7–219.0)	16/106
Species 7 (18, 39, 45, 59, 68, 70, 85)	8	21.1	11.2	5.2–17.2	2.87 (0.88–4.87)	8/2.778	110.7 (38.5–183.7)	8/72.3
Species 6 (30, 53, 56, 66)	6	15.8	14.7	4.9–24.4	2.15 (0.43–3.88)	6/2.778	68.1 (15.5–120.8)	6/88.2
Species 5 (26, 51, 69, 82)	1	2.6	3.9	–	0.36 (−0.03 to 1.06)	1/2.778	256.4 (−174.4 to 674.3)	1/3.9
					*RR		**RR	

IR = incident rate; pmr = person-month at risk; HPV = human papillomavirus; CI = confidence interval; RR = rate ratio.
* Comparison between species using RR: Sp9 vs Sp7: RR = 2.00 (0.87–4.59), $p = 0.107$; Sp9 vs Sp10: RR = 2.28 (0.96–5.41), $p = 0.063$; Sp9 vs Sp5: RR = 16.0 (3.59–71.25), $p = 0.0001$; Sp9 vs Sp6: RR = 2.66 (1.08–6.56), $p = 0.034$.
** Comparison between species: Sp9 vs Sp7: RR = 1.35 (0.58–3.16), $p = 0.491$; Sp9 vs Sp10: RR = 0.92 (0.38–2.25), $p = 0.848$; Sp9 vs Sp5: RR = 0.60 (0.08–4.45), $p = 0.592$; Sp9 vs Sp6: RR = 2.21 (0.88–5.52), $p = 0.091$.
[1] All baseline HPV-negative men at risk, months at risk calculated (1) until the first incident event or (2) as total follow-up months for those with no incident event (total pmr = 2778).
[2] Only men with an incident event (months at risk calculated until the first incident event).

Table 4 – Predictors of incident oral high-risk HPV infections in a univariate and multivariate Poisson regression model

Covariate	Incident* oral high-risk** HPV infections					
	Crude IRR	95% CI	*p*	Adjusted IRR	95% CI	*p*
Age (at each follow-up visit)	1.00	0.93–1.07	1.000	1.01	0.99–1.01	0.211
Father seroconverted to high-risk HPV (not ref)	1.00	0.41–2.38	1.000	*0.97*	*0.95–1.01*	*0.092*
Father seropositive to high-risk HPV at baseline (negative ref)	1.54	0.85–2.78	0.152	1.01	0.93–1.10	0.717
Baseline oral high-risk HPV DNA status (HPV- ref)	0.83	0.41–1.71	0.631	0.98	0.89–1.06	0.663
Marital status at baseline (single ref)	1.00	0.53–1.85	1.000	1.01	0.97–1.06	0.457
Employment status (employed; ref)	1.00	0.47–2.08	1.000	*0.96*	*0.93–1.01*	*0.090*
Age at onset of sexual activity (<13 yr of age ref)	1.00	0.13–7.27	1.000	**	–	–
No. of sexual partners until 20 yr of age (0–2 ref)	1.00	0.73–1.35	1.000	0.98	0.97–1.01	0.257
Lifetime no. of sexual partners (0–2 ref)	1.00	0.76–1.31	1.000	0.99	0.97–1.01	0.264
No. of weekly intercourse episodes (no trend)	1.00	0.60–1.64	1.000	1.01	0.97–1.03	0.964
No. of deliveries in all partnerships	1.00	0.75–1.33	1.000	1.01	0.99–1.02	0.274
Practices oral sex (no; ref)	1.00	0.39–2.55	1.000	1.01	0.95–1.07	0.765
Practices anal sex (never; ref)	1.00	0.46–2.17	1.000	0.98	0.95–1.01	0.228
Smoking habits (never; ref)	1.00	0.52–1.89	1.000	1.01	0.97–1.03	0.632
Initiation of smoking (10–13 yr of age ref)	1.00	0.57–1.72	1.000	**	–	–
History of STD (yes ref)	1.00	0.78–1.26	1.000	0.98	0.96–1.01	0.317
History of genital warts (no; ref)	1.00	0.75–1.33	1.000	1.03	0.98–1.08	0.173
History of oral warts (yes history; ref)	1.00	0.70–1.41	1.000	**	–	–
Change in marital status during follow-up	1.00	0.84–1.15	1.000	**	–	–
No. of current sexual partners (0 ref)	1.00	0.60–1.64	1.000	**	–	–

HPV = human papillomavirus; IRR = incidence rate ratio; CI = confidence interval; STD = sexually transmitted disease.
Poisson regression (population-averaged model) for panel data, clustered by male-ID, follow-up visits as time variable, independent within-group correlation structure, 95% CI calculated by robust variance estimator (vce); adjusted for age and all other covariates; dropped from the model because of co-linearity.
* Count outcome (incident events/person time at risk), as defined by the first incident event among baseline HPV-negative men during the follow-up.
** Species 7 (HPV genotypes: 18, 39, 45, 59, 68, 70, 85); Species 9 (HPV genotypes: 16, 31, 33, 35, 52, 58, 67); Species 6 (HPV genotypes 30, 53, 56, 66); Species 5 (HPV genotypes 26, 51, 69, 82).

cohort of fathers, of whom 10% acquired an incident high-risk HPV infection within 24 mo of follow-up [27]. In the absence of other studies reporting incident oral HPV infections in men, there is no reference with which to compare the current incidence times and rates. As expected, different genotypes showed considerable variation in both of these variables, which is commensurate with the results of our yet-unpublished data from the mothers in this cohort, showing that HPV16 and multiple-type combinations most frequently caused incident oral infections (65% and 12%, respectively) among the mothers within 13.3 and 17.1 mo, respectively. Second in order were HPV58, HPV18, and HPV6, causing incident infections within 11–24 mo (unpubl. data, Karolina Louvanto, Turku, Finland). As is always the case, two optional (not mutually exclusive) mechanisms can explain the newly detected (incident) HPV: (1) a new horizontal transmission or (2) reactivation of a prior infection. At present, there is no means to distinguish between these two mechanisms if the predefined definition used here is followed, namely, that incident events are only recorded in baseline HPV-negative subjects.

In the present cohort, HPV16 was the most frequent genotype at all follow-up visits, followed by multiple-type infections, which is in line with the report of Kreimer et al. [8]. The previously rare low-risk genotype HPV55 has recently been discovered with a higher prevalence in oral samples [8,28]. Although most previous studies have reported genotypes 6, 11, 16, 35, 39, 42, 51, 52, 56, 58, 59, 61, 62, 66, 68, 73, 84, and 89, we found the following HPV genotypes: 6, 11, 16, 18, 31, 33, 39, 43, 45, 51, 53, 56, 58, 59, 66, 70, and 82. Obviously, the genotype pattern detected depends on the coverage of the HPV genotyping method. Because the numbers of analysed oral samples of men are still limited, it is too early to conclude that new genotypes are conquering new anatomic sites. In our previous report, the spectrum of HPV genotypes was assessed in urethral, penile, and semen samples of this same cohort of fathers [26]. Compared with the present data, the HPV genotype spectrum in the oral samples seems to be wider than in the genital tract (HPV6, 11, 16, 18, 33, 43, 51, 53, 56, 66, and 70) [26]. Genotypes 31, 39, 45, 51, 56, 58, and 82 were detected only in the oral samples. However, these genotypes have previously been reported to affect genital sites, as well.

Immunodeficiency, smoking, French kissing, and oral sex seem to increase the risk for oral HPV infection, both in healthy men and in HIV-positive men [7,9,15]. In addition to sexual transmission, oral HPV can be transmitted from mouth to mouth during kissing or even in ordinary family life as well as vertically from a mother to her newborn during delivery [3,12,29]. Males in our cohort generally had had their sexual debut years before study enrolment, which contrasts with the study of D'Souza et al, which reported a low prevalence (2.9%) among young college students, of whom 9.0% had never engaged in open-mouthed kissing, 28% had never performed oral sex, and 30% had never had vaginal sex [9]. In a recent study consisting of 396 HIV-seronegative and 190 HIV-seropositive adults, HPV prevalence increased with age, male sex (odds ratio [OR]: 3.0), and genital herpes simplex-2 seropositivity (OR: 2.7), while oral–genital contact was not significantly associated with oral HPV infection among HIV-seronegative individuals [7]. This is consistent with the present observations, where multivariate analysis failed to disclose any significant association between the sexual habits (or any of the demographic characteristics) and incident oral high-risk HPV infections (Table 4).

As to the oral HPV infection, conflicting evidence on age dependence has been reported, in contrast with, for example, cervical HPV infections, with prevalence declining in parallel with increasing age [30]. In a cohort of 1688 healthy men 18–74 yr of age, HPV was present in 4% of all cases but in 6% of the older men (>55 yr of age) [8]. In another recent study, oral high-risk HPV infections showed a bimodal age pattern, with peak prevalence at 30–34 yr of age and another peak at 60–64 yr of age [10]. This finding is fully consistent with our observations on cervical HPV infections in a population-based cohort of women, where the second peak parallels the age of menopause [30]. This bimodal prevalence pattern allows speculations on the role of increased incidence, reactivation of latent infections linked with age-related changes in immunity, and increased persistence of oral high-risk HPV among older populations. The skewed age distribution of our cohort precludes any visualisation of such an eventual second peak prevalence of oral HPV infections in these males, however.

The morphologic manifestations of HPV infections in oral mucosa are well described in HPV textbooks [5]. To elucidate the clinical relevance of the HPV carriage detected in the present cohort, these men were also subjected to thorough clinical examination of their oral mucosa at the 7-yr follow-up visit. Of the 55 compliant fathers, 28 presented with some type of clinical lesion; all together, 17 different types of lesions were detected. These did not include a single case of oral wart, oral condyloma, or dysplasia, but one leukoplakia and five hyperkeratosis instances were found. Unfortunately, biopsies were not allowed to confirm the possible HPV association of these lesions. Given this meagre evidence on clinical lesions, it seems likely that the vast majority of the HPV-positive cases represent asymptomatic HPV carriage rather than clinically significant infections.

5. Conclusions

The present data substantiate our past hypothesis on oral mucosa as an important reservoir of HPV, where gingival pockets would represent the potential source of HPV transmission. In the present cohort of asymptomatic males, oral HPV DNA detection was common. Understanding the correlation between asymptomatic HPV carriage and HPV-associated disease is essential for accurately estimating the potential disease burden preventable by prophylactic HPV vaccines.

Author contributions: Katja Kero had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: S. Syrjänen, Grenman.
Acquisition of data: S. Syrjänen, Grenman.

Analysis and interpretation of data: Kero, Rautava.
Drafting of the manuscript: Kero, Rautava, K. Syrjänen.
Critical revision of the manuscript for important intellectual content: S. Syrjänen, K. Syrjänen, Grenman.
Statistical analysis: K. Syrjänen.
Obtaining funding: Kero.
Administrative, technical, or material support: None.
Supervision: S. Syrjänen.
Other (specify): None.

Financial disclosures: Katja Kero certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: The Finnish Family HPV study has been supported by the Academy of Finland, Finnish Cancer Foundation, Sohlberg Foundation, the Government Special Foundation (EVO) to Turku University Hospital, and the Finnish Society Against Sexually Transmitted Infections.

Acknowledgement statement: The authors would like to thank D. Marjut Rintala, Dr. Virpi Rantanen, and midwife Elisa Hovimäki for participating in the enrolment of the women in the Finnish Family HPV Study and their clinical follow-up. The skilful technical assistance of Mrs Tatjana Pescova, Mariia Henttinen, and Keitlin Adel is gratefully acknowledged.

References

[1] Kellokoski J, Syrjänen S, Syrjänen K, Yliskoski M. Oral mucosal changes in women with genital HPV infection. J Oral Pathol Med 1990;19:142–8.
[2] Syrjänen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. Crit Rev Oral Biol Med 2000;11:259–74.
[3] Rautava J, Syrjänen S. Human papillomavirus infections in the oral mucosa. J Am Dent Assoc 2011;142:905–14.
[4] D'Souza G, Dempsey A. The role in head and neck cancer and review of the HPV vaccine. Prev Med 2011;53(Suppl 1):S5–11.
[5] Syrjänen K, Syrjänen S. Papillomavirus infections in human diseases. New York: J. Wiley & Sons; 2000.
[6] Miller CS, White DK. Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:57–68.
[7] Kreimer A, Alberg A, Daniel R, et al. Oral human papillomavirus infection in adults is associated with sexual behaviour and HIV serostatus. J Infect Dis 2004;189:686–98.
[8] Kreimer AR, Villa A, Nyitray AG, et al. The epidemiology of oral HPV infection among a multinational sample of healthy men. Cancer Epidemiol Biomarkers Prev 2011;20:172–82.
[9] D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009;199:1263–9.
[10] Gillison ML, Broutian T, Pickard RK, et al. Prevalence of oral HPV infection in the United States, 2009–2010. JAMA 2012;15:693–703.
[11] D'Souza G, Fakhry C, Sugar EA, et al. Six-month history of oral versus cervical human papillomavirus infection. Int J Cancer 2007;121: 143–50.
[12] Rintala MA, Grénman SE, Puranen MH, et al. Transmission of high-risk human papillomavirus (HPV) between parents and infants: a prospective study of HPV in families in Finland. J Clin Microbiol 2005;43:376–81.
[13] Rintala MA, Grénman SE, Järvenkylä ME, Syrjänen KJ, Syrjänen SM. High risk types of human papillomavirus (HPV) DNA in oral and genital mucosa of infants during their first 3 years of life: experience from the Finnish HPV Family Study. Clin Infect Dis 2005;15: 1728–33.
[14] Parisi SG, Cruciani M, Scaggiante R, et al. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. BMC Infect Dis 2011;25:11–150.
[15] Beachler DC, Weber KM, Margolick JB, et al. Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative adults. Cancer Epidemiol Biomarkers Prev 2012;21:122–33.
[16] Castro TM, Bussoloti Filho I, Nascimento VX, Xavier SD. HPV detection in the oral and genital mucosa of women with positive histopathological exam for genital HPV, by means of the PCR. Braz J Otorhinolaryngol 2009;75:167–71.
[17] Termine N, Giovannelli L, Matranga D, et al. Low rate of oral human papillomavirus (HPV) infection in women screened for cervical HPV infection in southern Italy: a cross-sectional study of 140 immunocompetent subjects. J Med Virol 2009;81:1438–43.
[18] Xavier SD, Bussoloti Filho I, de Carvalho JM, Castro TM, Framil VM, Syrjänen KJ. Prevalence of human papillomavirus (HPV) DNA in oral mucosa of men with anogenital HPV infection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:732–7.
[19] Louvanto K, Rintala MA, Syrjänen KJ, Grenman SE, Syrjänen SM. Genotype-specific persistence of genital human papillomavirus (HPV) infections in women followed for 6 years in the Finnish Family HPV Study. J Infect Dis 2010;202:436–44.
[20] Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
[21] Snijders PJ, van den Brule AJ, Schrijnemakers HF, Snow G, Meijer CJ, Walboomers JM. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. J Gen Virol 1990;71:173–81.
[22] Anttila M, Syrjänen S, Ji H, Saarikoski S, Syrjänen K. Failure to demonstrate human papillomavirus DNA in epithelial ovarian cancer by general primer PCR. Gynecol Oncol 1999;72:337–41.
[23] Schmitt M, Bravo IG, Snijders PJ, Gissman L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol 2006;44:504–12.
[24] Zeger SL, Diggle PJ. Semiparametric models for longitudinal data with application to CD4 cell numbers in HIV seroconverters. Biometrics 1994;50:689–99.
[25] Diggle PJ, Liang K-Y, Zeger SL. Analysis of longitudinal data. ed. 1 Oxford, UK: Oxford University Press; 1994.
[26] Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Human papillomavirus genotypes in male genitalia and their concordance among pregnant spouses participating in the Finnish Family HPV Study. J Sex Med 2011;8:2522–31.
[27] Rintala M, Grénman S, Puranen M, Syrjänen S. Natural history of oral papillomavirus infections in spouses: a prospective Finnish HPV Family Study. J Clin Virol 2006;35:89–94.
[28] Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:4294–301.
[29] Sarkola ME, Grénman SE, Rintala MA, Syrjänen KJ, Syrjänen SM. Human papillomavirus in the placenta and umbilical cord blood. Acta Obstet Gynecol Scand 2008;87:1181–8.
[30] Syrjänen S, Shabalova I, Petrovichev N, et al., NIS Cohort Study Group. Age-specific incidence and clearance of high-risk human papillomavirus infections in women in the former Soviet Union. Int J STD AIDS 2005;16:217–23.